Impression cytology: a practical index of vitamin A status[1-3]

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ABSTRACT Impression cytology was performed on 148 Indonesian preschool children of whom half had mild xerophthalmia and half were age-matched control subjects. Subjects were divided into subgroups that reflected the degree of confidence in their true vitamin A status as determined by serum vitamin A levels, clinical examination, and response to therapy. Impression cytology was considered normal if goblet cells were present and abnormal if they were absent. Thirteen of 14 (93%) children with vitamin A-responsive Bitot's spots and night blindness with base-line serum vitamin A < 20 μg/dL (0.70 μmol/L) (group 1, definite deficiency) had abnormal cytology. In contrast, 17 of 18 (94%) children with normal ocular exam and serum vitamin A > 25 μg/dL (0.87 μmol/L) (group 7, least likely deficient) had normal cytology. Importantly, 12 of 26 (46%) clinically normal children with serum vitamin A levels < 20 μg/dL (0.70 μmol/L) had abnormal impression cytology. *Am J Clin Nutr* 1988;48: 695–701

KEY WORDS Vitamin A deficiency, diagnosis, surveys, xerophthalmia, keratinization, impression cytology

Introduction

Vitamin A deficiency is the major cause of childhood blindness in many developing countries (1, 2). Mild xerophthalmia consisting of night blindness and conjunctival xerosis is associated with increased morbidity and mortality (3–6). Vitamin A supplementation of nonxerophthalmic children reduces mortality, suggesting that subclinical vitamin A deficiency (ie, physiologic deficiency without ocular manifestations of xerophthalmia) is also associated with increased mortality (7). Previous studies demonstrated that conjunctival impression cytology correlated with vitamin A status in a vitamin A-deficient rabbit model (8) and was often abnormal in the presence of xerophthalmia (9) or depleted liver stores (10) in children. We herein report indices of sensitivity and specificity of impression cytology for the detection of early, physiologically significant vitamin A deficiency among young children.

Subjects and methods

The study was performed at the Cicendo Eye Hospital, Bandung, Indonesia. Children aged 36–72 mo with mild xerophthalmia, defined as a history of night blindness or the presence of conjunctival xerosis with Bitot's spots, were identified as cases in surrounding villages by a trained nurse. Whenever a case was identified, an age-matched control from the same village was also identified. All case and control subjects were brought to the hospital where they were examined by one of two ophthalmologists. Seventy-five cases and 73 age-matched controls were identified.

Each child's dietary and disease history was obtained from the parent or guardian. After parental consent was obtained, each child underwent base-line evaluation consisting of an ocular exam, anthropometry, and impression cytology. All ocular findings were drawn and photographed. Venous blood was obtained and promptly separated and the serum was frozen. Serum vitamin A was analyzed by high-performance liquid chromatography (HPLC) (11).

Conjunctival impression cytology was performed on each patient using our previously described technique (9, 12). Topical 0.5% proparacaine was applied to each eye. Precut 5 mm by 5 mm pieces of cellulose acetate filter paper (HAWP 304FO,

