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OCULAR SURFACE MICROBIOTA IN PEDIATRIC KERATOCONUS: A COMPARATIVE OBSERVATIONAL STUDY

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ABSTRACT

Aim. To compare the ocular surface microbiota composition in pediatric patients with keratoconus (KC) and healthy age-matched controls, and to assess the relationship between microbial dysbiosis and disease progression.
Materials and methods. A comparative observational study was conducted at the National Children's Specialized Hospital "Ohmatdyt". Twenty pediatric patients were enrolled: 10 with confirmed keratoconus (KC group) and 10 healthy age-matched controls (non-KC group). Conjunctival swab samples were collected for standard microbiological culture. Microbial diversity, prevalence of opportunistic and commensal microorganisms, and age-related trends were assessed using Spearman correlation analysis.
Results. Patients with KC demonstrated ocular surface microbiota alterations characterized by increased prevalence of opportunistic bacteria (Pseudomonas aeruginosa 50%, Ralstonia pickettii 40%, Staphylococcus aureus – elevated) and reduced abundance of commensal flora (Staphylococcus epidermidis 20% vs 100% in controls; Corynebacterium spp. 20% vs 90% in controls). Spearman correlation revealed positive associations between age and Pseudomonas aeruginosa (ρ=0.86, p=0.006) and Staphylococcus aureus (ρ=0.62, p=0.027), and negative correlations with Staphylococcus epidermidis (ρ=−0.66, p=0.015) and Corynebacterium spp. (ρ=−0.60, p=0.025).
Conclusions. Pediatric patients with keratoconus exhibit distinct ocular surface microbial alterations that may be associated with ocular surface instability. The observed associations may serve as a basis for further research. However, the cross-sectional design and limited sample size do not allow causal inference or claims regarding microbiota profiling as a clinical biomarker without validation in larger prospective studies.

Keywords: keratoconus; microbiota; dysbiosis; pediatrics; corneal diseases; biomarkers.

INTRODUCTION

Keratoconus (KC) is a progressive, multifactorial corneal ectatic disorder characterized by stromal thinning, irregular astigmatism, and protrusion of the corneal surface [1,9]. The disease commonly develops during adolescence and may progress rapidly in pediatric patients, leading to significant visual impairment and reduced quality of life [9]. Although the exact pathogenesis of keratoconus remains unclear, genetic predisposition, oxidative stress, chronic inflammation, environmental factors, and mechanical trauma associated with eye rubbing are considered important contributing factors [4]. In recent years, increasing attention has been directed toward the role of the ocular surface microbiota in maintaining ocular health and immune homeostasis [4-6]. The ocular microbiome consists primarily of commensal microorganisms that contribute to protection against pathogenic invasion and regulation of local inflammatory responses [4, 7]. Disturbances in this microbial balance, referred to as dysbiosis, have been associated with several ocular surface disorders, including blepharitis, conjunctivitis, and dry eye disease [8]. Recent studies suggest that alterations in the ocular microbiota may also be associated with the development and progression of keratoconus [1, 2]. Rocha de Lossada et al. demonstrated significant alterations in the ocular surface microbiota of patients with keratoconus, including an increased prevalence of opportunistic microorganisms such as Ralstonia pickettii [1]. However, data on pediatric populations remain limited, and the relationship between ocular microbiota dysbiosis and keratoconus in children remains insufficiently understood.

The present study evaluated the composition of the ocular surface microbiota in pediatric patients with keratoconus compared with healthy controls and assessed the potential association between microbial alterations and keratoconus. The study aimed to determine whether microbial dysbiosis [5,

10] is associated with keratoconus and to explore its potential relevance as a biomarker or therapeutic target.

AIM

The aim of this study was to compare ocular surface microbiota in pediatric patients with keratoconus and healthy controls and evaluate microbial alterations as a potential factor associated with disease progression.

