

CYTOLOGICAL MODIFICATION OF CONJUNCTIVA IN VERNAL KERATO CONJUNCTIVITIS AND ITS CLINICAL AND HEMATOLOGICAL CORRELATION

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ABSTRACT: AIM: To evaluate the cytological epithelial modification of conjunctiva in VKC and correlate with its various clinical features, hematological. Investigations and response to treatment. **MATERIALS AND METHOD:** This was a prospective study of 50 consecutive patients of VKC presenting to M.S Ramaiah Hospital, who were willing to undergo laboratory Investigations. A detailed preliminary eye examination, including Best Corrected Visual Acuity (BCVA), Tear film examination including Schirmer's test, Tear Break Up Time, Mucin ferning patterns, Tears pH, Conjunctival Impression Cytology (CIC), Skin Prick Test and Blood Investigations like Hemoglobin, Complete Blood Count, ESR, Absolute Eosinophil Count(AEC), Serum IgE Levels (S.IgE) were done. **RESULT:** Patients belonged to the age group of 4 to 25 years and M:F ratio was 4:1.Total number of patients with Palpebral VKC & mixed VKC were 13 And 37 respectively and none had limbal VKC. Total number of patients with VKC was 50 out of which Patients with normal AEC and normal S.IgE were 4(8%), with raised AEC & normal S.IgE Were 4(8%) and with raised or normal AEC & raised IgE were 42 (84%). All patients had alkaline tear film, abnormal ferning patterns were observed in 49 (98%), and abnormal TBUT in 37(74%) patients. 28(56%) had abnormal CIC grading.89.28 % (25/28) patients of abnormal CIC had Raised S.IgE levels. Only 37.5% (3/8) of VKC cases with normal S.IgE levels showed abnormal CIC. Only in 52.38% of patients with severe VKC could the allergen (skin prick test) be identified. 80. 95 b% of severe VKC cases had raised S.IgE. 59.95% (N: 25) cases had positive skin prick test out of 42 cases with raised S.IgE. VKC patients with Normal AEC &IgE levels were easily cured with mast cell stabilizers, antihistaminics and artificial tears VKC patients with raised AEC levels & Normal IgE levels were treated with topical steroids during the attacks and mast cell stabilizers and artificial tears, VKC patients with raised AEC &IgE were resistant to treatment with steroids and immunomodulators. **CONCLUSION:** VKC seems to be a strongly IgE mediated. There is need for a more robust skin prick test to identify the allergens/atopy. Immunotherapy should probably be considered early in the disease status reduce ocular morbidity due to VKC.

KEYWORDS: Vernal keratoconjunctivitis, CIC.

INTRODUCTION:

- **PURPOSE OF STUDY:** To evaluate the cytological epithelial modification of conjunctiva in VKC and correlate with its various clinical features, hematological investigations and response to treatment.
- Allergic rhinitis is a common clinical entity.

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- Seasonal and perennial allergic conjunctivitis are self-limiting, and cause less ocular surface damage.
- Vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis are complex chronic diseases that may be potentially blinding.¹
- Literature quotes that approximately 50 % of the patients with VKC do not have medical or family history of atopic diseases and do not show IgE sensitization. Thus suggesting that VKC is not solely IgE mediated Hence mainstay of treatment still remains corticosteroids and mast cell stabilizers(MCS).^{1,2}
- Immunotherapy with specific desensitization is not widely advocated unlike other allergic disorders in the body.³
- So understanding of the complex interactions and role of mediators is necessary to identify better therapeutic approaches.

MATERIALS AND METHODS: This was a prospective study of 50 consecutive patients of VKC presenting to M.S Ramaiah Hospital, who were willing to undergo laboratory investigation.

INCLUSION CRITERIA:

- VKC patients having involvement of both eyes.
- Itching.
- Scanty tenacious, ropy discharge.
- Tarsal papillae.
- Limbal elevation.
- Tranta's dots.

EXCLUSION CRITERIA:

- Any previously treated cases.
- Patients on systemic steroids and other systemic immunomodulating drugs.
- Patients above the age of 25 years.
- Patients with atopic keratoconjunctivitis and seasonal allergic conjunctivitis.