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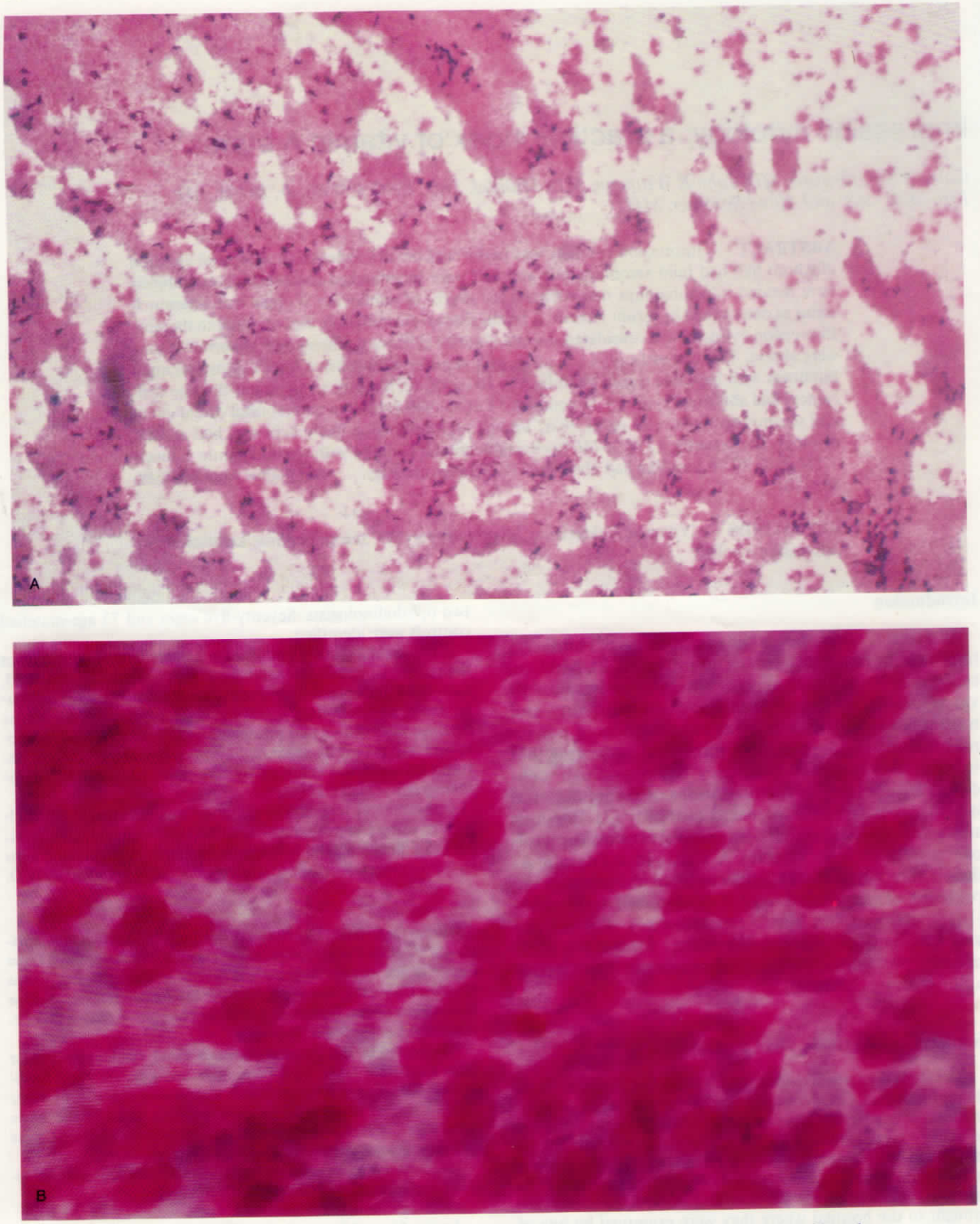

FIG 1. *A:* Low-power view of imprint from normal child. Sheets of small uniform epithelial cells are evident along with PAS positive goblet cells (periodic-acid Schiff and Harris hematoxylin, 100×). *B:* Higher-power view of same specimen showing mucin containing goblet cells densely stained with periodic-acid Schiff (400×).

