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Original Article

Is there is an association between the presence of *Staphylococcus* species and occurrence of vernal keratoconjunctivitis?

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Abstract

Purpose: The aim of this study was to identify the association of normal bacterial flora with vernal keratoconjunctivitis (VKC) occurrence in VKC and non-VKC groups.
Methods: Conjunctival specimens were collected from 18 VKC patients and 22 healthy controls, cultured and identified following standard methods. The association between the presence of bacteria and occurrence of VKC was analyzed using Chi square statistic.
Results: Comparable bacterial growth was observed in VKC (77.8%) as well as control group (77.2%) ($p = 0.970$). Analysis of individual bacterial revealed that *Staphylococcus aureus* was detected more frequently in VKC (27.78% vs. 4.55% in control, $p = 0.041$) and *Staphylococcus epidermidis* was found much more commonly in the control eyes (45.45% in control vs. 5.56% in VKC, $p = 0.005$).
Conclusions: An aggravating role of *S. aureus* colonization in the occurrence of VKC, and a possible role of *S. epidermidis* against the occurrence of VKC were concluded.

Keywords: Allergy, Conjunctivitis, Eyes, Staphylococci, Vernal keratoconjunctivitis

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Introduction

Vernal keratoconjunctivitis (VKC) is a bilateral allergic conjunctivitis, most commonly observed in young patients living in warm dry climate as in Saudi Arabia.[1] It is characterized by recurrent seasonal episodes of itching, tearing, burning, mucous stringy discharge, severe photophobia, blepharospasm and foreign body sensation.[2,3] Although in early phase of the allergic reaction, antihistamines are effective treatment options, and severe allergic reaction necessitates cytotoxic and immunosuppressant treatment for a long time.[4]

The pathophysiology of VKC seems to be multifactorial, as several different mechanisms involving immune, nervous, and endocrine systems have been proposed. Earlier, it was believed that the expression of a classical type I IgE-mediated hypersensitivity reaction at the conjunctival level is involved in the immunopathogenesis of VKC.[5] However, since nearly half of VKC cases are not associated with positive RAST or skin prick test, it is unlikely to be solely an IgE-mediated disease.[6] Other studies demonstrated the involvement of neural factors such as substance P (a neuropeptide) and nerve growth factor in the pathogenesis of VKC. The over-expression of estrogen and progesterone receptors in the conjunctiva of VKC patients points toward the possible involvement of endocrine system.[5] Detection of Toll-like receptor (TLR) expression at the ocular surface

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during VKC led to the suggestions about possible role of TLRs in VKC.[7] As such, the etiology and pathophysiology of VKC remain unclear.

Conjunctival sac is colonized by different species of gram positive as well as gram negative normal bacterial flora,[8] of which staphylococci form the majority.[8,9] There is evidence to indicate the possible role of normal bacterial flora, particularly *Staphylococcus aureus* in triggering and exacerbation of different forms of allergies. Staphylococcal enterotoxin A (SEA) and B (SEB)-specific IgE antibodies have been detected in the tears of patients with allergic conjunctival disorders, particularly during exacerbation.[10,11] Staphylococcal enterotoxins have also been linked to disease severity of skin allergy and asthma as well.[12,13] In this study, we aim to find out the association between the presence of normal bacterial flora (particularly *S. aureus* and *S. epidermidis*) and occurrence of VKC.

Materials and methods

The study included 18 VKC patients (13 males, 5 females) and 22 healthy volunteers (13 males and 9 females) as controls. Healthy volunteers were selected from subjects who came to have refractive examinations to receive spectacles and contact lenses and who consented to cultures and to the protocol of the study. The study was approved by Institutional Review Board and written informed consent was obtained from all adult subjects and in the case of children from their parents.

Sample collection and transportation

Upper tarsal conjunctiva, lower conjunctival sac, and upper lid margin skin were swabbed for bacterial cultures in all subjects using sterile swabs under strict aseptic conditions. Each swab was immediately inserted in a tube containing Brain Heart infusion broth (Oxoid, Basingstoke, Hampshire, England) which served as a transport medium. Inoculated specimens were transported to the laboratory for bacteriological analysis.

Isolation of bacterial agents

A swab from each specimen was streaked onto a blood agar plate and MacConkey agar plates. Inoculated plates were incubated aerobically at 37 °C for 24 h. Plates were examined for bacterial growth. Number and types of colonies were labeled and recorded till further identification.

Identification of bacterial agents

Bacterial growth was subjected to an identification scheme using morphological and biochemical tests following standard procedures.[14] Isolates were first examined microscopically using Gram stained slides to look for gram positive cocci that usually arranged in clusters. Isolates were then examined for hemolysis after overnight incubation at 37 °C on sheep blood agar. DNase production was tested on DNase test agar as per the manufacturer's recommendations (Difco). Coagulase test was done with rabbit plasma following the procedure described by the manufacturer (BioMerieux). Catalase test was done by transferring a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick. A drop of 3% H_2O_2 was paced on to the slide and the preparation was mixed. A positive result is the rapid evolution of oxygen (within 5–10 s.) as evidenced by bubbling. A negative result is shown when no bubbles are evident. Oxidase test was done by soaking a small piece of filter paper in 1% Kovács oxidase reagent which was left for to dry. A loopful of a well-isolated bacterial colony from a fresh bacterial plate was picked and rubbed onto the filter paper. Oxidase positive organisms produced show color changes to dark purple within 5–10 s.

Identities of organism were then confirmed using Vitek II automated identification system following procedures described by manufacturer (BioMérieux SA, Marcy, France). The VITEK card contains 64 wells which comprise many fluorescent biochemical tests: 20 of which are carbohydrate assimilation; 4 are phosphatase, urea, nitrate, and actidione tests. When a test outcome is documented as ''low discrimination,'' this indicates that the result is doubtful. In these cases, the previously mentioned morphological and biochemical tests were repeated to come to a decision on such uncertain results. The VITEK 2 scheme managed card automatically from filling, sealing and then transferring them into the connected incubator (35 °C). The cards are filled automatically every 15 min by a fluorescence system. Each resulting profile is decoded according to a precise algorithm. The acquired results were compared to the ID-GP (identification of gram positive bacteria) database. In the majority of the cases the recognized gram positive bacteria are identified with high percentages of certainties.

Statistical analysis

Collected patients' data and results of cultured samples were analyzed, using Statistical Package for Social Sciences program (SPSS; Version 16). The association between the presence of bacteria and occurrence of VKC was analyzed using Chi square statistic. *P*-values < 0.05 were considered statistically significant.

Results

Table 1 demonstrates the bacterial growth of samples from both vernal keratoconjunctivitis (VKC) group and control group. Fourteen out of 18 VKC samples (77.8%) vs. 17 out of 22 control samples (77.27%) revealed bacterial growth ($p = 0.970$). *Staphylococcus* species were the most common bacterial isolates representing 50% (9/18) of the VKC samples and 54.55% (12/22) of the control samples ($p = 0.775$). *Streptococcus* species were the next most common bacteria constituting 11.11% (2/18) of the bacterial isolates in VKC samples and 18.18% (4/22) in controls ($p = 0.533$).

Staphylococci detected in this study (50%) grew aerobically on blood agar. All strains produced catalase, which differentiated them from the catalase-negative streptococci. *S. aureus* was coagulase positive; this test distinguished it from the other staphylococci. *S. aureus* produced opaque, smooth, circular, colonies that were yellow (golden) to white with beta hemolysis whereas *S. epidermidis* were white in color with alpha- or no hemolysis.