PARAMETERS ASSESSED:

- Detailed preliminary eye examination, including Best Corrected Visual Acuity (BCVA)
- Tear film examination
 - Schirmer's test
 - Tear Break Up Time
 - Mucin ferning patterns
 - Tears pH
 - Conjunctival Impression Cytology(CIC)
 - Skin Prick Test
- Blood Investigations
- Haemoglobin
- Complete Blood Count

- ESR
- Absolute Eosinophil Count(AEC)
- Serum IgE Levels (S.IgE)

CONJUNCTIVAL IMPRESSION CYTOLOGY

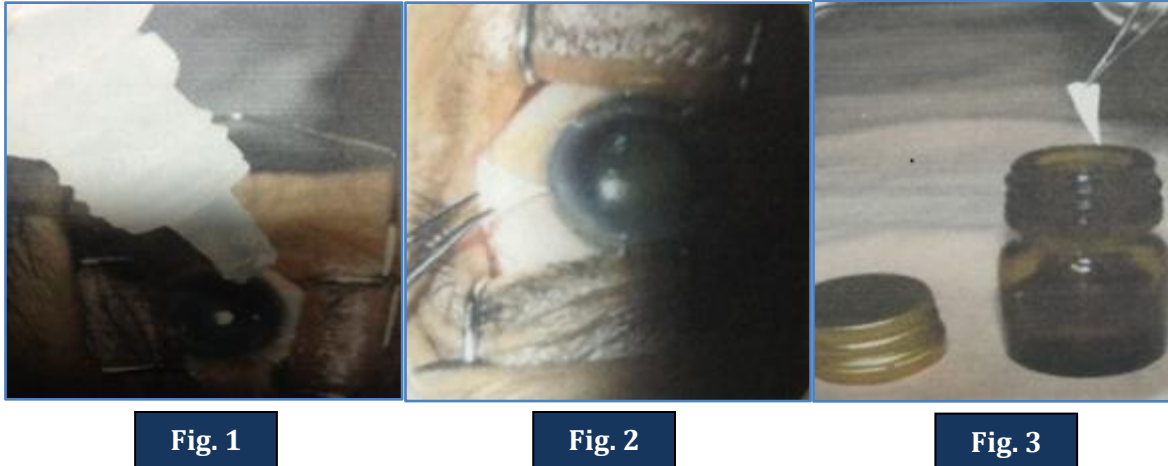


Fig. 1

Fig. 2

Fig. 3

Figure 1: Done under topical anaesthesia.

Figure 2: Triangular shaped cellulose acetate filter papers (pore size 0.22 μm) were applied over nasal and temporal conjunctiva (2mm away from limbus) and pressed by a sterile glass rod.

Figure 3: They were fixed with 95 % ethyl alcohol & stained with periodic acid Schiff and Haematoxylin – Eosinophilic stains. Conjunctival goblet cells and epithelial cells were studied under light microscope at 400x magnification.

NELSON'S CLASSIFICATION FOR CONJUNCTIVAL IMPRESSION CYTOLOGY^{4,5}

GRADE	FEATURES
0	>500 Goblet cells/ mm^2 (plump oval shape), Nucleus: Cytoplasm(N:C) ratio = 1:2 Small, round epithelial cells with large nuclei
1	350-500 goblet cells/ mm^2 (plump oval shape), N:C ratio 1:3 large, polygonal epithelial cells with small nuclei
2	100-350 goblet cell/ mm^2 (poorly defined cell borders) N:C ratio 1:4-1.5
3	<100 goblet cells/ mm^2 , N:C ratio =6 Large, polygonal epithelial cells with small/ absent nuclei (pyknotic)
	GRADE 2 or more is considered abnormal

Table 1

Figure 4: normal CIC Nelson's grade 0.

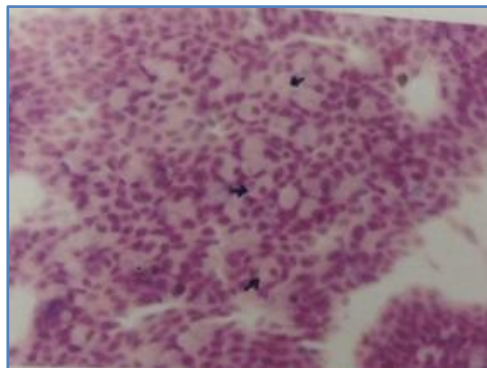


Fig. 4: Normal smear

Figure 5: Nelson's grade 1: altered N: C ratio and few scattered goblet cells.

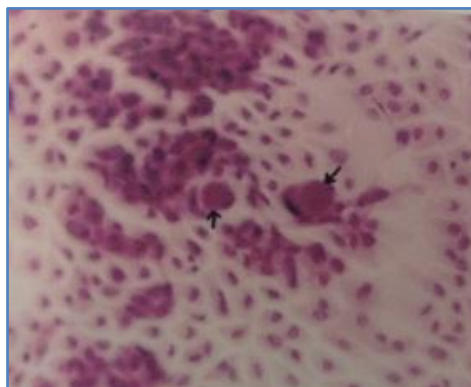


Fig. 5: Early dry eye state, Altered N:C ratio, few scattered goblet cells

Figure 6: Nelson's grade 2: altered N: C ratio. Very few scattered goblet cells.