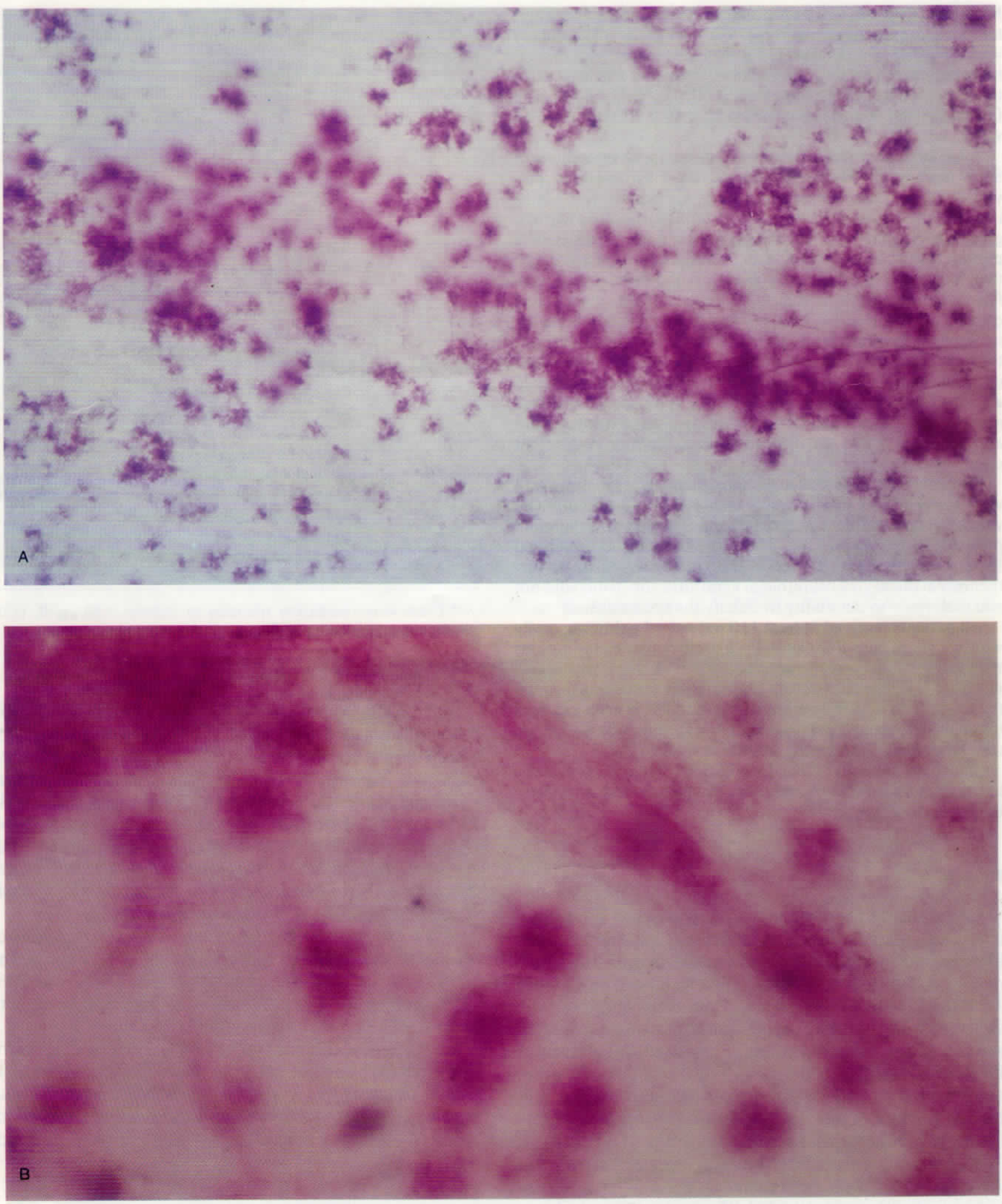

FIG 2. *A:* Low-power view of imprint from normal child. Note absence of epithelial cell sheets but abundant PAS-positive mucin spots, presumably the contents of adherent but broken goblet cells. These are a definite sign of normally differentiated epithelium (periodic-acid Schiff and Harris hematoxylin, 160×). *B:* Higher-power view of imprint from another normal child. The specimen contain few if any intact cells of any type but PAS-positive, smudged, mucin spots particularly in the lower left half of the figure. This specimen is graded as normal (periodic-acid Schiff and Harris hematoxylin, 400×).

Millipore, Bedford, MA) were applied to the bulbar nasal and temporal conjunctiva of each eye. The filter paper was removed with a peeling motion after 3–5 s. The filter paper with the adherent epithelial cells was immediately placed in a fixative solution prepared by mixing 750 mL 70% ethyl alcohol, 50 mL 37% formaldehyde, 50 mL glacial acetic acid, and 150 mL distilled water.

