CLINICAL MEDICINE

Medical Science of Ukraine
Медична наука України

ISSN: 2664-472X
e-ISSN: 2664-4738
Vol. 22, No. 2, 2026

UDC 617.713-002.28-022.6:616.379-008.64:577.112:355.01(477) Journal homepage: https://nmuofficial.com/zhurnal-medychna-nauka-ukrayiny/
DOI: https://doi.org/10.32345/2664-4738.2.2026.01 Received: 16.01.2026 Revised: 29.04.2026 Accepted: 28.05.2026 Published: 30.06.2026

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Tear fluid levels of interleukin-6 and tight junction protein ZO-1 and their association with the course of bacterial keratitis in type 2 diabetes mellitus under martial law in Ukraine

Abstract

Relevance. Bacterial keratitis is one of the most common infectious diseases of the cornea and may lead to severe complications, persistent visual impairment, and disability.

Aim. To investigate the levels of interleukin-6 and the tight junction protein zonula occludens-1 in the tear fluid of patients with bacterial keratitis according to the presence of type 2 diabetes mellitus and to assess their association with the clinical course and development of disease complications.

Materials and methods. A prospective comparative multicentre cohort study involving 88 participants was conducted, including patients with bacterial keratitis with and without type 2 diabetes mellitus, as well as healthy controls. Concentrations of interleukin-6 and the tight junction protein zonula occludens-1 in tear fluid were determined using enzyme-linked immunosorbent assay (ELISA).

Results. Significantly elevated concentrations of interleukin-6 and zonula occludens-1 were detected in patients with bacterial keratitis compared with the control group. The highest levels of both biomarkers were observed in patients with concomitant type 2 diabetes mellitus. The concentration of interleukin-6 in patients with bacterial keratitis without diabetes mellitus was 245 [170;390] pg/mL, whereas in patients with concomitant type 2 diabetes mellitus it reached 430 [310;610] pg/mL. The level of zonula occludens-1 was 6.8 [4.9;9.5] ng/mL and 9.6 [7.2;13.4] ng/mL, respectively. Statistically significant positive correlations were found between the levels of the investigated biomarkers and the thickness, width, and area of corneal infiltrates. The strongest correlations were observed between interleukin-6 concentration and infiltrate thickness ($\rho = 0.74$; $p < 0.001$), as well as between zonula occludens-1 level and infiltrate thickness ($\rho = 0.76$; $p < 0.001$) in patients with type 2 diabetes mellitus. Patients with infiltrate ulceration, delayed epithelialisation, and stromal opacity demonstrated significantly higher levels of both biomarkers compared with those without a complicated disease course.

Conclusions. Bacterial keratitis is associated with increased levels of interleukin-6 and the tight junction protein zonula occludens-1 in tear fluid. The investigated biomarkers were associated with morphometric characteristics of corneal infiltrates and the development of clinical complications of the disease.

Keywords: infectious corneal disease; diabetic keratopathy; proinflammatory cytokines; corneal infiltrate; biomarkers.

Suggested Citation:
Hudz A, Kadubets S. Tear fluid levels of interleukin-6 and tight junction protein ZO-1 and their association with the course of bacterial keratitis in type 2 diabetes mellitus under martial law in Ukraine. Med Sci Ukr. 2026;22(2):5–13. DOI: https://doi.org/10.32345/2664-4738.2.2026.01

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INTRODUCTION

Bacterial keratitis is one of the most common and potentially sight-threatening infectious diseases of the cornea, as it may lead to the development of corneal ulceration, scarring, opacification, and even corneal perforation, resulting in permanent impairment of visual function. Patients with type 2 diabetes mellitus represent a particular clinical challenge because disturbances in glucose metabolism, chronic inflammation, and delayed tissue repair are associated with a more severe disease course and an increased risk of complications. The problem has become even more relevant in Ukraine under martial law, where restricted access to timely specialist ophthalmic care and pathogenetically targeted treatment may adversely affect the course of bacterial keratitis, promote the development of complications, and worsen functional treatment outcomes, particularly in patients with concomitant type 2 diabetes mellitus. Consequently, investigating the mechanisms of local inflammation and disruption of corneal epithelial integrity, which may determine the clinical course of bacterial keratitis and the development of its complications, is of considerable importance.

