

## Conjunctival Impression Cytology in Vit-A Deficient Children & It's Role In Therapeutic Assessment.

Dr.RaisaTandon,<sup>1</sup>Dr.Deepanjan Ghosh<sup>2</sup>

<sup>1</sup>(DepartmentofOphthalmology,Assam Medical College and Hospital, India)

<sup>2</sup>(DepartmentofOphthalmology,Assam Medical College and Hospital, India)

### Abstract:

**Background:**Vitamin A deficiency and resultant ocular diseases are among the most common debilitating nutritional disorders that affect the rural children in India. Our aim was to study Conjunctival Impression Cytology(CIC) in children presenting with history of Night Blindness, signs and symptoms of Xerophthalmia before and after Vitamin A administration.

**Materials and methods:** In this case control study, 20 cases(age group 1-12 years) with history of Night Blindness, signs and symptoms of xerophthalmia were enrolled in the study and 20 healthy children were recruited as age matched control group. All were clinically evaluated, underwent CIC( technique suggested by AmedeeManesome), followed up after Vitamin A supplementation with repeat CIC.

**Results:** 90% cases and 6 % controls had abnormal CIC with a higher percentage in 1-4 years age group with male preponderance. 10 % children with history of night blindness and 94% in controls showed normal CIC thus attributing a strong relationship between Vit. A deficiency and abnormal CIC(  $p < 0.0001$ ).Male preponderance was seen that was not statistically significant.67% children in the study group with h/o diarrhea showed clinical manifestation of xerophthalmia which is statistically important cause of vitamin-A deficiency and abnormal CIC .After 4 weeks, following Vit.A supplementation, 67% reverted to normal CIC.

**Conclusion:** CIC can be used as a safe, non invasive, cost effective method for screening as well as a diagnostic tool to detect and assess response to Vit A therapy.

**Key Word:**Vitamin A deficiency, Night Blindness, Xerophthalmia, Conjunctival Impression Cytology(CIC).

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### I. Introduction

Vitamin A deficiency and resultant ocular diseases are among the most common debilitating nutritional disorders that affect the rural children in India. An estimated 5-7% children in India suffer from eye signs of Vitamin A deficiency. VAD causes more than 250,000 children to go blind in Asia every year with 52,500 such cases per year being from India.<sup>1</sup> Vitamin A deficiency also leads to retarded growth and development, decreased resistance to infections as well as increased child morbidity and mortality.<sup>2</sup> Conjunctival Impression Cytology using cellulose acetate filter paper is a histological technique based on reduction of goblet cell number and abnormal epithelial cell characteristics of cellular impression obtained from conjunctiva. It has proved to be a reliable and valid measure of physiologically significant Vit. A deficiency, especially in view of serious shortcomings associated with all other approaches available for assessing Vit A deficiency.<sup>3</sup>

### II. Materials and Methods

**Study design:** Case control study

**Study location:** This was a tertiary care teaching hospital based study done in Department of Ophthalmology, at Assam Medical College and Hospital, Dibrugarh.

**Study Duration:** April 2018 to April 2019.

**Selection of cases:**

**Sample size:** 20 cases with H/O night blindness and/or showing sign and symptoms of xerophthalmia, attending Department of Ophthalmology OPD/admitted in ward, AMCH, Dibrugarh.

**(A) Inclusion criteria:**

1. Cases with h/o night blindness and/or showing sign and symptoms of xerophthalmia, attending Department of Ophthalmology OPD/admitted in ward, AMCH, Dibrugarh.

2. Age group of the patients between 1-12 years.

3. Patients and their parents willing to give consent to participate in the study.

**(B) Exclusion criteria:**

1. Patients less than 1 year and above 12 years of age.
2. Patients suffering from other ocular surface disorders like cicatricial ocular pemphigoid, OSSN, keratoconjunctivitis sicca.
3. Patients whose parents are not willing to participate in the study.