MATERIALS AND METHODS

Study Design. This was a cross-sectional, comparative observational study designed to investigate differences in ocular surface microbiota between children with keratoconus and healthy controls.

Setting. The study was conducted at the National Specialized Children's Hospital "Ohmatdyt", involving pediatric patients aged 6 to 15 years.

Participants. Study Group (Keratoconus group): 10 children clinically diagnosed with keratoconus (KC). Control Group: 10 age- and gender-matched children without any clinical signs of keratoconus or other ocular surface diseases.

Inclusion Criteria: Age between 6 and 15 years. No recent use of topical or systemic antibiotics (within the past 2 weeks). No history of ocular surgery or chronic eye disease (other than keratoconus for the study group).

Exclusion Criteria: Recent ocular infections or trauma. Contact lens wear during the previous 30 days. Use of immunosuppressive medications. Systemic inflammatory conditions.

Clinical assessment of keratoconus. Keratoconus was confirmed using Pentacam HR corneal tomography according to the Belin ABCD classification. The following tomographic parameters were recorded: maximum keratometry (Kmax), thinnest corneal pachymetry, posterior corneal elevation, disease stage, and evidence of progression based on serial examinations (increase in Kmax ≥1.0 D and/or reduction in minimum corneal thickness during follow-up). Previous treatment and contact lens wear, was documented.

Ocular sampling. Conjunctival swabs were collected from the inferior fornix of one eye using sterile cotton swabs without prior topical anesthesia or antiseptics. Sampling was performed in the morning, before any diagnostic procedures. Swabs were immediately placed into Amies transport medium and delivered to the laboratory within a maximum of 2 hours at +4…+8 °C.

Culture examination. All samples (n=20) were examined by culture methods. Inoculation was performed on blood agar, chocolate agar, Sabouraud agar, and MacConkey agar. Incubation was carried out at 37 °C under aerobic conditions for 48-72 hours (for bacteria) and up to 5 days (for fungi). Quantitative assessment of microbial load was performed by colony counting and expressed as CFU/ml. A titer of $\geq 10^3$ CFU/ml was considered clinically significant. Identification of isolated microorganisms was performed based on morphological, cultural, and biochemical characteristics using standard test systems.

Data Analysis. Descriptive statistics to compare prevalence and load of microbial species. Chi-square test to evaluate statistical differences in bacterial detection between groups. Spearman correlation analysis to assess the relationship between age and frequency of microorganism detection. The unit of analysis was the patient (one eye per patient). Given the small sample size (n=10 per group), correlation analyses should be interpreted with caution. No correction for multiple comparisons was applied.

Ethical Considerations. Written informed consent was obtained from parents or legal guardians. The study adhered to the Declaration of Helsinki and was approved by the Bioethics Commission (Protocol dated 23 December 2024).

RESULTS

A total of 20 pediatric patients were included: 10 with keratoconus (KC group) and 10 healthy controls. The KC group demonstrated elevated microbial titers ($>10^3$-10^6 CFU/ml) compared to the normal range ($\leq 10^3$ CFU/ml), reduced biodiversity with monoculture or single-species dominance, and presence of opportunistic pathogens. Table 1.

Table 1. Comparison of normal ocular surface microbiota and dysbiosis parameters in keratoconus (KC)

Parameter	Normal	Dysbiosis (KC)
Quantity (CFU/ml)	$\leqslant 10^3$ CFU/ml	$>10^3$ – 10^6 CFU/ml
Diversity	2–3 species	Often monoculture or dominated by 1 species
Flora Type	Commensals	Opportunistic pathogens
Antibiotic Response	Weak, often unnecessary	Broad testing (8–15 antibiotics) recommended

Pseudomonas aeruginosa was detected in 5 of 10 KC patients (50%) and in none of the controls. Ralstonia pickettii was identified in 4 KC patients (40%) and was absent in the non-KC group. Corynebacterium spp. and Staphylococcus epidermidis were markedly reduced in the KC group (20% each) compared to controls (90% and 100%,

respectively). Staphylococcus aureus demonstrated elevated presence in the KC group. Table 2.