After fixation the specimens were stained with periodic-acid Schiff and modified Papanicolaou's stain as described previously (9, 12). All impression-cytology specimens were examined by one investigator in blinded fashion. Specimens were graded as normal if goblet cells or abundant mucin spots were present (previous stages 0 and 1) or abnormal if enlarged epithelial cells were present and goblet cells and mucin spots were few or absent (previous stages 2–5). Mucin spots are discrete clusters of PAS-positive granules of variable size. Each cluster is slightly larger than an intact goblet cell and represents secretions from a goblet cell that failed to adhere to the filter paper (13). The child was graded normal if any of the specimens taken at that exam was normal and abnormal if all specimens were abnormal. **Figures 1 and 2** show imprints from a normal child and **Figure 3** shows an imprint from a vitamin A-deficient child.

These figures were collected subsequent to this study and were stained by a modification of our earlier technique (8), which we find simpler to carry out and interpret and therefore presently recommend (14). The more complicated modified Papanicolaou's stain (9) used in this study was chosen to further reveal the degree of epithelial keratinization, which did not add materially to our ability to classify the specimens.

All patients received at least 110 mg retinyl palmitate orally within 1 wk of base-line exam. Follow-up ocular examinations with impression cytology were performed 2 wk, 2 mo, and 6 mo after the base-line exam; 121 children remained through the 6-mo follow-up. Examiners were blinded at each exam as to treatment group, previous diagnosis, impression-cytology results, and base-line serum vitamin A level.

Statistical analyses utilized binomially derived confidence limits about proportions and a nonparametric test for monotonic trends (15).

Results

The ability of impression cytology to detect physiologically significant vitamin A deficiency was determined by subdividing case and control subjects into subgroups reflecting our degree of confidence in their true vitamin A status (**Table 1**). At one extreme were children who were certainly deficient with vitamin A-responsive Bitot's spots and night blindness and with serum vitamin A levels < 20 μg/dL (0.70 μmol/L) (group 1). At the other extreme were those children least likely to be deficient having normal ocular examinations, a negative history for night blindness, and serum vitamin A of ≥ 25 μg/dL (0.87 μmol/L) (group 7). (Although clinically significant deficiency has been occasionally reported at a serum level of 25–30 μg/dL [0.87–1.05 μmol/L], few Indonesian children have serum levels above this range.) The remaining children had various clinical signs, symptoms, and serum levels and were of less certain, intermediary status.

TABLE 1
Subdivision of patients by their combined clinical and biochemical vitamin A status

Vitamin A status	Group
Deficient	1. Definite vitamin A deficiency Night blindness (XN) with Bitot's spot (XIB) responding to treatment and serum vitamin A < 20 μg/dL (0.70 μmol/L)
	2. Probable vitamin A deficiency Bilateral X1B responding to treatment
	3. Probable vitamin A deficiency XN responding to treatment with base-line serum vitamin A < 20 μg/dL (0.70 μmol/L)
	4. Possible vitamin A deficiency Unilateral X1B (without XN) responding to treatment
	5. Possible vitamin A deficiency Normal exam with base-line serum vitamin A < 20 μg/dL (0.70 μmol/L)
	6. Borderline vitamin A status XN with base-line serum ≥ 20 μg/dL (0.70 μmol/L) or normal subjects with base-line serum between 20 and 25 μg/dL (0.70–0.87 μmol/L)
Normal	7. Normal vitamin A Normal exam with base-line serum vitamin A > 25 μg/dL (0.87 μmol/L)*

* There were insufficient numbers to include > 30 μg/dL (1.05 μmol/L) as a criterion for normal.