Current knowledge regarding the epidemiology, risk factors, and clinical characteristics of infectious keratitis has been summarised by D. Ting *et al*. [1]. The authors emphasised that disease severity is determined not only by the virulence of the causative pathogen but also by the characteristics of the host local immune response. Similar conclusions were reached by M. Cabrera-Aguas *et al*. [2], who demonstrated that the intensity of the inflammatory response directly influences the extent of corneal damage and the risk of adverse clinical outcomes. One of the principal mechanisms of tissue damage in bacterial keratitis is local inflammation. Pro-inflammatory cytokines play a central role in this process, with interleukin-6 (IL-6) being of particular importance. H. Alenezi *et al*. [3], investigating the tear cytokine profile in bacterial keratitis, reported a marked increase in the levels of pro-inflammatory mediators, including IL-6. They concluded that IL-6 concentration reflects the activity of the local inflammatory response and may be associated with the severity of the infectious process.

An equally important component of the pathogenesis of infectious corneal disease is the integrity of the epithelial barrier. The protective function of the ocular surface is maintained by a system of tight junctions, which regulate epithelial permeability and preserve structural integrity. S. Kaur *et al.* [4] demonstrated that disruption of tight junction architecture is associated with impairment of the corneal barrier function and facilitates the penetration of pathogenic microorganisms into corneal tissue. W. Kuo *et al.* [5] established that the zonula occludens-1 (ZO-1) protein is one of the key regulators of intercellular junctions, supporting epithelial cell proliferation, survival, and functional activity. Alterations in ZO-1 levels may therefore indicate disruption of epithelial structural integrity and the extent of epithelial damage. These mechanisms are of particular importance in patients with diabetes mellitus. L. Ladea *et al.* [6] demonstrated that chronic hyperglycaemia is associated with structural alterations of the corneal epithelium, impairment of barrier function, and delayed regenerative processes. Similar findings were reported by F. Buonfiglio *et al*. [7], who showed that oxidative stress and chronic inflammation are the principal mechanisms underlying diabetic keratopathy and contribute to the progression of corneal tissue damage. Thus, concomitant diabetes mellitus may exacerbate both inflammatory and structural alterations in bacterial keratitis.

Considerable attention has recently been devoted to the identification of non-invasive biomarkers for ocular surface diseases. Tear fluid represents a promising biological medium because its composition reflects local pathological processes. In a systematic review and meta-analysis, M. Polkamp *et al*. [8] demonstrated that changes in tear fluid composition may reflect the development of diabetic ophthalmic complications. Likewise, N. Gospodarczyk *et al*. [9] highlighted the potential of tear biomarkers for assessing ocular surface status and monitoring the course of pathological processes. Collectively, current evidence indicates that local inflammation and disruption of the corneal barrier function play a pivotal role in the pathogenesis of bacterial keratitis. Interleukin-6 reflects inflammatory activity, whereas the ZO-1 protein characterises the integrity of intercellular junctions and the structural condition of the epithelial barrier. Simultaneous assessment of these biomarkers may therefore provide a comprehensive evaluation of both inflammatory activity and the extent of structural corneal damage. However, data regarding their concurrent measurement in the tear fluid of patients with bacterial keratitis remain limited. The relationship between IL-6 and ZO-1 levels, the influence of concomitant type 2 diabetes mellitus on these biomarkers, and their value in predicting the clinical course, complications, and treatment outcomes of bacterial keratitis have not yet been sufficiently investigated.

The aim of this study was to determine the concentrations of interleukin-6 and the ZO-1 protein in the tear fluid of patients with bacterial keratitis with and without type 2 diabetes mellitus and to evaluate their association with the clinical course and complications of the disease.

MATERIALS AND METHODS

A prospective comparative multicentre cohort study was conducted. The clinical investigation was performed in accordance with the principles of the World

Medical Association Declaration of Helsinki, the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine, and Order No. 690 of the Ministry of Health of Ukraine [10-12]. The study protocol was approved by the Bioethics Committee of Danylo Halytsky Lviv National Medical University (Protocol No. 12, 16 December 2024). Written informed consent was obtained from all participants before enrolment. The diagnosis of bacterial keratitis was established on the basis of a comprehensive ophthalmological examination, including medical history taking, slit-lamp biomicroscopy of the anterior segment, fluorescein staining, and assessment of stromal infiltrate, epithelial defect, conjunctival injection, and other clinical signs of infection. The bacterial aetiology of the disease was confirmed by bacterioscopic and bacteriological examination of corneal ulcer specimens obtained before the initiation of antibacterial therapy.