Table 2. Prevalence of microorganisms in pediatric patients with and without keratoconus (n=10 per group)

Microorganism	With KC (n=10)	Without KC (n=10)	Interpretation
Pseudomonas aeruginosa	5 (50%)	0 (0%)	Opportunistic; strongly associated with KC
Ralstonia pickettii	4 (40%)	0 (0%)	Absent in normal flora
Corynebacterium spp.	2 (20%)	9 (90%)	Reduced in KC
Staphylococcus epidermidis	2 (20%)	10 (100%)	Nearly absent in KC
Staphylococcus aureus	Elevated presence	Low presence	Common in KC

Average microbial titers by group are illustrated in Figure 1. The KC group showed substantially higher titers of Pseudomonas aeruginosa, Ralstonia pickettii, Staphylococcus aureus, Enterococcus faecalis, and Streptococcus viridans compared to controls.

The KC group showed substantially higher titers of Pseudomonas aeruginosa, Ralstonia pickettii, Staphylococcus aureus, Enterococcus faecalis, and Streptococcus viridans compared to controls.

Age-related analysis showed a positive correlation between age and Pseudomonas aeruginosa (ρ=0.86, p=0.006) and Staphylococcus aureus (ρ=0.62, p=0.027), and negative correlations with Staphylococcus epidermidis (ρ=−0.66, p=0.015) and Corynebacterium spp. (ρ=−0.60, p=0.025), indicating progressive reduction of commensal flora with age in KC patients. Ralstonia pickettii showed a tendency toward a negative correlation (ρ=−0.45, p=0.095). Table 3.

Table 3. Spearman correlation coefficients between patient age and presence of microorganisms in the keratoconus group

Microorganism	Spearman's ρ	p-value	Interpretation
Staph. aureus	0.62	0.027	Moderate positive correlation
Pseudomonas aeruginosa	0.86	0.006	Strong positive correlation
Staph. epidermidis	−0.66	0.015	Negative correlation
Corynebacterium spp.	−0.60	0.025	Negative correlation
Ralstonia pickettii	−0.45	0.095	Tendency toward negative correlation

Spearman correlation analysis was performed to assess the relationship between patient age and microbial presence in the KC group. Results are summarized in Table 3 and illustrated in Figure 2.

Clinical topographic characteristics of the KC group (Kmax, thinnest pachymetry, Belin/ABCD Staging) are presented in the Appendix (Table a4). Clinical topographic characteristics of the control group (Kmax, thinnest pachymetry, Belin/ABCD staging) are presented in the appendix (Table a3).

DISCUSSION

The results of this study demonstrate significant alterations in the ocular surface microbiota of pediatric patients with keratoconus compared with healthy controls. The higher prevalence of