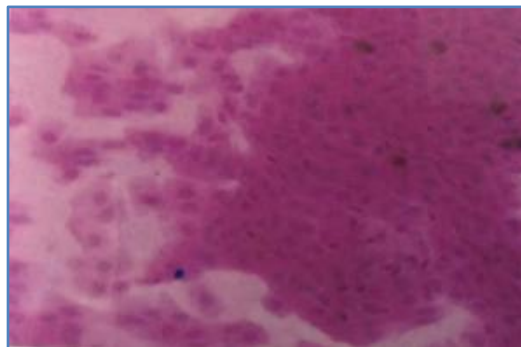


Fig. 6: Grade 1-2 dry eye state: Very few goblet cells

SKIN PRICK TEST:

To identify the causative allergen:

- Involves pricking skin surface (forearm in adults, back in children) with few drops of purified allergen.
- Measurement of indurations was quantified by shivapuri's scale and response was noted as compared to control value.^{6,7}
- Sensitivity for 123 allergens was tested in two sittings:
 - Sitting 1: Pollen, dust, insects, animal dander, and non-vegetarian food.
 - Sitting 2: Vegetarian food.



Fig. 7: Skin prick test various allergens in a box

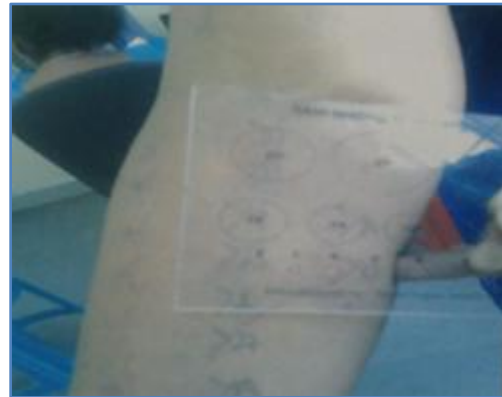


Fig. 8: Measurement of induration With shivapuri's scale

TEST FOR FERNING:

- About 2 μ l tears were collected from the inferior tear menisci of both eyes using a glass capillary.
- Tears were transferred to glass slide & allowed to dry at room temperature.
- These slides were examined under microscope and graded according to Rolando's classification.
- Ferning patterns of type III and IV were considered abnormal.⁸

Fig. 9: I: uniform arborisation in the entire field of observation without spaces between the ferns. Single fern are big and closely branched.



Fig. 9

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Fig.10: II: arborisation is abundant, but the single fern are smaller and have a lower frequency of branching than in grade 1; empty spaces appear between the fern.

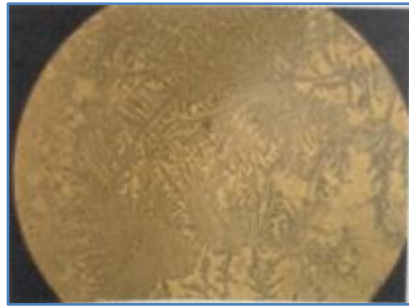


Fig. 10

Fig. 11: III: Single ferns are little and incompletely formed with rare or no branching.



Fig. 11

Fig. 12: IV: No ferning is present; mucus may appear in cluster and threads



Fig. 12

RESULTS:

AGE AND SEX DISTRIBUTION:

- Age Distribution - patients belong to the age group of 4 to 25 years
- Gender distribution - M:F = 4:1

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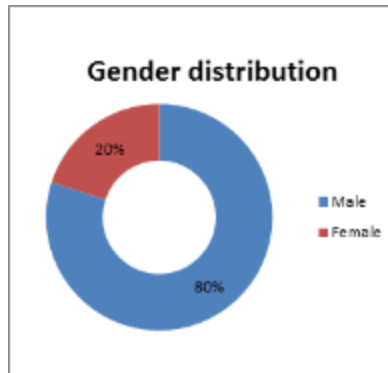


Chart 1

- Palpebral VKC: 13
- Limbal VKC: None
- Mixed VKC: 37

	NUMBER	PERCENT
Normal AEC & Normal S. IgE	4	8%
Raised AEC & Normal S.IgE	4	8%
Raised/Normal AEC & Raised S.IgE	42	84%
Total patients of VKC	50	100%

