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REVIEW ARTICLE

Tear Biomarker Utility in Pediatric Vernal Keratoconjunctivitis

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Abstract

Vernal keratoconjunctivitis (VKC) is a chronic, vision-threatening ocular allergy that primarily affects children in warm and dry climates. Diagnosis in pediatric populations is frequently delayed due to symptom overlap with other allergic conjunctivitis, lack of pediatric tools and difficulty obtaining precise histories. These delays contribute to the risk of developing shield ulcers, corneal scarring, keratoconus, and eventual blindness.

Tear film analysis offers a minimally invasive solution to detect and monitor VKC by analyzing ocular surface inflammation markers. This narrative review summarizes recent literature on tear biomarkers in pediatric VKC. Some key markers include Th2 cytokines (IL-4, IL-5, IL-10), eosinophil cationic protein (ECP), matrix metalloproteinases (MMP-1, MMP-9), tear-derived micro-RNAs, and acute phase proteins like hemopexin and transferrin. These correlate with disease severity and can be used to reflect efficacy of therapy.

Collection via Schirmer strips or microcapillary tubes is rapid and well-tolerated, enabling repeated monitoring. Standardization of pediatric-specific reference ranges and guidelines, and large-scale validation could potentially establish tear biomarker analysis as a gold-standard tool for VKC detection and treatment monitoring, reducing preventable vision loss in children.

Keywords

Vernal Keratoconjunctivitis, Preventative care, Tear biomarkers, Biomarkers, Pediatrics, Standard of Care

Introduction

Pediatric ocular allergy is an underrecognized contributor to ocular morbidity worldwide; among its many manifestations, vernal keratoconjunctivitis (VKC) represents one of the most severe and vision-threatening forms, particularly in children and

adolescents residing in warm and dry climates [1,2]. VKC is characterized by photophobia, intense pruritus, tearing, mucous discharge and its pathomnemonic presence of giant papillae of the upper tarsal conjunctiva and limbal hypertrophy [3]. Due to its development during key periods of visual development, it often renders pediatric patients particularly vulnerable to long-term consequences if early recognition and management are not achieved.

The global prevalence of VKC varies greatly with the climate and geography of the reporting region. In developed regions VKC is rather rare, approximately accounting for less than 1% of ocular diseases; it is estimated that the prevalence in Western Europe is 3.2 per 10,000 individuals and 1.24 per 10,000 children in North America [4]. Conversely, VKC is significantly more prevalent in tropical and subtropical regions, with studies reporting rates between 3% and 6% among pediatric patients. Studies from Ethiopia and Egypt report prevalence rates of 5.8% and 3.9% respectively [5-8]. Despite these analyses, true incidence is likely underestimated due to diagnostic challenges, lack of standardized criteria in children, and overlap with other allergic conjunctivitis [9].

Early VKC is frequently underdiagnosed or misclassified, especially in its subclinical and early stages. The nonspecific symptoms overlap with other allergic eye diseases like seasonal and perennial allergic conjunctivitis [10]. Children, particularly toddlers and younger, struggle to articulate exact symptoms thus adding another layer of complexity when diagnosing VKC [9]. The episodic and transient nature of the disease may further delay presentation, creating optimal conditions for subclinical inflammation to remain unchecked.



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The burden of VKC exceeds ocular discomfort. Prolonged inflammation can lead to corneal epithelial defects, shield ulcers, limbal stem cell deficiency and permanent corneal scarring [3,11]. Mechanical eye trauma caused from eye rubbing also predisposes children to the development of keratoconus [12]. These vision-threatening complications underscore the need for objective, reproducible, and minimally invasive diagnostic and monitoring tools in this vulnerable population.

In recent years, diagnostic technology has evolved to create tear film analysis, which is a promising avenue for the diagnosis of VKC. Tear film represents a uniquely accessible, disease-relevant biofluid that reflects the localized immunopathology of VKC. Immunoassay-based analyses have demonstrated elevated levels Th2 immunological cytokines, like IL-4, IL-5 and IL-10, along with eosinophil derived mediators such as eosinophil cationic protein (ECP), matrix metalloproteinases (MMP-1 and MMP-9), and acute phase proteins hemopexin and transferrin in VKC tears compared with healthy controls [9,13,14]. Multiple studies have shown that these biomarkers correlate directly with clinical grading scales, such as the Bonini severity score, and decline with effective anti-inflammatory therapy [1,9,10,15,16]; Demonstrating both diagnostic and therapeutic monitoring potential.