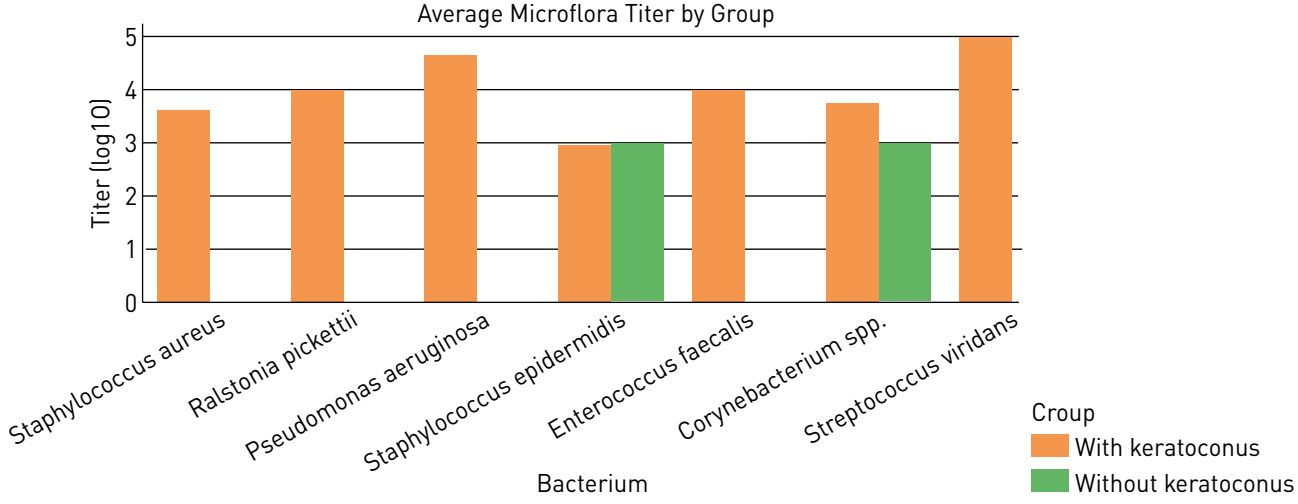

Fig. 1. Average microflora titer (log10) by bacterium and group

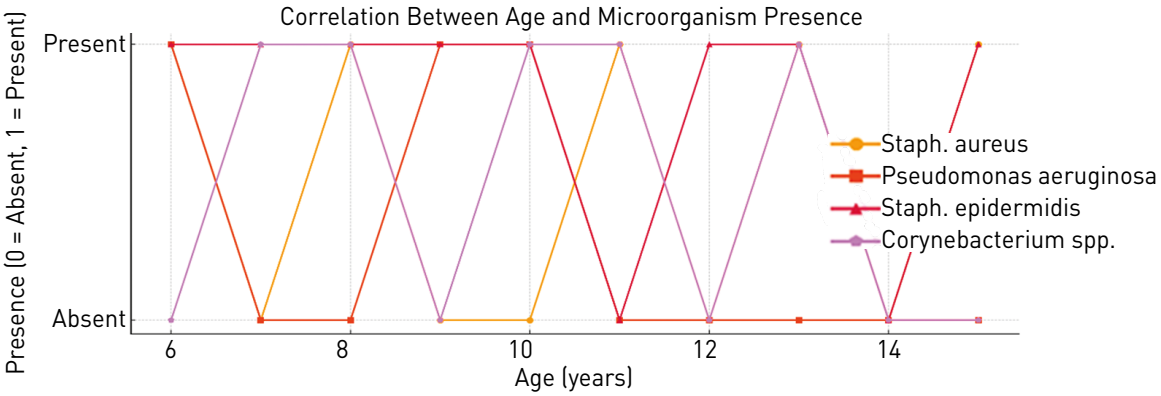

Fig. 2. Correlation between patient age and microorganism presence in the keratoconus group

opportunistic microorganisms, such as *Pseudomonas aeruginosa* and *Ralstonia pickettii*, together with the marked reduction in commensal microorganisms (*Staphylococcus epidermidis* and *Corynebacterium spp.*), is consistent with the findings of Rocha de Lossada et al. [1], who reported similar microbial alterations in adults with treatment-naïve keratoconus.

The strong positive correlations between age and the prevalence of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, together with the negative correlations observed for commensal microorganisms, may indicate age-related changes in the ocular surface microbiota in pediatric patients with keratoconus. These microbial alterations could potentially contribute to chronic low-grade inflammation, impaired epithelial barrier function, and increased oxidative stress, mechanisms previously implicated in the pathogenesis of keratoconus [3, 4]. However, the present study cannot determine whether these microbial alterations are involved in disease progression or reflect age-related changes or consequences of the disease.

Nevertheless, the cross-sectional design and small sample size preclude causal inference. Culture-based methods, although clinically practical, capture only a subset of the ocular microbiota compared with molecular and metagenomic sequencing approaches [9, 10]. Future longitudinal studies combining culture-based and molecular methods in larger pediatric cohorts are needed to determine whether microbial alterations precede, accompany, or result from keratoconus progression.

