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Role of conjunctival impression cytology in detecting sub-clinical vitamin A deficiency

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Abstract: Introduction: Vitamin A is an essential nutrient required for normal vision, epithelial maturation, and immunological responses. Vitamin A deficiency (VAD) is a major public health problem in many developing countries, including India, but only 5%-10% show clinical evidence. Studies have shown that subclinical vitamin A deficiency is associated with increased mortality and morbidity.

Materials and Methods: Children suspected of VAD, who were attending the Pediatric OPD at VIMS, Ballari, were studied. Those children with classical features of VAD were excluded. Conjunctival impression was taken using cellulose acetate filter paper, stained, and studied for epithelial changes, which were graded according to the Nelson grading system.

Results: Out of 250 children, 140 (56%) were male and 110 (44%) were female. All age groups were equally affected and had subclinical VAD with an average of 81.2%. The occurrence of subclinical VAD was significantly associated with severe grades of PEM, recurrent LRTI, diarrhea, UTI, measles, etc. Nelson's grading for Conjunctival imprint cytology (CIC) was effective in detecting more than 80% of all cases of sub-clinical VAD.

Conclusion: Subclinical VAD without ocular manifestations was largely prevalent in a suspected group of children, more so in children of low socio-economic status. Conjunctival Impression cytology was able to detect the majority of these cases, initiate early management of these subclinical cases, and thus reduce the mortality and morbidity associated with VAD.

Keywords: Vitamin A deficiency; Subclinical; Conjunctival impression cytology; Childhood malnutrition; Public health.

1. Introduction

Vitamin A deficiency (VAD) is a public health problem in more than half of all countries, particularly in South-East Asia and Africa, affecting young children and pregnant women the most. An estimated 250 million preschool children are Vitamin A deficient, and between 250,000 to 500,000 of them die every year due to various infections caused by VAD [1–8].

Vitamin A is an essential nutrient for the proper differentiation and maintenance of mucosal epithelium, immunological responses, etc [3]. The absence of Vitamin A leads to a loss of goblet cells and squamous metaplasia [2,5,9].

The measurement of Vitamin A levels requires invasive sampling, sophisticated lab equipment, trained personnel, and proper transport [3]. Serum Vitamin A levels also suffer from poor correlation with body stores, except under conditions of severe depletion, and are not a direct indicator of physiological status. Subclinical cases would also be missed on serum values [2,3,9]. Hence, there is a need for a relatively simple yet objective test for Vitamin A status to identify communities with VAD [2,4].

Conjunctival impression cytology (CIC) takes advantage of the changes in certain cells of the eye that occur due to VAD and reflects the physiologic function as it relates to vitamin A status [3,4]. CIC is a relatively simple and practical technique for documenting physiologically significant VAD [7–13].

CIC was first described by Larmande and Timsit for the diagnosis of squamous neoplasia of the ocular surface in 1954. Haller-Schober et al. documented the use of impression cytology for examining ocular surface disorders and goblet cell densities in 1977 [8].

The applications of CIC include etiologic diagnosis of various ocular surface disorders, documenting sequential changes in the conjunctival and corneal surface over time, monitoring the effects of treatment, staging conjunctival squamous metaplasia, etc [9].

The main advantage of CIC is the loss of goblet cells, which can be assessed and graded as efficiently as in biopsy samples [9–11].

2. Objectives

1. To determine the efficacy of Conjunctival impression cytology in detecting subclinical VAD in predisposed children.
2. To determine the prevalence of subclinical VAD in susceptible populations.

3. Methodology

All patients aged 1-12 years who presented with malnutrition, recurrent URTI, measles, recurrent diarrhea, and failure to gain weight at the Pediatric OPD at VIMS, Ballari were included in the study.

Exclusion criteria included children with classical signs and symptoms of VAD, acute eye infections, those with immunodeficiency, uncooperative children, and those who had already started vitamin A supplements.