The study included 88 participants (88 eyes), comprising 60 patients with bacterial keratitis and 28 apparently healthy individuals in the control group. The participants were divided into three groups. Group 1 consisted of 32 patients with bacterial keratitis without disturbances of glucose metabolism. Group 2 included 28 patients with bacterial keratitis and confirmed type 2 diabetes mellitus. The control group comprised 28 apparently healthy individuals without corneal pathology, diabetes mellitus, or other systemic diseases. The inclusion criteria were age over 18 years, clinically and laboratory-confirmed bacterial keratitis, the ability to undergo a complete ophthalmological examination, and the availability of tear fluid samples for laboratory analysis. For the main study group, confirmed type 2 diabetes mellitus was an additional inclusion criterion. Exclusion criteria included viral, fungal, Acanthamoeba, or autoimmune keratitis, type 1 diabetes mellitus, severe concomitant systemic diseases, immunosuppressive therapy, and recent corneal surgery.

All participants underwent a standard ophthalmological examination, including assessment of visual acuity, refraction, intraocular pressure, slit-lamp biomicroscopy, and fundus examination. Optical coherence tomography of the anterior segment was used to evaluate the morphometric characteristics of the cornea. Corneal infiltrate thickness, width, and area were measured. Tear fluid samples were collected following instillation of 60 μL of sterile 0.9% sodium chloride solution into the conjunctival sac. After 30 seconds, tear fluid was collected using sterile capillary tubes, transferred into microcentrifuge tubes, and stored at -70°C until laboratory analysis. IL-6 concentrations were determined using a solid-phase enzyme-linked immunosorbent assay (ELISA) with the High Sensitivity Human Interleukin-6 ELISA Kit (MyBioSource, USA). The results were expressed in pg/mL. ZO-1 concentrations were measured using ELISA with the Human Tight Junction Protein 1 (ZO-1) ELISA Kit (MyBioSource, USA) and expressed in ng/mL.

To evaluate the pathogenetic significance of the investigated biomarkers, their relationships with the morphometric characteristics of the corneal infiltrate (thickness, width, and area) and with the development of clinical complications, including delayed epithelialisation, stromal opacity, and corneal ulceration, were analysed. Statistical analysis was performed using Statistica version 13.3 (StatSoft Inc., USA) and IBM SPSS Statistics version 27.0. Normality of data distribution was assessed using the Shapiro-Wilk test. Quantitative variables with a non-normal distribution are presented as the median and interquartile range (Me [Q1; Q3]). Comparisons between independent groups were performed using the Kruskal-Wallis test followed by Dunn's post hoc test or the Mann-Whitney U test, as appropriate. Correlation analysis was performed using Spearman's rank correlation coefficient. Differences were considered statistically significant at $p < 0.05$.

The authors declare that the views expressed in this article are their own and do not represent the official position of their institution.

RESULTS AND DISCUSSION

To evaluate the characteristics of the local inflammatory response and the integrity of the corneal epithelial barrier, the concentrations of interleukin-6 (IL-6) and the tight junction protein ZO-1 were analysed in the tear fluid of patients with bacterial keratitis with and without type 2 diabetes mellitus. Tear fluid IL-6 concentrations differed significantly between the study groups (Table 1). In the control group, the median IL-6 concentration was 18 [12;25] pg/mL. In patients with bacterial keratitis without concomitant type 2 diabetes mellitus, the IL-6 concentration was 245 [170;390] pg/mL, representing a 13.6-fold increase compared with the control group ($p < 0.001$). In patients with bacterial keratitis and type 2 diabetes mellitus, the median IL-6 concentration reached 430 [310;610] pg/mL, which was 23.9-fold higher than in the control group ($p < 0.001$) and 1.8-fold higher than in patients with bacterial keratitis without type 2 diabetes mellitus ($p = 0.02$). The highest IL-6 concentrations were observed in patients with bacterial keratitis and type 2 diabetes mellitus.