CONCLUSIONS

Pediatric patients with keratoconus demonstrated significant ocular surface microbiota dysbiosis characterized by increased prevalence of opportunistic microorganisms and reduced commensal flora.

Compared to healthy controls, children with KC exhibit: a higher prevalence of opportunistic bacteria (Pseudomonas aeruginosa, Ralstonia pickettii, Staphylococcus aureus); a notable reduction in beneficial commensal flora (Staphylococcus epidermidis, Corynebacterium spp.); a trend of increasing pathogenic bacterial presence with age, coupled with declining commensals.

Alterations in microbial composition may contribute to ocular surface instability and inflammatory processes associated with keratoconus progression.

Ocular microbiota profiling may serve as a potential biomarker for early diagnosis and individualized management of pediatric keratoconus [1, 9]. The observed associations between microbial composition and age may reflect progressive destabilization of ocular surface homeostasis. However, due to the cross-sectional design and limited sample size, these findings should be interpreted as associations rather than evidence of a causal relationship or a validated diagnostic biomarker. Further prospective studies with larger cohorts are required.

Article declarations

Perspectives for further research. Further studies should include larger pediatric cohorts, longitudinal follow-up to assess the relationship between microbiota changes and keratoconus progression, and evaluation of potential microbiota-targeted interventions.

Study limitations. The main limitations include the small sample size (n=10 per group), cross-sectional design that precludes causal inference, and the use of culture-based methods only, which may not fully capture the entire microbial community.

Primary data and materials. The authors confirm that primary medical documentation and statistical databases of individual patients were used in accordance with ethical approval. Anonymized summary data are available from the corresponding author upon reasonable request.

Research ethics. The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Commission (Protocol dated 23 December 2024). Written informed consent was obtained from parents or legal guardians of all participants.

Declaration of AI use. Artificial intelligence tools were used solely for language editing and proofreading. The authors confirm that all scientific content, data interpretation, and conclusions are entirely their own. The authors take full responsibility for the accuracy and integrity of the work.

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Conflict of Interest. The authors declare no conflict of interest related to the preparation, conduct, or publication of this study.

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APPENDIX. RAW DATA

Table A1. Raw microbiological data – Keratoconus group (n=10)

#	Age	Microflora (titer)	Group
1	6	Staph. aureus 10^4, Ralstonia pickettii	With keratoconus
2	6	Pseudomonas aeruginosa 10^4	With keratoconus
3	7	Enterococcus faecalis 10^4, Corynebacterium spp.	With keratoconus
4	7	Staph. epidermidis 10^3, Corynebacterium spp.	With keratoconus
5	8	Streptococcus viridans 10^5	With keratoconus
6	9	Pseudomonas aeruginosa 10^5	With keratoconus
7	10	Pseudomonas aeruginosa 10^5	With keratoconus
8	11	Staph. aureus 10^3, Ralstonia pickettii	With keratoconus

#	Age	Microflora (titer)	Group
9	13	Staph. aureus 10^4, Corynebacterium spp.	With keratoconus
10	15	Staph. aureus 10^4, Staph. epidermidis	With keratoconus

Table A2. Raw microbiological data – Control group without keratoconus (n=10)

#	Age	Microflora (titer)	Group
11	6	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus
12	6	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus
13	7	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus
14	11	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus
15	13	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus
16	9	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus
17	10	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus
18	11	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus
19	13	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus
20	15	Staph. epidermidis 10^3, Corynebacterium spp.	Without keratoconus

Table A3. Clinical topographic characteristics of the control group (n=10)

