

EVALUATION OF VITAMIN A STATUS BY CONJUNCTIVAL IMPRESSION CYTOLOGY AMONG SCHOOL AGED POPULATION

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ABSTRACT

Conjunctival impression cytology was used to determine the extent of preclinical and clinical vitamin A deficiency in school children aged 8-13 years. Nearly 23% of the examined children had an abnormal cytology reflecting a poor vitamin A status. The differences in cytology results were not significantly affected by either age or sex.

Key words: Xerosis, Conjunctival impression cytology.

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Vitamin A deficiency is a major nutritional problem in developing countries. In countries like India and Bangladesh, 0.4% of pre-school children are estimated to be suffering from nutritional blindness(1). Prevalence of vitamin A deficiency by clinical examination, in different parts of India has been estimated to vary from 4.4% to 34.1%(2,4). However, the criteria used for clinical diagnosis have been variable, with some workers also including X1A in the diagnosis.

Studies conducted among school children in India have indicated that prevalence of mild clinical ocular signs of vitamin A deficiency is high in this population(5,7). Pratinidhi *et al.*(4) observed that 55.9% of children aged 5-13 years showed signs or symptoms of vitamin A deficiency, while 34.88% of rural children aged 5-15 years, from Vidarbha also showed similar abnormalities(8).

Diagnosing vitamin A deficiency in a population, based on clinical signs and symptoms poses problems due to the criteria followed for the clinical diagnosis, as well as the skill of the observer. Thus, overestimation due to the inclusion of X1A in the clinical diagnosis is a relatively common problem. At the same time assessments based on serum samples are fraught with technical, analytical and cultural problems(9).

Impression cytology of the conjunctiva is gaining acceptance as a highly sensitive (93%) and specific (94%) test for detection of vitamin A deficiency(10). The test has been found to be very effective in the preclinical stages also(11,12). The test has also gained acceptance as a method for establishing the community vitamin A status(13).

Since blinding malnutrition is seen almost exclusively in the under five age group,

this segment of the population has always been the major focus group for intervention programmes, while the school aged population has generally received less importance. But recent evidence linking vitamin A deficiency to diarrhea, measles, acute respiratory infections and growth status could mean that even the school aged child may require more attention in the future. The present study was thus undertaken to establish whether vitamin A deficiency in school aged populations was indeed a public health problem.

Materials and Methods

One hundred and fifty six children of both sexes, aged 8-13 years were evaluated for evidence of vitamin A deficiency by conjunctival impression cytology (CIC) and both eyes were examined. CIC was performed by standard procedure(10) using HAWG filter paper 0.45 μ m pore size. The filter paper along with the cells were immediately transferred to a fixative solution containing 70% ethyl alcohol, 37% formalin and glacial acetic acid in the ratio of 20 : 1 : 1 by volume(11). Minimum time allowed for fixation was 10 minutes. Staining with PAS and hematoxylin(12) was done at any convenient time thereafter and they were mounted under DPX solution.

All the slides were read by one of the investigators (NG) who was blind to the collection of specimens and clinical assessment of vitamin A deficiency. The slides were examined for PAS positive goblet cells and epithelial cellular morphology. The results were interpreted as per the criteria adopted by Amedee-Manesme O *et al.*(1).

Results

One hundred and fifty six children aged 8-13 years were examined for evidence of vitamin A deficiency. Nearly, half (85 cases;

54.49%) were boys and 45.51% (n = 71) were girls.

Of the 156 children examined, conjunctival impression cytology detected 23.08% (n=36) as abnormal (deficient vitamin A status). The proportion of boys showing abnormal impression cytology was 22.35% as against 23.94% among girls (*Table I*). These differences in sex wise prevalence were however not statistically significant ($\chi^2=0.0524$; $p>0.05$).

Comparing impression cytology findings in different age groups, irrespective of their gender (*Table II*), it was observed that older children had a higher prevalence of abnormal cytology (27.72%) compared to the younger children (14.55%). Although the difference was more marked compared to the gender differences, the difference was

TABLE I—*Impression Cytology of School Children of Both Sexes*

Sex	Labelled abnormal by impression cytology (%)	Labelled normal by impression cytology (%)	Total
Males	19 (22.35)	66 (77.65)	85
Females	17 (23.94)	54 (76.06)	71
Total	36 (23.08)	120 (76.92)	156

TABLE II—*Impression Cytology Findings in Different Age Groups*

Age group (yrs)	Labelled abnormal by impression cytology (%)	Labelled normal by impression cytology (%)	Total
8-10	8 (14.55)	47 (85.45)	55
11-13	28 (27.72)	73 (72.28)	101
Total	36 (23.08)	120 (76.92)	156

not statistically significant ($\chi^2=3.4940$; $p>0.05$).

The prevalence of abnormal cytology was thus found to indicate a public health problem of preclinical vitamin A deficiency even among the school going population.