**Selection of controls:**

**Sample size:** 20 healthy children of same age and sex without any history of night blindness, and/or showing sign and symptoms of vitamin-A deficiency and with no xerophthalmia.

**(A) Inclusion criteria:**

1. Children without any history of night blindness, and/or showing sign and symptoms of vitamin-A deficiency and with no xerophthalmia.
2. Age group of the patients between 1-12 years.
3. Patients and their parents willing to give consent to participate in the study.

**(B) Exclusion criteria:**

1. Patients less than 1 year and above 12 years of age.
2. Patients suffering from other ocular surface disorders like cicatricial ocular pemphigoid, OSSN, keratoconjunctivitis sicca.
3. Patients whose parents are not willing to participate in the study.

**Procedure Methodology**

Every patient's parents were explained the nature of the study and prior written informed consent was taken from each one of them before their inclusion in the study. In this case control study, patients presenting with history of night blindness and/or showing symptoms of xerophthalmia within age group of 1-12 years were included in the study after applying inclusion and exclusion criteria and one control of each case of same age and sex without any history of night blindness and signs and symptoms of xerophthalmia attending the outpatient department of Department of Ophthalmology, Assam Medical College and Hospital, Dibrugarh were taken. After clinical evaluation including history, general and local examination, the patients were subjected to laboratory investigation and Conjunctival Impression Cytology using a technique suggested by Amedee Manseme (1988) was done. The eyes were anaesthetized with 0.5% proparacaine. Conjunctival impressions were obtained using cellulose acetate filter paper strips (5×5mm). A blunt smooth edged forceps was used to grasp the filter paper at one end and then applied on temporal bulbar conjunctiva. A smooth glass rod held in other hand was used to press the paper gently for 5-10 seconds. The filter paper was transferred to a slide and fixated with 95% ethyl alcohol. They were then transferred to the laboratory for staining. Stains used were haematoxylin, eosin, PAS and PAP. The slides were examined under light microscope using 400 magnification. Impression cytology specimens were graded based on epithelial cell morphology and goblet cell density according to Nelson's grading. Those patients whose CIC was suggestive of positive findings were then given Vitamin A treatment according to the WHO/UNICEF protocol and a repeat CIC was done after a follow up of 2<sup>nd</sup> and 4<sup>th</sup> week.

**III. Result**

1.90% children in the study group had abnormal CIC and 6% in the control group had abnormal CIC. A higher percentage of children between 1-<4 years of age had abnormal CIC in both study group (93%) and control group (7%) respectively. In the study group 10% children with h/o night blindness showed normal CIC as compared to the control group where 94% children showed normal CIC, attributing a strong relationship between vitamin-A deficiency and abnormal CIC (P<0.001).

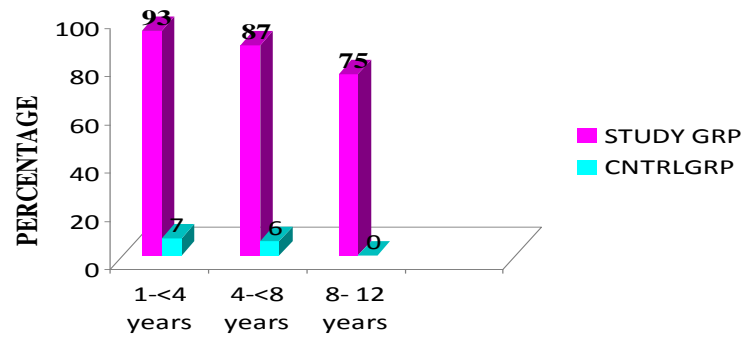


Fig 1

2. Number of males in the study group were 10 and in control group was 1 whereas number of females in the study group were 8 and in the control group was 1. The male children were 56% and female children were 44% showing a male preponderance but the difference was not found to be statistically significant ( $p > 0.05$ ).