Similar changes were observed in the analysis of ZO-1 levels in tear fluid (Table 2). In patients with bacterial keratitis without type 2 diabetes mellitus, the median ZO-1 level was 6.8 [4.9;9.5] ng/mL, which was significantly higher than that of the control group ($p < 0.001$). In patients with bacterial keratitis and type 2 diabetes mellitus, the ZO-1 level increased to 9.6 [7.2;13.4] ng/mL, exceeding the corresponding value in the control group by 4.6-fold and that in the group without type 2 diabetes mellitus by 1.4-fold ($p = 0.01$).

Table 1. IL-6 levels in the tear fluid of patients in the study groups, Me [Q1; Q3]

Study group (number of eyes)	IL-6, pg/mL	p
Control group (n = 28)	18 [12;25]	–
Bacterial keratitis (n = 32)	245 [170;390]	p < 0.001[1]
Bacterial keratitis + type 2 diabetes mellitus (n = 28)	430 [310;610]	p < 0.001[1]; p = 0.02[2]

Note: normality of data distribution was assessed using the Shapiro-Wilk test. As the data were not normally distributed, non-parametric statistical methods were applied. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's post hoc test. Data are presented as the median and interquartile range, Me [Q1; Q3]. [1] Statistically significant differences compared with the control group; [2] statistically significant differences compared with the bacterial keratitis group without concomitant type 2 diabetes mellitus
Source: authors' own research

Table 2. ZO-1 concentration in the tear fluid of patients in the study groups, Me [Q1;Q3]

Study group (number of eyes)	ZO-1, ng/mL	p
Control group (n = 28)	2.1 [1.4;3.2]	–
Bacterial keratitis (n = 32)	6.8 [4.9;9.5]	p < 0.001[1]
Bacterial keratitis + type 2 diabetes mellitus (n = 28)	9.6 [7.2;13.4]	p < 0.001[1]; p=0.01[2]

Note: normality of data distribution was assessed using the Shapiro-Wilk test. As the data were not normally distributed, non-parametric statistical methods were applied. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's post hoc test. Data are presented as the median and interquartile range, Me [Q1; Q3]. [1] Statistically significant differences compared with the control group; [2] statistically significant differences compared with the bacterial keratitis group without concomitant type 2 diabetes mellitus
Source: authors' own research

To assess the relationship between tear fluid IL-6 levels and the clinical characteristics of the disease, a correlation analysis was performed with the morphometric parameters of the corneal infiltrate (thickness, width, and area) (Table 3). Statistically significant positive correlations were identified between IL-6 levels and all investigated parameters. In patients with bacterial keratitis without type 2 diabetes mellitus, the correlation coefficients ranged from 0.45 to 0.52, whereas in the group with type 2 diabetes mellitus they ranged from 0.66 to 0.74. The strongest association was observed between IL-6 levels and corneal infiltrate thickness in patients with type 2 diabetes mellitus ($\rho = 0.74$; $p < 0.001$). Analysis of the relationship between tear fluid ZO-1 levels and the morphometric characteristics of the corneal infiltrate also revealed statistically significant positive correlations (Table 4). In the bacterial keratitis group without type 2 diabetes mellitus, the correlation coefficients ranged from 0.47 to 0.55, whereas in patients with type 2 diabetes mellitus they ranged from 0.69 to 0.76. The highest correlation coefficient was recorded between ZO-1 levels and corneal infiltrate thickness in the bacterial keratitis with type 2 diabetes mellitus group ($\rho = 0.76$; $p < 0.001$).

Table 3. Correlation between tear fluid IL-6 levels and the morphometric characteristics of the corneal infiltrate at initial presentation in patients with bacterial keratitis and type 2 diabetes mellitus