№	Eye (age)	Kmax (D)	Thinnest pachy (мкм)	Belin/ABCD
1	6	43.8	533	A0 B0 C0 D
2	6	44.9	513	A0 B0 C0 D
3	7	47.7	601	A0 B0 C0 D
4	11	44.1	558	A0 B0 C0 D
5	13	46.2	530	A0 B0 C0 D
6	9	46.9	557	A0 B0 C0 D
7	10	46.5	583	A0 B0 C0 D
8	11	45.2	529	A0 B0 C0 D
9	13	44.9	523	A0 B0 C0 D
10	15	42.0	596	A0 B0 C0 D

Table A4. Clinical topographic characteristics of the keratoconus group (n=10)

№	Eye (age)	Kmax (D)	Thinnest pachy (мкм)	Belin/ABCD
11	6	50.4	592	A0 B1 C0 D
12	6	49.0	541	A0 B2 C0 D
13	7	53.2	544	A0 B1 C0 D
14	8	53.4	566	A0 B1 C0 D
15	9	52.4	538	A2 B2 C0 D
16	10	53.6	360	A0 B2 C4+ D
17	11	49.5	518	A2 B2 C0 D
18	12	54.0	490	A0 B4+ C1 D
19	13	53.8	493	A2 B2 C1 D1
20	15	56.7	449	A2 B4+ C2 D

МІКРОБІОТА ПОВЕРХНІ ОКА ПРИ ПЕДІАТРИЧНОМУ КЕРАТОКОНУСІ: ПОРІВНЯЛЬНЕ ОБСЕРВАЦІЙНЕ ДОСЛІДЖЕННЯ

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РЕЗЮМЕ

Вступ. Це дослідження вивчає зв'язок між мікробіотою поверхні ока та розвитком кератоконусу (КК), особливо у дітей і підлітків. Кератоконус – це прогресуюче захворювання рогівки, що характеризується порушенням і конічною деформацією рогівки, що часто призводить до порушення зору. Останні дослідження свідчать, що очний мікробіом може відігравати роль у патогенезі цього захворювання.

Мета. Мета дослідження порівняти склад мікробіоти поверхні ока у педіатричних пацієнтів із кератоконусом (КК) та здорових дітей відповідного віку, а також оцінити зв'язок між мікробним дисбіозом та прогресуванням захворювання.

Матеріали та методи. Було проведено порівняльне обсерваційне дослідження в Національній дитячій спеціалізованій лікарні «Охматдит». До дослідження включено 20 педіатричних пацієнтів: 10 із підтвердженим кератоконусом (група КК) та 10 здорових дітей контрольної групи. У всіх учасників взято мазки з кон'юнктиви для стандартного мікробіологічного дослідження. Мікробне різноманіття, поширеність опортуністичних та коменсальних мікроорганізмів і вікові тенденції оцінювали за допомогою кореляційного аналізу Спірмена.

Результати. У пацієнтів із КК виявлено значний дисбіоз мікробіоти поверхні ока, що характеризувався підвищеною поширеністю опортуністичних бактерій (Pseudomonas aeruginosa – 50%, Ralstonia pickettii – 40%, Staphylococcus aureus – підвищений рівень) та зниженою кількістю коменсальної флори (Staphylococcus epidermidis – 20% проти 100% у контрольній групі; Corynebacterium spp. – 20% проти 90%). Кореляційний аналіз Спірмена виявив сильні позитивні зв'язки між віком і P. aeruginosa (ρ=0,86, p=0,006) та S. aureus (ρ=0,62, p=0,027), а також значущі від'ємні кореляції з S. epidermidis (ρ=−0,66, p=0,015) і Corynebacterium spp. (ρ=−0,60, p=0,025).

Висновки. Педіатричні пацієнти з кератоконусом мають характерний дисбіоз мікробіоти поверхні ока, що може сприяти нестабільності поверхні ока та запальним механізмам прогресування захворювання. Профілювання мікробіоти ока може слугувати потенційним біомаркером для ранньої діагностики та індивідуалізованого ведення педіатричного кератоконусу за умови валідації у подальших дослідженнях.

Ключові слова: кератоконус; мікробіота; дисбіоз; педіатрія; захворювання рогівки; біомаркери.

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