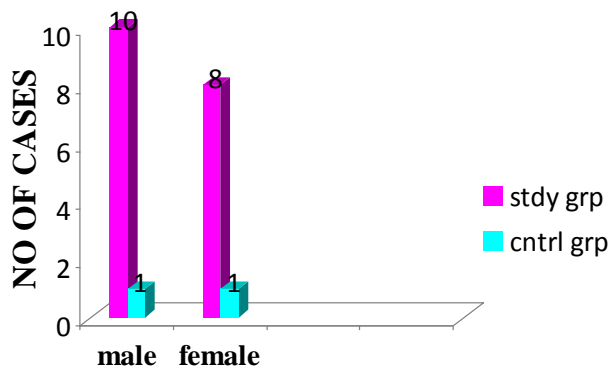


Fig 2

3. 6.7% children in the study group with h/o diarrhea showed clinical manifestation of xerophthalmia which is statistically important cause of vitamin-A deficiency and abnormal CIC ( $P < 0.01$ ). For all infections and diseases  $p < 0.001$ .

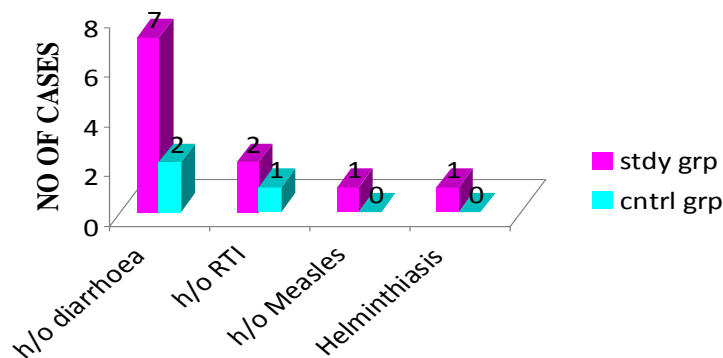


Fig - 3

4. Incidence of night blindness and ocular changes in the study group with low immunization coverage (66%) was found to be higher than those children in the control group with (90%) coverage of immunization ( $P < 0.001$ ).

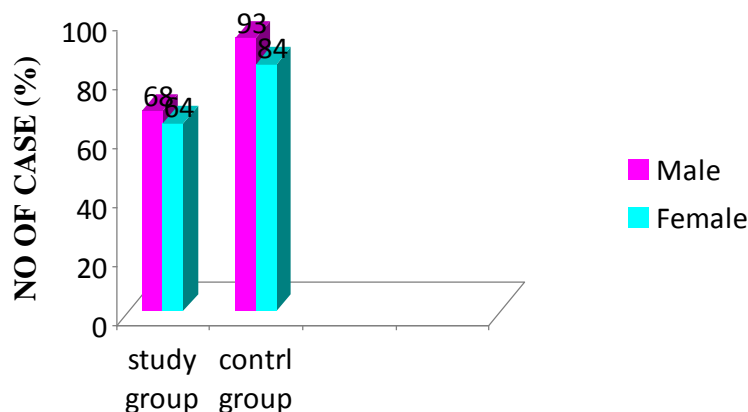


Fig – 4

SEX WISE IMMUNIZATION STATUS

5. At 4 weeks of follow up after giving vitamin-A supplementation, 67% reverted to normal and 33% continued to show abnormal CIC.