The proportion of subjects with abnormal impression cytology was directly related to the likelihood that they were vitamin A deficient (Table 2, **Fig 4**): 93% of children definitely vitamin A deficient (group 1) had abnormal cytologic impressions whereas 6% of children least likely to be vitamin A deficient (group 7) had abnormal cytology. The single exception in group 7 became normal after receiving vitamin A, suggesting he was also deficient despite a serum vitamin A level > 25 μg/dL (0.87 μmol/L) and the absence of clinical xerophthalmia.

With the extreme groups taken as approximations of true deficiency (group 1) and normality (group 7), the sensitivity for detecting vitamin A deficiency was 93% (95% confidence limit [CL] of 66–100%) and specificity at least 94% (95% CL of 73–100%).

Importantly, 12 of 26 (46%; 95% CL of 27–65%) seemingly normal control subjects whose serum vitamin A was < 20 μg/dL (0.70 μmol/L) (group 5) had abnormal cytology. Follow-up cytology was available on 10 of these 12 abnormal subjects: all became normal by 6 mo.

Discussion

Vitamin A deficiency in developing countries is the major cause of blindness and increases both morbidity and mortality among preschool children (1–6). Recent reports documenting improved mortality among nonxe-

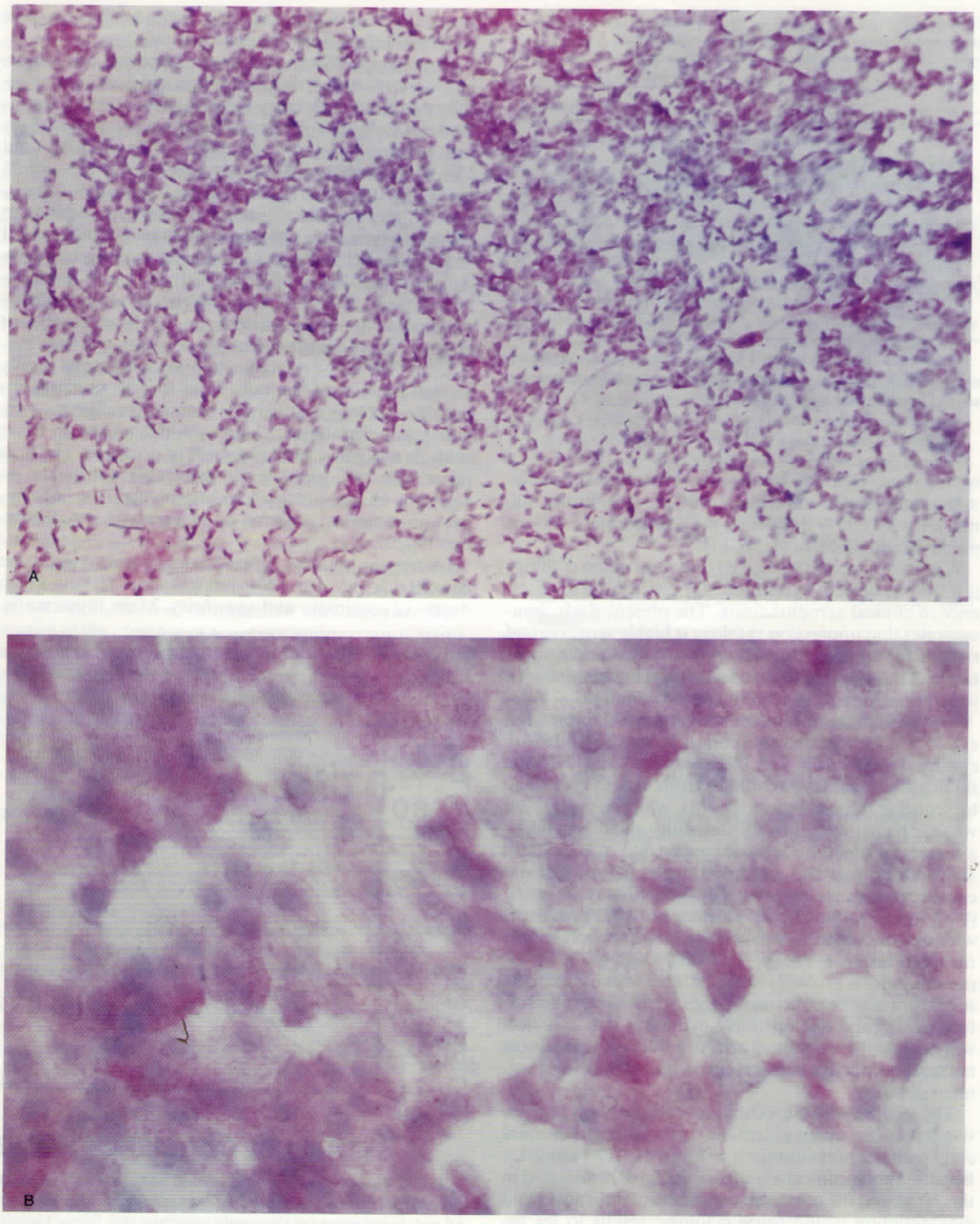

FIG 3. *A:* Low-power view of imprint from vitamin A-deficient child with clinical xerophthalmia (X1B and XN). Note irregularly shaped, separating epithelial cells and lack of goblet cells (periodic-acid Schiff and Harris hematoxylin, 100×). *B:* Higher-power view of same specimen (400×).

TABLE 2
Impression cytology results for each group of subjects

Group	Number of subjects	Impression cytology			
		Normal		Abnormal	
		n	%	*n*	%
1 (Deficient)	14	1	7	13	93
2	22	4	18	18	82
3	15	5	33	10	67
4	8	4	50	4	50
5	26	14	54	12	46
6	43	37	86	6	14
7 (Normal)	18	17	94	1	6
Total	146*	82	56	64	44

* One study child who lacked a serum vitamin A level and one on whom follow-up was inadequate to determine clinical response are omitted from the analysis.