Tear collection via Schirmer strips or microcapillary tubules is rapid, non-invasive and well-tolerated in children. This method avoids discomfort and systemic confounders of venipuncture. Sample volumes of less than 1 µL can yield robust multiplex immunoassay and proteomic profiles. Tear-based diagnostics align with the goals of precision, speed, and cost-effectiveness in pediatric ophthalmology. The specificity of tear-derived biomarkers to ocular surface inflammation enhances their utility over systemic biomarkers, enabling a more targeted intervention.

This article examines the current evidence supporting tear film biomarkers as diagnostic and monitoring tools in pediatric VKC, evaluates their clinical validity and utility, identifies gaps in the need for standardization of tear film analysis reference ranges in children, and explores methods of how to integrate personalized approaches into routine pediatric eye care to reduce vision-threatening sequelae.

Methodology

This study was conducted as a narrative review to evaluate the utility of tear film biomarkers in pediatric cases of VKC. A comprehensive literature search was performed in Web of Science, PubMed, Scopus, and Google Scholar using the following key words: “tear biomarkers,” “tear cytokines,” “tear proteins,” “vernal keratoconjunctivitis,” “pediatric vernal keratoconjunctivitis,” “pediatric tear biomarkers,”

“ocular allergy.” Additional references were identified through manual searches of citation lists from relevant articles.

We included studies that included human participants, assessed tear film biomarkers through biochemical, immunoassay, or proteomic techniques; and reports of VKC cases with disease diagnosis, severity, and treatment response, with an emphasis on pediatric populations. We excluded conference abstracts without full data, articles not available in English, and papers prior to 1990.

This review aimed to evaluate the role of tear film biomarkers in the diagnosis and monitoring of pediatric VKC. We sought to identify the biomarkers most consistently linked with disease presence and severity, and whether tear film analysis was a feasible solution for diagnosing and monitoring VKC in the pediatric population.

Review

Current diagnostic challenges in pediatric ocular surface disease

Beyond the challenges outlined in the introduction, pediatric ocular surface disease (OSD), particularly VKC, presents additional unique diagnostic hurdles due to limited validation of pediatric-specific tools and practical challenges during examination. Instruments like the slit-lamp biomicroscopy, and adult-centered symptom questionnaires, may be less effective in children who commonly struggle to articulate their symptoms. These limitations, along with the lack of standardized pediatric guidelines and logistical demands of confirmatory tests, can lead to delayed or inaccurate diagnosis [17,18].

The gold standard for ocular surface examination is slit-lamp biomicroscopy, it serves to evaluate tear film integrity, corneal and conjunctival staining and pathologies. While effective in adults, its utility is limited in pediatrics due to fear of instrumentation, poor positioning and concentration [18]. These limitations impair visualization of subtle pathological findings, resulting in underdiagnosis or misclassification of pediatric OSD and treatment.

Lack of validated pediatric diagnostic tools

The current diagnostic tools for OSD are predominantly derived from adult studies. Clinical instruments such as the ocular Surface Disease index, standard patient evaluation of eye dryness questionnaire, Schirmer's test and fluorescein tear break up time lack formal validation for use in children [19]. Moreover, diagnostics tools such as Impression cytology, while minimally invasive and clinically informative, require topical anesthesia, patient cooperation, a multidisciplinary team of pathologist, microbiologist, and the immunologist, posing logistical challenges [20]. Similarly, in vivo confocal microscopy provides high resolution images of the ocular surface

but has a small field of view, limited standardization, expensive equipment and additional expertise [21].

Tear film biomarkers offer a practical and non-invasive alternative as it does not involve needles, imaging machines or contact with the cornea. Tear collection is manageable in pediatric patients as it causes minimal discomfort using schirmer strips or capillary tubes and with even a <1 µL volume, proteins, lipids and metabolic signals can be measured revealing early ocular surface pathology . An optimized tear film collection protocol showed 1 µL volume lead to the identification of an average of 361 ± 63 proteins in pooled samples and 525 ± 123 proteins in individual small-volume tear fluid samples [15]. Tear sampling is low risk, cost-effective and suitable for repeated monitoring [16]. However, the relationship between clinical disease and biomarkers remains understudied in pediatrics. Reference data are essential for diagnosis, monitoring treatments and personalized therapy.