Morphometric characteristic of the infiltrate (μm)	Group	IL-6 (pg/mL)	Value	Spearman's ρ	p
Infiltrate thickness (μm)	Bacterial keratitis (n = 32)	245 [170;390]	378.4 ± 83.2	0.52	0.003
	Bacterial keratitis + type 2 diabetes mellitus (n = 28)	430 [310;610]	401.8 ± 82.9	0.74	<0.001
Infiltrate width (μm)	Bacterial keratitis (n = 32)	245 [170;390]	1,655.2 ± 162.1	0.48	0.006
	Bacterial keratitis + type 2 diabetes mellitus (n = 28)	430 [310;610]	1,728.6 ± 168.4	0.69	<0.001
Infiltrate area	Bacterial keratitis (n = 32)	245 [170;390]	0.53 ± 0.15	0.45	0.009
	Bacterial keratitis + type 2 diabetes mellitus (n = 28)	430 [310;610]	0.57 ± 0.14	0.66	<0.001

Note: data are presented as the median and interquartile range, Me [Q1; Q3]. The morphometric parameters of the infiltrate are presented as the mean ± standard deviation (M ± SD). Correlation analysis was performed using Spearman's rank correlation coefficient (ρ)
Source: authors' own research

Table 4. Correlation between tear fluid ZO-1 levels and the morphometric characteristics of the corneal infiltrate at initial presentation in patients with bacterial keratitis and type 2 diabetes mellitus

Morphometric characteristic of the infiltrate (μm)	Group	ZO-1 (ng/mL)	Value	Spearman's ρ	p
Infiltrate thickness (μm)	Bacterial keratitis (n = 32)	6.8 [4.9;9.5]	378.4 ± 83.2	0.55	0.002
	Bacterial keratitis + type 2 diabetes mellitus (n = 28)	9.6 [7.2;13.4]	401.8 ± 82.9	0.76	<0.001

Table 4. *Continued*

Morphometric characteristic of the infiltrate (μm)	Group	ZO-1 (ng/mL)	Value	Spearman's ρ	p
Infiltrate width (μm)	Bacterial keratitis (n = 32)	6.8 [4.9;9.5]	1,655.2 ± 162.1	0.51	0.004
	Bacterial keratitis + type 2 diabetes mellitus (n = 28)	9.6 [7.2;13.4]	1,728.6 ± 168.4	0.71	<0.001
Infiltrate area	Bacterial keratitis (n = 32)	6.8 [4.9;9.5]	0.53 ± 0.15	0.47	0.007
	Bacterial keratitis + type 2 diabetes mellitus (n = 28)	9.6 [7.2;13.4]	0.57 ± 0.14	0.69	<0.001

Note: data are presented as the median and interquartile range, Me [Q1; Q3]. The morphometric parameters of the infiltrate are presented as the mean ± standard deviation (M ± SD). Correlation analysis was performed using Spearman's rank correlation coefficient (ρ)
Source: authors' own research

Analysis of tear fluid IL-6 levels according to the development of clinical complications demonstrated statistically significant differences between patients with and without complications. Patients who developed complications had significantly higher IL-6 levels than those without the corresponding clinical manifestations. In the group without type 2 diabetes mellitus, the highest IL-6 levels were recorded in patients with corneal infiltrate ulceration, reaching 420 [340;520] pg/mL compared with 250 [180;345] pg/mL in patients without ulceration (p = 0.018). In the group with type 2 diabetes mellitus, the corresponding values were 660 [520;780] pg/mL and 375 [290;490] pg/mL, respectively (p < 0.001) (Tables 5 and 6).

Table 5. Development of complications in patients with bacterial keratitis without type 2 diabetes mellitus according to tear fluid IL-6 levels (n = 32)

Complication	Complication present	Complication absent	Mann-Whitney U	p
Delayed epithelialisation (>10 days)	365 [290;470] (n = 8)	228 [170;310] (n = 24)	52.0	0.010
Development of stromal opacity	385 [300;490] (n = 11)	235 [175;325] (n = 21)	46.0	0.006
Corneal infiltrate ulceration	420 [340;520] (n = 5)	250 [180;345] (n = 27)	28.0	0.018

Note: data are presented as the median and interquartile range, Me [Q1; Q3]. Between-group comparisons were performed using the Mann-Whitney U test. Differences were considered statistically significant at p < 0.05
Source: authors' own research

Table 6. Development of complications in patients with bacterial keratitis and type 2 diabetes mellitus according to tear fluid IL-6 levels (n = 28)