rophthalmic children who received vitamin A supplementation suggest subclinical but physiologically significant vitamin A deficiency may constitute a serious public health problem (7, 16, 17).

A previous report (9) showed that impression cytology is a simple, objective technique for confirming the presence of clinical xerophthalmia. The present study demonstrates that impression cytology is highly sensitive and specific for the detection of physiologically significant vitamin A deficiency and that such deficiency may affect a large proportion of apparently normal children.

The percentage of subjects with abnormal impression cytology corresponded to vitamin A status defined on clinical, biochemical, and therapeutic grounds because no single test defines physiologic truth. Bitot's spots may be sequelae of past deficiency (18), which is the reason we gave higher weighting to bilaterality and reversibility; a history of nightblindness is never 100% specific (19); and both normal and abnormal ocular appearance are consistent with serum levels between 10 and 30 μg/dL (0.32 and 1.05 μmol/L) (2). Thirteen of 14 (93%) patients with definite vitamin A deficiency (group 1) had abnormal impression cytology. The single exception in the present series is probably attributable to variations in the severity of histologic abnormalities across the ocular surface (3, 18) and our operative definition that a subject was normal if even a small area of goblet cell-containing normal conjunctiva was present on any of the four specimens obtained.

The specificity of the technique was an equally impressive 94%. The single exception was a clinically normal child with a serum vitamin A > 25 μg/dL (0.87 μmol/L). Because subtle clinical abnormalities have been noted in this range and the time required for cellular turnover and differentiation would cause cytologic results to lag behind acute changes in biochemical status, this child may indeed have been deficient, either at the time of base-line examination or in the recent past. Indeed, his impression cytology became normal after treatment.