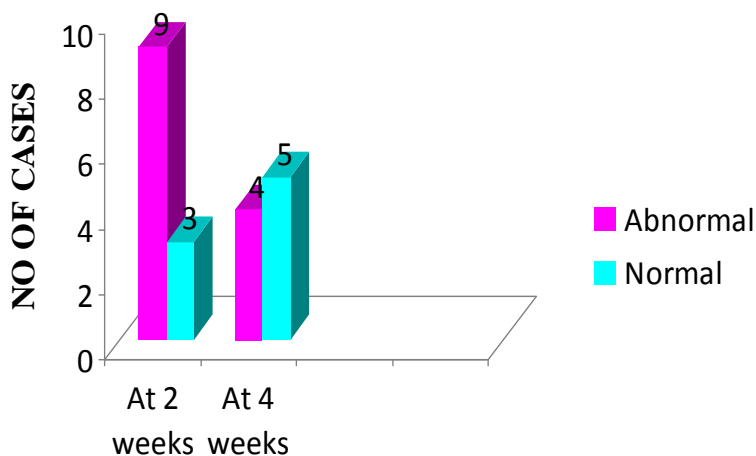


Fig 5

IV. Discussion

Preschool age children had been found to be especially vulnerable to Vitamin A deficiency and xerophthalmia, probably due to the relatively high requirements for growth and relatively low body stores of vitamin A in younger children.<sup>4</sup>The peak prevalence of xerophthalmia had been estimated to be approximately 4 years of age.<sup>5-7</sup>

In a study conducted by **Natadisastra G et al**, 93% children with vitamin A-responsive Bitot's spots and night blindness with base-line serum vitamin A less than 20 micrograms/dL (definite deficiency) had abnormal cytology. In contrast, 94% children with normal ocular exam and serum vitamin A greater than 25 micrograms/dL (least likely deficient) had normal cytology.<sup>8</sup> These findings were consistent with our study that revealed 90% children in the study group to have abnormal CIC and 6% in the control group had abnormal CIC. A higher percentage of children between 1-4 years of age had abnormal CIC in both study group (93%) and control group (7%) respectively. In the study group 10% children with h/o night blindness showed normal CIC as compared to the control group where 94% children showed normal CIC, attributing a strong relationship between vitamin-A deficiency and abnormal CIC.

Numerous studies had indicated negligible differences in prevalence estimates of Vitamin A deficiency according to gender.<sup>6,9,10</sup> Men had been found to have a higher prevalence of XN and X1B and that might be reflect gender differences in susceptibility to diarrhoea, infections and malnutrition.<sup>11</sup> The present study too had a male preponderance but the results were not statistically significant.

VitA deficiency was found to be associated with an increased risk of several infections. Such infections included diarrhoeal diseases and respiratory tract infections that result from loss of integrity of mucosal tissues, all of which had been associated with an increased incidence of mortality.<sup>12</sup> Vit A deficiency had also been seen to be associated with marked susceptibility to severe measles.<sup>13</sup> In our study, 67% children in the study group with h/o diarrhea showed clinical manifestation of xerophthalmia which is statistically important cause of vitamin-A deficiency and abnormal CIC (P<0.01). Also, incidence of night blindness and ocular changes in the study group with low immunization coverage (66%) was found to be higher than those children in the control group with (90%) coverage of immunization (P<0.001).

In a study conducted by **Natadisastra G et al** following vitamin A treatment, impression cytology detected improvement in vitamin A status in 95% of the subjects who had abnormal results at baseline examination.<sup>14</sup> This variation was consistent with that noted in previous clinical studies describing patients with conjunctival xerosis or Bitot's spots requiring two weeks to more than two months to return to normal.<sup>15</sup> The present study indicated 4 weeks of follow up after giving vitamin-A supplementation, 67% reverted to normal and 33% continued to show abnormal CIC.

**Fuchs GJ** concluded in his study that although CIC was a poor indicator of an individual child's vitamin A status, it accurately characterized the risk of vitamin A deficiency of communities.<sup>16</sup>

## V. Conclusion

Vitamin-A deficiency was found to be more prevalent in the pre-school children with a male preponderance and diarrhea is one of the main predisposing etiologic factors for night blindness and xerophthalmia along with lack of immunization.

The technique of conjunctival impression cytology (CIC) can be used as a safe, non-invasive, cost effective method for screening of subclinical cases and also as a diagnostic tool to detect and assess the response to Vitamin-A therapy, by observing the conjunctival changes in Vitamin-A deficiency.

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