Tear Film Biomarkers: Pathophysiology and Monitoring

Vernal keratoconjunctivitis

VKC is driven by Th2 immunopathy which recruits mast cells, eosinophils, and lymphocytes leading to the hyper production of cytokines. Research has demonstrated these Th2-type cytokines, IL-4, IL-5 and IL-10 were detected only in tears of VKC patients and absent in healthy controls [22]. In a study using prometric profiling, levels of albumin, transferrin, and hemopexin were found up to be 100 times higher in VKC tears than control tear levels and correlated to the severity of disease. Hemopexin, transferrin, mammaglobin B, and secretoglobulin 1D were also found significantly upregulated in VKC samples compared with the control samples. VKC patients were evaluated post topical cyclosporine or corticosteroids, tear samples revealed a drastic reduction in the protein levels [23]. Therefore, noting the change in biomarkers correlate with treatment progression and can be used for quick and non-invasive progress monitoring (Figure 1).

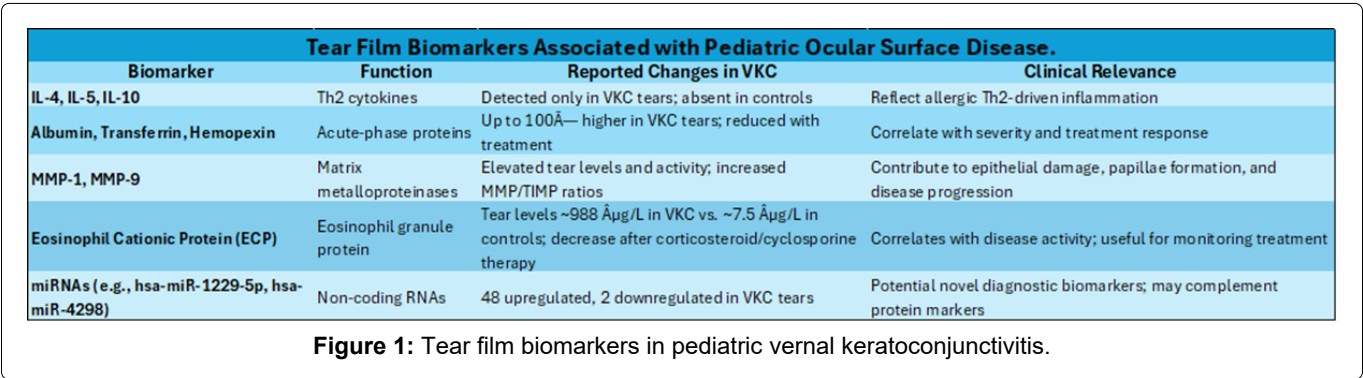
Matrix Metalloproteinase proteins (MMP) and tissue inhibitor of metalloproteinases (TIMPs) play an important role in in the evolution of eye diseases

and contribute to the development of angiogenesis, epithelial - mesenchymal transition, cell invasion and migration in the pathogenesis of ocular diseases, specifically giant papillae formation in VKC [24,25]. A study by Dr. Leonardi highlights tear levels of matrix metalloproteinase (MMP)-1 and -9 as being significantly upregulated (P < 0.001) in patients with VKC compared to control subjects. MMP-1/TIMP-1 and MMP-9/TIMP-1 rations were also significantly increased (P < 0.001) in VKC tear samples. The levels of the protein was measured but activity of them is just as important as MMP-1 and MMP-9 activities were significantly increased in VKC tears compared with control samples (P < 0.005) [24].

Eosinophil Cationic Protein (ECP) is a potent granular protein derived from the degradation of eosinophils that has been shown to be an important tear biomarker [24,25]. In measuring the mean ECP levels in control tear samples were found to be 7.5 +/- 0.4 microgram/l while in VKC tears 988.3 +/- 128 micrograms/l before therapy (P < 0.001). When VKC patients were treated with 0.1% dexamethasone or 2% cyclosporin A for 7-14 days, tear ECP decreased significantly upon retesting tear samples [26]. These findings support that ECP levels tear testing can be an effective way to diagnose, reflect the clinical status and monitor treatment in patients with VKC [26].

Beyond protein based biomarkers, emerging research has identified tear derived microRNAs (miRNAs) as a potential promising class of diagnostic and prognostic indicators for OSDs. miRNAs are small, non-coding RNAs that regulate gene expression by inducing transcription degradation or inhibiting translation [27]. They act as the “fine-tuning forks” of gene expression, influencing up to 30% of human protein coding genes [27,28]. Emerging evidence suggests that miRNAS play a role in conjunctival inflammation.