After a detailed history and examination at the Pediatric OPD, relevant investigations were performed to rule out VAD. Cases that were not detectable by any of these but had clinical suspicion of subclinical VAD were subjected to Conjunctival Impression Cytology (CIC). The obtained data was entered into Microsoft Excel and analyzed with SPSS software version 27 for prevalence, proportion, chi-square test, and trend analysis.

4. Procedure

Children and parents were informed about the procedure, and informed consent was taken. A drop of Proparacaine (0.5%) was instilled into both eyes, and the children were asked to keep their eyes closed for 2 minutes. Children under 6 years of age were held in place by being seated on their mother's lap, and older children were asked to sit on a chair/stool and lean back.

A cellulose acetate paper disc (pore size 0.45 μm; Sartorius GmbH.3400 Gottingen, W.Germany) was cut into 4 symmetrical parts, diametrically. Blunt forceps were used to hold the filter paper to avoid contamination with desquamated cells from our fingers.

Subjects were distracted and asked to look laterally at a distant object, and the eyelids were kept wide open by an assistant. The rough side of the filter paper was pressed to the temporal aspect of the bulbar conjunctiva, and slight pressure was applied using a sterile cotton bud. The filter paper was then lifted off and pressed over a labeled and numbered clean, dry glass slide, helping to transfer the collected desquamated cells onto the glass slide. A similar procedure was repeated on the other eye.

The slides were fixed in isopropyl alcohol for 15 minutes and stained with PAS stain. The cytopathologist then reviewed the slides and graded them according to the Nelson's grading system [9].

Grade 0: small round epithelial cells with eosinophilic cytoplasm and large basophilic nucleus with N/C ratio 1:2. Abundant goblet cells ($>$500cells/mm^2).

Grade 1: slightly larger, polygonal cells, smaller nucleus with N/C ratio 1:3. Slightly reduced goblet cells (350-500 cells/mm^2), oval, plump with intensely PAS+ cytoplasm.

Grade 2: larger polygonal epithelial cells, occasional multinucleate cells with variably stained cytoplasm, N/C ratio 1:4 to 1:5. Markedly reduced goblet cells (100-150 cells/mm^2), smaller in size, and less intense PAS.

Grade 3: large polygonal cells with basophilic cytoplasm and small pyknotic nucleus with N/C ratio $>$1:6. Very scant goblet cells ($<$100cells/mm^2).

Grade 0 and 1 were considered normal, while grade 2 and 3 were considered abnormal.

5. Results

A total of 250 children suspected of having subclinical vitamin A deficiency and attending the Department of Pediatrics at VIMS, Ballari, from January 2012 to December 2012, were studied. They ranged in age from 1 to 12 years, of whom 140 (56%) were male and 110 (44%) were female. All age groups were equally affected by vitamin A deficiency (80%). Children in the age group of 4 to 6 years were the most affected (91 cases), followed by 1-3 years with 46 cases and 33 cases in the 7-9 years category.

Both male and female children were equally affected, and the prevalence of VAD in this study was ≥80%.

In the present study, 148 cases from rural areas accounted for 59.2% of total admissions. 27.2% were from urban slums, and 13.6% were from urban areas, indicating a higher incidence of VAD in rural and low socio-economic status populations.

The CIC grading according to Nelson's grading system showed that the majority were of grade 3 and grade 2, accounting for 42% and 39.2%, respectively. Less than 20% were of grade 1 and grade 0 together (Table 2).

In the present study, the prevalence of subclinical Vitamin A deficiency in cases subjected to CIC was 81.20%, i.e., the efficacy of CIC in detecting subclinical VAD was >80%.

The present study found significant subclinical VAD. 88% of affected cases were in children with recurrent diarrhea, 82.2% in children with recurrent LRTI, 65.5% in children with recurrent UTI, 100% in children with measles, 62.6% in children with post-measles bronchopneumonia, and 83.3% in children with severe grades of PEM had subclinical VAD. This was statistically significant with a p-value < 0.05.