Equally importantly, impression cytology was abnormal in a large proportion of children at risk of physiologically significant deficiency but with normal ocular examinations. Fully 46% of clinically normal children with serum vitamin A levels < 20 μg/dL (0.70 μmol/L) (and 15% of those with vitamin A levels between 20 and 25 μg/dL [0.70 and 0.87 μmol/L]) had abnormal impressions. Almost half of all preschool-age rural Indonesian children have serum vitamin A levels < 20 μg/dL (16). If 46% of these children have abnormal impression cytology (as well as 15% of children with vitamin A levels between 20 and 25 μg/dL) the prevalence of physiologically significant vitamin A deficiency probably approaches or exceeds 25% of the preschool-age population. The prevalence of clinically detectable mild xerophthalmia is only a small fraction of this rate. Field surveys utilizing impression cytology should more accurately reflect the vitamin A status of the population while requiring far fewer subjects to achieve similar degrees of precision. The high prevalence of metabolically detectable deficiency revealed by impression cytology helps explain the large impact on mortality achieved by vitamin A supplementation (7, 20).

Impression cytology differentiates vitamin A status (as defined on clinical and biochemical grounds) with a high degree of sensitivity and specificity. More importantly,

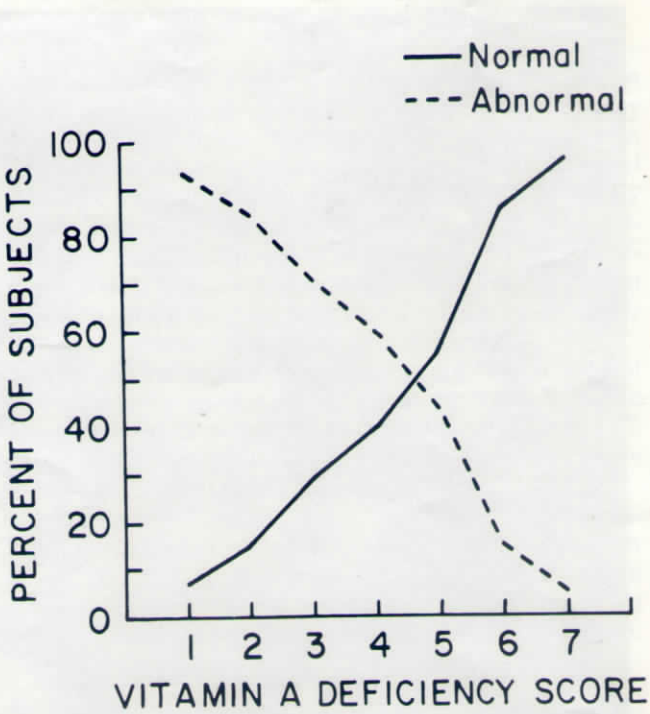

FIG 4. Percent of subjects with normal and abnormal impression cytology by degree of vitamin A deficiency as determined by biochemical and clinical status and therapeutic responsiveness. Group 1 consists of children almost surely deficient and group 7 children least likely deficient.

this technique identifies early, physiologically significant vitamin A deficiency not apparent by standard methods of assessment. Impression cytology is well suited for population surveys to determine the prevalence of vitamin A deficiency or to monitor the impact of supplementation programs. Its great strength is simplicity, logistical practicality, and close association with apparent vitamin A status. Sensitivity and specificity of course are < 100%, especially given the confidence limits in this study. It should prove a useful and more convenient gauge of vitamin A status of the community than other approaches but is not infallible in individual subjects. The more areas sampled per eye, however, the more likely normal conjunctiva will be identified and the higher the specificity will be. Specimens are obtained easily and atraumatically, can remain in fixative indefinitely, and require only an ordinary microscope for interpretation. Simpler staining techniques to determine the presence or absence of goblet cells without needing to delineate keratin (for which the more complicated modified Papanicolaou's stain [9] was adopted in this study) will facilitate objective determinations of vitamin A status. A further, large scale field evaluation utilizing a modification of our original simplified PAS and hematoxylin staining process (8, 9) for goblet cells is presently in progress (Figs 1–3).

Clinical studies suggest reversal of keratinization in the presence of conjunctival inflammation (21). Sensitivity and specificity derived in this study may not, therefore, be valid in the presence of acute or chronic conjunctivitis and other external ocular inflammation. Until more data and experience are available, interpretation of impression cytology, particularly for gauging a community's vitamin A status, should be limited to noninflamed eyes.

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