In a study by Syed, et al. 51 tear miRNA (48 upregulated and 2 downregulated) were differently expressed in the tears of VKC children. The three most significant upregulated miRNAs were hsa-miR-1229-5p, hsa-miR-6821-5p, and hsamiR-6800-5p [28]. The three most significant downregulated genes were hsa-miR7975, hsa-miR-7977, and hsa-miR-1260a. Although the small sample size limited the statistical power and several miRNAs demonstrated low area under the



curve (AUC) values, suggesting limited discriminatory potential, two miRNAs, hsa-miR-1229-5p and hsa-miR-4298, remained significantly upregulated after multiple testing correction [28]. These two remarkable miRNAs may serve as potential diagnostic biomarkers for VKC.

Despite the multiple challenges the study faced, low RNA yield, instability during storage and having multiple techniques, it slightly underscores the need for standardized protocols in miRNA analysis. Integrating miRNA profiling with established protein biomarkers could enhance early detection of subclinical inflammation, improve disease monitoring and offer a more comprehensive molecular signature for VKC.

Considering all the presented biomarkers with varying specificities and sensitivities, there needs to be a unified guideline with adequate reference ranges, scaling with age to provide cut-off values for detecting VKC. The current literature underscores the significant potential for tear film biomarkers for diagnosing and monitoring VKC. Beyond their diagnostic accuracy these biomarkers provide significant advantages: they allow for minimally invasive sampling, provide rapid results which correlate with clinical severity, and can be monitored over time to assess treatment response. The ability to track disease activity in real-time is essential in clinical practices, as it gives clinicians the opportunity to intervene earlier, prevent complications and allow for more unique tailoring of treatment regimens for patients. Tear biomarkers can complement or even replace more invasive procedures, reducing the burden on children and improving compliance with office visits and therapy.

While tear biomarker analysis sounds promising, the integration of these findings into clinical practice remains limited. Existing studies have identified key biomarker signatures like elevated Th2 cytokines, ECP, and MMP activity; there is a need to contextualize and generate interpretive guidelines for pediatric and adult populations. Comparison between biomarkers of pediatric and adult VKC patients suggest potential differences in immune profile intensity and biomarker presence, potentially indicating immunological variations between both populations. This observation warrants further age-stratified studies.

A primary barrier to clinical application of tear fluid biomarker analysis is the lack of standardized tear collection and analysis protocols. Variability in sampling techniques, like using Schirmer strips vs. microcapillary tubes, and variability in assay analyses can potentially introduce analytical noise, thus complicating cross-study comparisons. Additionally, the lack of age-adjusted reference ranges limits the ability to clinically interpret the validity of results in the pediatric setting. Inter-laboratory variability in analyses performance and

biomarker quantification further highlights the need for standardized guidelines.

From a practical point of view, the integration of tear biomarker testing into routine pediatric ophthalmology care, faces unique logistical and economic challenges. The development of microfluidic devices capable of analyzing biomarkers could bridge the gap, effectively allowing for rapid bedside diagnostics. These innovations, combined with the non-invasive and well-tolerated nature of tear collection, create an environment that promotes frequent, child-friendly monitoring without compromising comfort [29]. It is possible that the ease of repeated sampling can increase clinician and patient engagement, allowing the patient and families to be more proactive in disease management, fostering an overall more positive experience. Furthermore, simplified protocols and portable diagnostic materials hold promise for extending advanced and modern care to underserved and remote areas; ultimately reducing the burden of blindness VKC has on children across diverse healthcare settings.

Future research should prioritize large-scale, multicenter pediatric studies to validate biomarker panels, establish consistent guidelines, and assess biomarker changes throughout disease progression and treatment. Combining tear biomarker profiling with other supportive diagnostic modalities, could further enhance diagnostic precision and allow for personalized disease monitoring.

Conclusions

Tear film biomarkers hold significant promise as a minimally invasive, rapid, and precise diagnostic tool for pediatric VKC. They uniquely offer the dual advantage of aiding in early diagnosis while enabling ongoing monitoring of disease activity and treatment response. However, to fully utilize their clinical value, standardization of collection protocols, establishment of pediatric-specific reference ranges, and large-scale validation studies are necessary. With continued research and advancements in technology, tear biomarker analysis has the potential to become the gold standard for diagnosing and monitoring pediatric VKC.

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Conflict of Interests

The authors have no conflicts of interest.

Previous Presentation

The content of this manuscript has not been previously presented.

Ethical Approval

Not applicable- study is a literature review and did not involve human subjects or identifiable patient data.

Author Contributions

- Conceptualization: Martena Grace, Amir A. Estil-las, Camelia Arsene
- Methodology: Martena Grace, Amir A. Estil-las, Camelia Arsene
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- Writing- Review & Editing: Martena Grace, Amir A. Estil-las, Camelia Arsene
- Supervision: Camelia Arsene

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