Table 1. Relation between subclinical VAD and certain demographics

Age group	**Sub clinical VAD**		**Percentage**
	Present	**Absent**	
1 - 3 years	46 (80.7%)	11 (19.3%)	57 (100%)
4 – 6 years	91 (84.2%)	17 (15.8%)	108 (100%)
7- 9 years	33 (71.7%)	013(28.3%)	46 (100%)
10 -12 years	33 (84.6%)	06 (15.4%)	39 (100%)
Total	203 (81.2%)	47 (18.8%)	250 (100.0%)
Gender	**Sub clinical VAD**		**Percentage**
	Present	**Absent**	
Male	115 (82.1%)	25 (17.9%)	140 (100%)
Female	088 (80.0%)	22 (20.0%)	110 (100%)
Total	203 (81.2%)	47 (18.8%)	250 (100.0%)
Locality	**Frequency**		**Percentage**
Rural	148		59.2%
Urban	034		13.6%
Urban slum	068		27.2%
Total	250		100%

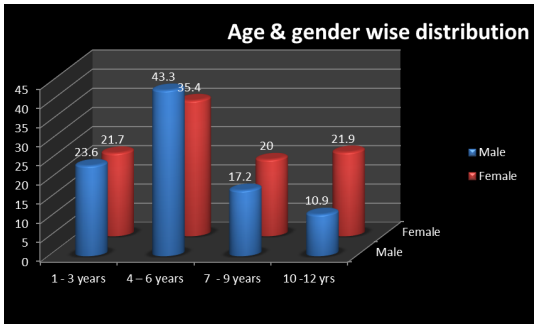

Figure 1. Age & Gender wise distribution of cases

Table 2. Distribution based on CIC grading

CIC grading	**Frequency**	**Percentage**
Grade 0	020	08.0%
Grade 1	027	10.8%
Grade 2	098	39.2%
Grade 3	105	42.0%
Total	250	100%

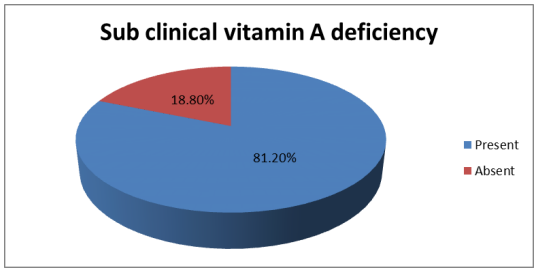

Figure 2. Distribution of cases based on detection of Sub clinical VAD

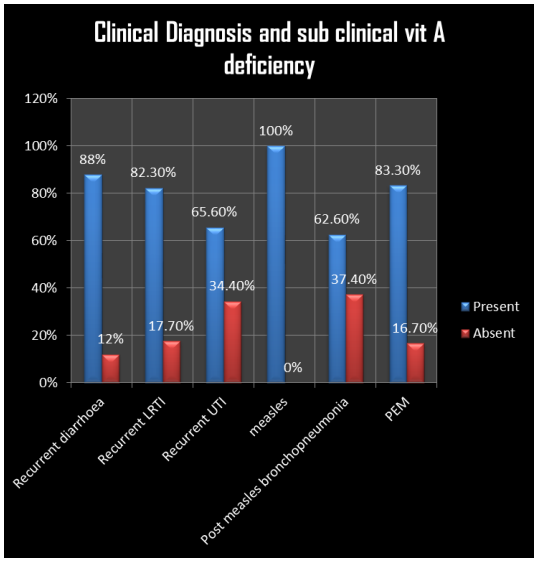

Figure 3. Relation between clinical diagnosis and subclinical VAD

6. Discussion

Vitamin A deficiency (VAD) among preschool children in developing countries is associated with increased morbidity and mortality from respiratory and diarrheal diseases and can cause blindness. Many studies indicate that vitamin A supplementation of non-Xerophthalmic children reduced mortality and morbidity, suggesting that subclinical VAD (without ocular manifestations of Xerophthalmia) is also associated with increased mortality.

In the present study, the male to female ratio was 1:0.78, which was statistically comparable to other studies. The presence of subclinical VAD in the suspected group of children was 82.1% in males and 80% in females.