Complication	Complication present	Complication absent	Mann-Whitney U	p
Delayed epithelialisation (>10 days)	595 [470;710] (n = 12)	330 [250;440] (n = 16)	31.0	<0.001
Development of stromal opacity	610 [490;735] (n = 14)	345 [260;460] (n = 14)	26.0	<0.001
Corneal infiltrate ulceration	660 [520;780] (n = 8)	375 [290;490] (n = 20)	19.0	<0.001

Note: data are presented as the median and interquartile range, Me [Q1; Q3]. Between-group comparisons were performed using the Mann-Whitney U test. Differences were considered statistically significant at p < 0.05
Source: authors' own research

A similar pattern was observed for the ZO-1 protein (Tables 7 and 8). In patients without type 2 diabetes mellitus, the highest ZO-1 level was detected in those with corneal infiltrate ulceration, reaching 11.6 [9.4;13.8] ng/mL compared with 6.1 [4.6;7.8] ng/mL in patients without this complication (p = 0.006). In the group of patients with type 2 diabetes mellitus, the highest ZO-1 levels were likewise recorded in those with infiltrate ulceration, reaching 15.8 [12.7;18.4] ng/mL compared with 8.4 [6.7;10.6] ng/mL, respectively (p < 0.001).

Table 7. Development of complications in patients with bacterial keratitis without type 2 diabetes mellitus according to tear fluid ZO-1 levels (n = 32)

Complication	Complication present	Complication absent	Mann-Whitney U	p
Delayed epithelialisation (>10 days)	8.9 [7.2;10.8] (n = 8)	5.9 [4.3;7.6] (n = 24)	48.0	0.008
Development of stromal opacity	9.8 [8.0;11.9] (n = 11)	6.0 [4.5;7.8] (n = 21)	41.0	0.003
Corneal infiltrate ulceration	11.6 [9.4;13.8] (n = 5)	6.1 [4.6;7.8] (n = 27)	18.0	0.006

Note: data are presented as the median and interquartile range, Me [Q1; Q3]. Between-group comparisons were performed using the Mann-Whitney U test. Differences were considered statistically significant at p < 0.05
Source: authors' own research

Table 8. Development of complications in patients with bacterial keratitis and type 2 diabetes mellitus according to tear fluid ZO-1 levels (n = 28)

Complication	Complication present	Complication absent	Mann-Whitney U	p
Delayed epithelialisation (>10 days)	13.1 [10.4;15.6] (n = 12)	8.0 [6.4;10.2] (n = 16)	29.0	<0.001
Development of stromal opacity	14.0 [11.2;16.5] (n = 15)	8.2 [6.6;10.4] (n = 13)	22.0	<0.001
Corneal infiltrate ulceration	15.8 [12.7;18.4] (n = 8)	8.4 [6.7;10.6] (n = 20)	12.0	<0.001

Note: data are presented as the median and interquartile range, Me [Q1; Q3]. Between-group comparisons were performed using the Mann-Whitney U test. Differences were considered statistically significant at $p < 0.05$
Source: authors' own research

One of the principal findings of the present study was the significant increase in IL-6 and ZO-1 concentrations in the tear fluid of patients with bacterial keratitis, with the highest levels of both biomarkers observed in the presence of concomitant type 2 diabetes mellitus. Specifically, the IL-6 concentration in patients with bacterial keratitis and type 2 diabetes mellitus was almost 1.8-fold higher than that in patients without diabetes. These findings indicate a more pronounced activation of the local inflammatory response in patients with metabolic disturbances. Similar observations have been reported by A. Konstantopoulos *et al*. [13], who demonstrated an association between the cytokine profile and the clinical characteristics of bacterial keratitis. Likewise, K. Cheng *et al.* [14] emphasised that excessive production of pro-inflammatory cytokines represents one of the key mechanisms underlying corneal tissue damage during infectious keratitis. The clinical relevance of the observed immunoinflammatory alterations is further supported by studies investigating predictors of bacterial keratitis severity and risk factors for the development of disease complications [15].

Another important finding of this study was the identification of positive correlations between IL-6 concentration and the morphometric characteristics of the corneal infiltrate. The strongest association was observed between IL-6 concentration and infiltrate thickness in patients with type 2 diabetes mellitus ($\rho = 0.74$; $p < 0.001$). This finding suggests that the intensity of the local inflammatory response is directly related to the extent of structural corneal damage. Of particular interest is the observation that these correlations were stronger in patients with type 2 diabetes mellitus than in those without diabetes. This may be explained by the presence of chronic low-grade inflammation, oxidative stress, and impaired reparative processes, which are characteristic features of diabetic keratopathy. A similar interpretation has been proposed by F. Buonfiglio *et al.* [7].