Discussion

CIC is a relatively newer technique for assessing marginal vitamin A status. Impression cytology is studied using cellulose acetate millipore filter paper which is applied by finger pressure over the bulbar conjunctiva and the cells adhere to the filter paper which are then transferred to a slide, fixed and stained. Generally the test has been shown to have a high sensitivity (93%) and specificity (94%)(11,13) and only rarely has a low sensitivity (26%) been reported(14). With CIC, with normal vitamin A status, goblet cells are abundant and epithelial cells are normal, while in those with a low serum retinol level, goblet cells are reduced or absent and epithelial cells are abnormal (Figs. 1 & 2). Impression cytology has also been found to be safe, non-invasive and easy to perform(10).

In the present study, 23.08% of school children aged 8 to 13 years had an abnormal cytology. Sex and age did not seem to significantly affect prevalence. Clinical surveys among school children have consistently documented vitamin A deficiency in such children(2-8). Results of the present study show that impression cytology has also been able to detect a high prevalence of Vitamin A deficiency in school children. As in the earlier clinical surveys(7), impression cytology has also documented higher prevalence rates in older children. Thus, it is evident that vitamin A deficiency is a significant problem in school aged populations also. It has been recently stated that CIC can be conveniently used to define vitamin A deficiency as a public health problem(14). It has recently been estimated that when abnormal CIC findings are seen in 10.8% of a population, 5% of this sample would have serum retinol levels of less than 10 $\mu\text{g}/\text{dl}$ (14). The WHO (1982) has recommended that when serum retinol levels are less than 10 $\mu\text{g}/\text{dl}$ in more than 5% of a population, it indicates the presence of a significant public health problem of vitamin A deficiency.

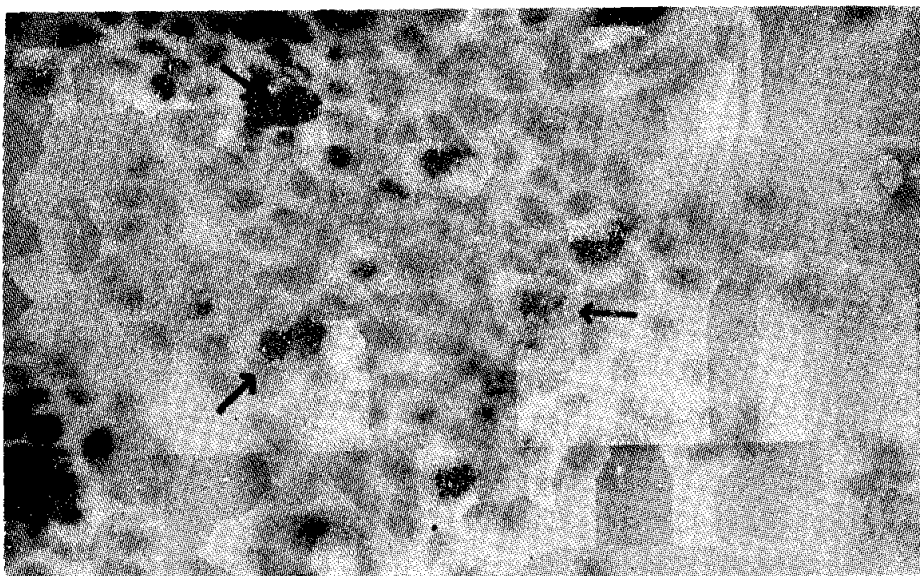


Fig. 1. Normal conjunctival impression cytology, with PAS +ve material (400 X).



Fig. 2. Conjunctival impression cytology in vitamin A deficiency, flattened pleomorphic squamous cells, no goblet cells (400 X).

These estimates are however valid only for preschool children, and their significance in terms of older children needs to be established.

The schoolaged child had generally received less attention in terms of prophylaxis programmes, mainly because blinding malnutrition is a spectre that haunts only the pre-school child. However, recent evidence has pointed to a more comprehensive role for vitamin A, in addition to the ocular effects, including the effect on growth and reproduction and sexual maturation. It has also been hypothesised that clinical signs seen during school age are only reflective of past and not active vitamin A deficiency(15). However, the abnormal CIC findings in the present study indicate that active marginal vitamin A deficiency is a problem in the school aged child also. This is the first study in India in this specific population.

Thus, it is observed that subclinical vitamin A deficiency is seen in approximately 1 out of 4 school aged children, and the prevalence is similar in boys and girls. However, the consequences of the subclinical defi-

ciency in the school aged child needs to be adequately established.

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NOTES AND NEWS

XIII ANNUAL CONVENTION OF NATIONAL NEONATOLOGY FORUM

The XIII Annual Convention of National Neonatology Forum is to be held at Baroda from December 17 to 19, 1993. This Convention is organized jointly by the Department of Pediatrics, Medical College, Baroda and the Indian Academy of Pediatrics, Baroda Branch.

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