In a study from the eye hospital of Dr. Yap KieTiong in Jogjakarta, Indonesia, male preponderance was found in cases of VAD in children admitted to the hospital with various illnesses. Among the 6300 cases, the male-to-female ratio varied with age. It was 1.4:1.0 in the preschool age period and 6.0:1.0 at around 10 years of age. This could be due to various sociocultural and demographic factors such as more only males attending school and the general male population being higher.

Out of the 250 cases, 66% were below 6 years, whereas in other studies, it varied. In the studies by Ausayakhun et al. [4] and Reddy et al. [5], the cases were more in children older than 6 years. This could probably be due to the sampling effect as the studies done by Reddy et al. [5] and Ausayakhun et al. [4] were community-based, whereas the present study was hospital-based and considered those cases with multiple predisposing conditions.

Though there are many other studies [4,5,10] that are in contrast to our findings, i.e., the incidence of VAD is more common among school-going children above 6 years of age, what is comparable is the increased prevalence of subclinical VAD and the public health problem posed by it.

Comparison was not possible with many studies as the study populations were different, and most of them chose school-going children, whereas our study also focused on under 6 years as VAD is a known factor for chronic illnesses like PEM, respiratory diseases, measles, etc., early in childhood.

A total of 81.2% of cases in the present study showed abnormal grading on CIC, which is much higher compared to the other 2 studies. This difference could be because the present study was conducted in a suspected group of children, whereas in the other studies, the subjects were from the general population where both normal and Xerophthalmic children were studied.

Cases considered in the present study were not having established eye features of VAD but were subclinical cases where the underlying VAD has predisposed them to recurrent infections and poor growth.

From the above, it is seen that the majority of the studies considered only one clinical parameter, i.e., persistent diarrhea, to perform CIC. When Natadisastra et al. [2] included severe PEM cases along with persistent diarrhea, the detection rate of CIC increased exponentially.

Table 3. Age distribution compared to other studies

Age group	Present study	Reddy et al., [5]	Ausayakhun et al., [4]
$\leq$ 6yrs	66%	37.8%	51%
$>$ 6yrs	34%	62.2%	49%

Table 4. Abnormal CIC comparison to other studies

Age group	Present study	Reddy *et al.*, [5]	Ausayakhun *et al.*, [4]
$\leq$6yrs	84.8%	35.5%	40.2%
$>$6yrs	77.6%	25.5%	17.3%
Total	81.2%	30.5%	28.75%

7. Conclusion

Subclinical VAD without ocular manifestations is highly prevalent in developing countries. Although predisposing conditions are more common in rural populations with low economic backgrounds, all children with predisposing factors are equally affected. Many studies have documented significant improvement in mortality and morbidity of non-xerophthalmic patients receiving vitamin A supplements, suggesting the existence of subclinical VAD.

CIC was found to play an important role in the early detection and management of subclinical VAD. Many studies have shown that CIC (post-supplements) correlates well with improved clinical findings in cases of VAD and subclinical VAD on Vitamin A supplementation.

CIC is a relatively simple, rapid, atraumatic, cost-effective, and objective method of assessing Vitamin A deficiency. This technique is much favored, especially in developing countries with limited resources.

Hence, we conclude that by increasing the purview of diseases under subclinical VAD, the scope of detection of VAD by CIC increases.

Author Contributions: All authors contributed equally to the writing of this paper. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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Appendix Images

Figure 4. Shows the process of sample collection from the lateral part of the bulbar conjunctiva