TABLE 2: CORRELATION OF VKC WITH AEC, S.IgE LEVELS AND SKIN PRICK TEST

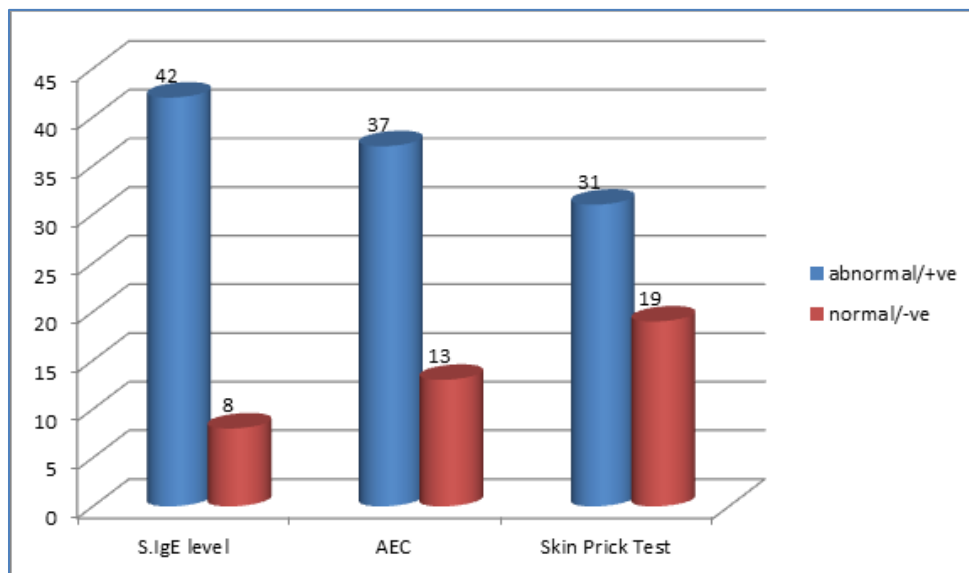


Chart 2

- Only 16% of the patients had a positive history of systemic atopy.
- Raised S.IgE& AEC levels prove that VKC has a strong association with systemic allergy, though the allergen was not identifiable with routine skin prick test in all patients.

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TEAR FILM ABNORMALITIES ASSOCIATED WITH VKC:

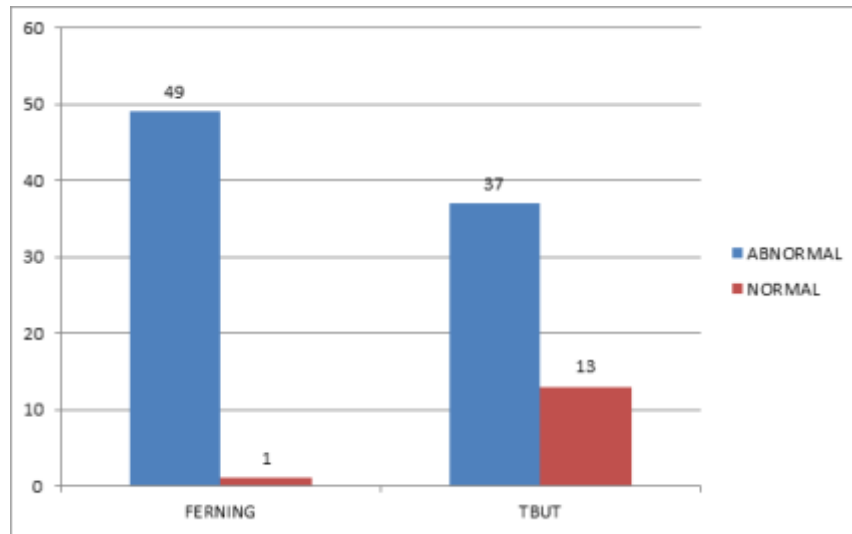


Chart 3

- All patients had alkaline pH
- Abnormal ferning patterns:98%
- Abnormal TBUT 74%

ASSOCIATION OF ABNORMAL CIC GRADING IN VKC:

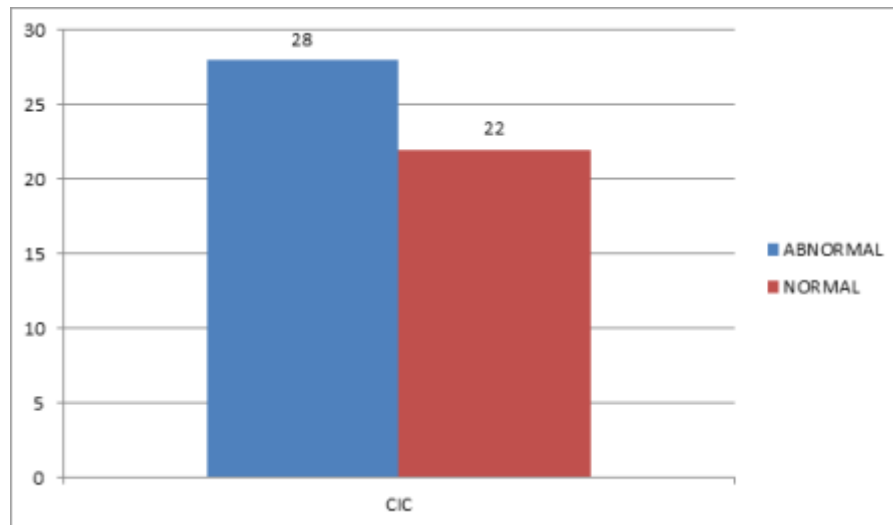


Chart 4

- Abnormal CIC grading: 56%.
- These specimens showed decrease in epithelial and goblet cells, abnormal nucleus: cytoplasmic ratio and presence of inflammatory cells, confirming the progression of VKC to ocular surface disorders (OSD) over a period of time.

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CORRELATION OF ABNORMAL CIC PATTERNS WITH RAISED AEC & S.IgE LEVELS

	NORMAL CIC	ABNORMAL CIC	TOTAL CIC
Normal AEC & Normal S.IgE levels	2	2	4
Raised AEC & Normal S.IgE levels	3	1	4
Raised/Normal AEC & Raised S.IgE levels	17	25 (89.28%)	42
Total	22	28	50

Table 3

CORRELATION BETWEEN SEVERE VKC AND ABNORMAL AEC, S.IgE LEVELS, CIC, & SKIN PRICK TEST

SEVERE VKC: 21/50 (GRADE IV TARSAL PAPILLAE, GRADE III LIMBAL PAPILLAE)

	SEVERE VKC (N:21)
POSITIVE SKIN PRICK TEST	11 (52.38%)
RAISED/NORMAL AEC and RAISED IgE LEVELS	17 (80.95%)
ABNORMAL CIC	14 (66.66%)

Table 4

- Only in 52.38% of the patients with severe VKC could the allergen (skin prick test) be identified.
- 80.95% of severe VKC cases had raised S. IgE.
- Odd's ratio: 2.1429 (significant).

ASSOCIATION BETWEEN SKIN PRICK TEST AND RAISED S.IgE

- 59.95 % (N:25) cases had positive skin prick test out of 42 cases with raised S.IgE
- Common allergens identified to skin prick test:
 - Dustmite
 - Pollen
 - Dust

RESPONSE TO TREATMENT:

GROUP 1	VKC patients with normal AEC & IgE levels: Easy to treat, MCS &/or Anti Histaminics and artificial tears(AT)
GROUP 2	VKC patients with Raised AEC levels & Normal IgE levels: Topical steroids during attacks; In between attacks – MCS & AT
GROUP 3	VKC patients with raised AEC & IgE levels: Resistant to treatment – Topical Systemic/supratarsal steroids during attacks; Immunomodulators in some cases. In between attacks – MCS & AT

Table 5

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DISCUSSION: Literature state that VKC is IgE and non IgE mediated disease and approximately 50 % of the VKC patients do not show IgE sensitization.^{1,2,3}

Some studies showed more positive results when specific IgE was measured in Tear after conjunctiva provocation test suggesting a more localized conjunctival hypersensitivity^{9, 10, 11}

However our study shows:

- Raised S IgE level in 84 % cases (only serum IgE)
- Patient prone to IgE – mediated allergic reactions are said to be ATOPIC
- Only 62 % cases had a positive skin prick test (Sensitivity for only 123 allergens) – thus, a more robust skin prick test is required to identify more allergens
- Increased role of artificial tears – severe VKC progresses very rapidly to severe ocular surface disorder as shown by abnormal CICs – Including dry eyes.
- VKC patients with raised S.IgE levels were also more resistant to treatment.
- Antigen – injection immunotherapy is very effective in treating inhalant allergens (not been studied widely in VKC).

CONCLUSIONS:

- VKC seems to be a strongly IgE mediated.
- There is need for a more robust skin prick test to identify the allergens/atopy.
- Immunotherapy should probably be considered early in the disease status to reduce ocular morbidity due to VKC.

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