The findings regarding the ZO-1 protein are equally important. In the present study, ZO-1 concentrations were significantly higher in patients with bacterial keratitis, with the highest values observed in those with concomitant type 2 diabetes mellitus. Moreover, strong positive correlations were identified between ZO-1 levels and the thickness, width, and area of the corneal infiltrate. These findings suggest that disruption of intercellular junctions and impairment of the epithelial barrier constitute important components of the pathogenesis of bacterial keratitis. S. Alfuraih *et al.* [16] demonstrated that hyperglycaemia adversely affects tight junction proteins and the barrier function of the ocular surface epithelium. Similar findings were reported by S. Casarella *et al.* [17] and A. Ebrahim *et al*. [18], who described structural and functional alterations of the corneal epithelium in diabetic keratopathy. Furthermore, M. Yu *et al.* [19] showed that activation of pro-inflammatory signalling pathways is accompanied by disruption of corneal epithelial barrier integrity and contributes to the progression of structural corneal damage. The clinical significance of these pathogenetic alterations is further supported by epidemiological studies demonstrating an increased susceptibility of patients with diabetes mellitus to severe corneal disease [20].

Particular attention should be paid to the observed association between elevated IL-6 and ZO-1 levels and the development of clinical complications. Patients with infiltrate ulceration, delayed epithelialisation, and stromal opacity exhibited significantly higher concentrations of both biomarkers than patients with an uncomplicated disease course. The highest levels were consistently observed in patients with type 2 diabetes mellitus. These findings indicate that local inflammation and impairment of the corneal barrier function may represent interrelated mechanisms contributing to the development of complications in bacterial keratitis. Similar conclusions regarding risk factors for adverse outcomes in bacterial keratitis have been reported by C. Bertret *et al*. [15], A. Nayel *et al.* [21], and E. Österhed *et al*. [22], who highlighted the importance of clinical, microbiological, and therapeutic factors in predicting treatment outcomes. Particular emphasis has also been placed on the integrity of the corneal epithelial barrier, the disruption of which is regarded as one of the key factors promoting disease progression and the development of complications [23].

Thus, the findings of the present study expand current understanding of the pathogenetic mechanisms underlying bacterial keratitis in patients with type 2 diabetes mellitus. Concomitant type 2 diabetes mellitus was associated with greater increases in IL-6 and ZO-1 concentrations, larger corneal infiltrates, and a higher incidence of clinical complications. Determination of these biomarkers in tear fluid may therefore have

practical value for assessing disease severity and predicting the risk of developing complications in patients with bacterial keratitis.

CONCLUSIONS

1. It was established that the median tear fluid IL-6 level in patients with bacterial keratitis and type 2 diabetes mellitus was 1.8-fold higher than in patients without diabetes and 23.9-fold higher than in the control group. The ZO-1 level in this group was also significantly elevated, exceeding that of the bacterial keratitis group without type 2 diabetes mellitus by 1.4-fold and the control group by 4.6-fold. These findings indicate more pronounced disruption of the corneal epithelial barrier and a more intense local inflammatory response in the presence of concomitant metabolic disturbances.

2. Statistically significant positive correlations were identified between IL-6 and ZO-1 levels and the morphometric characteristics of the corneal infiltrate. In patients with bacterial keratitis and type 2 diabetes mellitus, strong correlations were observed between the investigated biomarkers and infiltrate thickness, width, and area ($\rho = 0.66$-0.76; $p < 0.001$), whereas in patients without diabetes the correlations were of moderate strength ($\rho = 0.45$-0.55; $p < 0.01$). These findings demonstrate a close relationship between the severity of local inflammation, the degree of disruption of intercellular junctions, and the extent of structural corneal changes.