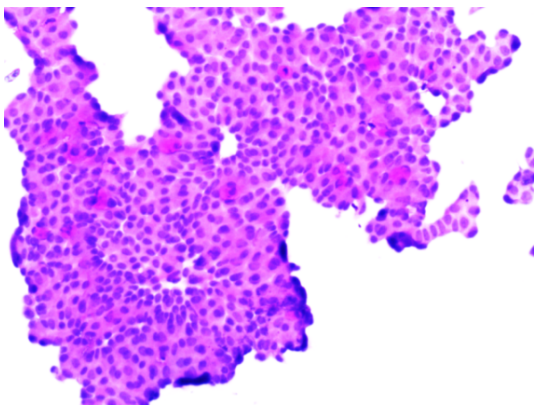

Figure 5. Nelson's Grade 0 – shows abundant goblet cells & occasional squamous cells PAS 100x

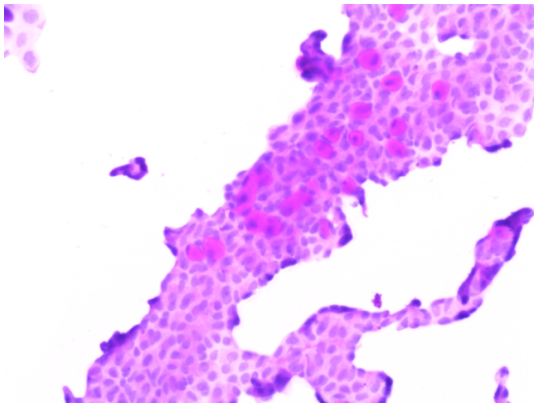

Figure 6. Nelson's Grade 1 – shows many goblet cells & occasional squamous cells PAS 100x

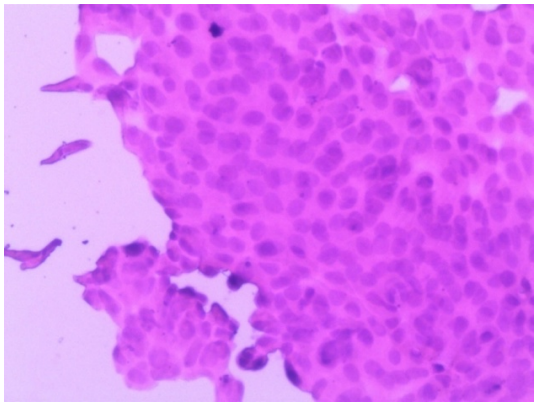

Figure 7. Nelson's Grade 2 – shows increase in squamous cells with nuclear atypia and reduced goblet cells PAS 100x

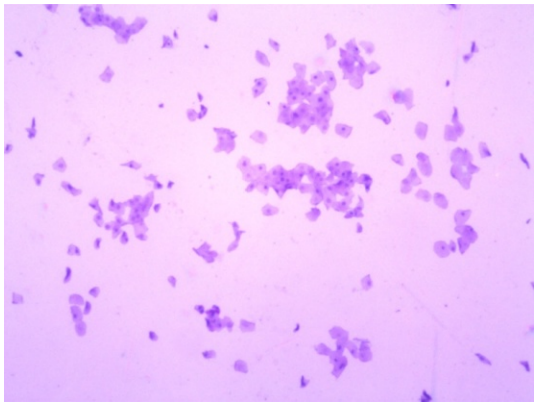

Figure 8. Nelson's Grade 3 – shows abundant squamous cells and reduced to absent goblet cells PAS 40x

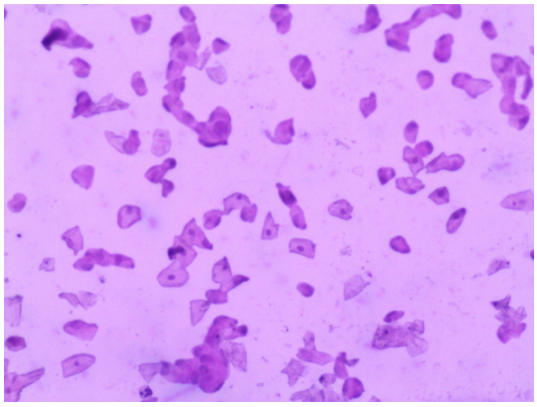

Figure 9. Nelson's Grade 3 – shows abundant squamous cells, few with increased atypia and reduced to absent goblet cells PAS 100x