3. Elevated IL-6 and ZO-1 levels were shown to be associated with the development of clinical complications of bacterial keratitis. The highest levels of both biomarkers were recorded in patients with corneal infiltrate ulceration, whereas lower levels were observed in patients without complications. Similar patterns were identified for delayed epithelialisation and the development of stromal opacity. These changes were more pronounced in patients with type 2 diabetes mellitus, confirming the adverse effect of metabolic disturbances on the course of the infectious process and the reparative capacity of the cornea.

4. The obtained findings support the potential use of IL-6 and ZO-1 as promising biomarkers of inflammatory activity, epithelial barrier damage, and the risk of a complicated course of bacterial keratitis, particularly in patients with type 2 diabetes mellitus.

5. Future research should focus on investigating the dynamics of IL-6 and ZO-1 levels in response to treatment, evaluating their prognostic value for long-term functional outcomes, and developing models for the early prediction of bacterial keratitis complications based on a combination of clinical and laboratory parameters.

6. Study limitations. The findings should be interpreted in the context of the relatively small sample size of the study groups and the absence of long-term follow-up to assess changes in the investigated biomarkers over time.

ACKNOWLEDGEMENTS

The authors have no specific acknowledgements related to the conduct of this study.

FUNDING

No funding was received.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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Вміст інтерлейкіну-6 та білка щільних контактів ZO-1 у слізній рідині та їх зв'язок із перебігом бактеріального кератиту при цукровому діабеті 2 типу в умовах воєнного стану в Україні

Анотація

Актуальність. Бактеріальний кератит є одним із найбільш поширених інфекційних захворювань рогівки та може призводити до розвитку тяжких ускладнень, стійкого зниження гостроти зору й інвалідизації пацієнтів.

Мета. Вивчити вміст інтерлейкіну-6 та білка щільних міжклітинних контактів zonula occludens-1 у слізній рідині пацієнтів із бактеріальним кератитом залежно від наявності цукрового діабету 2 типу та оцінити їх зв'язок із клінічним перебігом і розвитком ускладнень захворювання.

Матеріали та методи. Проведено проспективне порівняльне когортне мультицентрове дослідження за участю 88 осіб, серед яких пацієнти з бактеріальним кератитом за наявності та відсутності цукрового діабету 2 типу, а також особи контрольної групи. Концентрації інтерлейкіну-6 та білка щільних міжклітинних контактів zonula occludens-1 у слізній рідині визначали методом імуноферментного аналізу.

Результати. Встановлено достовірне підвищення концентрацій інтерлейкіну-6 та білка щільних міжклітинних контактів zonula occludens-1 у пацієнтів із бактеріальним кератитом порівняно з контрольною групою. Найвищі значення обох показників визначалися у пацієнтів із супутнім цукровим діабетом 2 типу. Концентрація інтерлейкіну-6 у пацієнтів із бактеріальним кератитом без цукрового діабету становила 245 [170;390] пг/мл, тоді як у хворих із супутнім цукровим діабетом 2 типу досягала 430 [310;610] пг/мл. Вміст білка щільних міжклітинних контактів zonula occludens-1 становив відповідно 6,8 [4,9;9,5] нг/мл та 9,6 [7,2;13,4] нг/мл. Виявлено статистично значущі прямі кореляційні зв'язки між рівнями досліджуваних біомаркерів і товщиною, шириною та площею інфільтрату рогівки. Найбільш сильні кореляції встановлено між концентрацією інтерлейкіну-6 та товщиною інфільтрату ($\rho = 0,74$; $p < 0,001$), а також між рівнем білка щільних міжклітинних контактів zonula occludens-1 та товщиною інфільтрату ($\rho = 0,76$; $p < 0,001$) у пацієнтів із цукровим діабетом 2 типу. У хворих із виразкуванням інфільтрату, уповільненою епітелізацією та стромальним помутнінням рівні обох біомаркерів були достовірно вищими порівняно з пацієнтами без ускладненого перебігу захворювання.

Висновки. Бактеріальний кератит супроводжується підвищенням вмісту інтерлейкіну-6 та білка щільних міжклітинних контактів zonula occludens-1 у слізній рідині. Встановлено зв'язок досліджуваних показників із морфометричними характеристиками інфільтрату рогівки та розвитком клінічних ускладнень захворювання.

Ключові слова: інфекційне ураження рогівки; діабетична кератопатія; прозапальні цитокіни; корнеальний інфільтрат; біомаркери.