

Relationship of conjunctival impression cytology with clinical and biochemical assessment of vitamin A status of preschoolers

CC Tanchoco¹ MPH, MP Rodriguez¹ BSMT, E Olivar-Santos² MD, FV Velandria¹ Dip FRRCD and JA Magbitang¹ MSPH

¹Science Research Specialist, Food and Nutrition Research Institute, Manila, Philippines

²Consultant, Institute of Ophthalmology, University of the Philippines–Philippines General Hospital, Manila, Philippines

Comparison of conjunctival impression cytology (CIC) with ocular signs and vitamin A levels was done based upon a national nutrition survey involving 427 randomly selected subjects aged 6 months to 6 years. Medical nutritionists looked for ocular signs and performed conjunctival impressions. Imprints were submitted to the Institute of Ophthalmology, University of the Philippines–Philippines General Hospital, for analysis. Fixing, staining and reading of all imprints was performed in accordance with the procedures of the International Centre for Epidemiologic and Preventive Ophthalmology (ICEPO). Comparison of the biochemical determination of serum vitamin A (SVA) with CIC results revealed that irrespective of SVA levels, more than 50% of the subjects were noted to have abnormal CIC results. The highest proportion of abnormal imprints was seen among those with low SVA. The relationship, however, was found not to be significant. Of the 247 subjects examined, 95% had a normal clinical and biochemical assessment of vitamin A; of these, 50.2% had abnormal cytology imprints while 45.3% had normal imprints. Using sensitivity and specificity analyses, CIC was compared with SVA levels. The computed sensitivity, specificity and positive predictive value (PPV) were 60.5, 45.2 and 60.0%, respectively. The lack of significant difference in mean serum retinol levels between normal and abnormal CIC groups may partly explain the low sensitivity and PPV of the CIC. In this study population, CIC failed to identify children with subclinical vitamin A deficiency as established by biochemical examination.

Key words: vitamin A deficiency, preschoolers, conjunctival impression cytology, serum vitamin A, Philippines, low diagnostic value.

Introduction

Vitamin A deficiency has long been recognized as a major public health problem in the Philippines,^{1–3} as in many other developing countries. The most obvious and clinically important manifestation of vitamin A deficiency is xerophthalmia, which can lead to permanent blindness.⁴ Most surveys show that ocular signs and serum vitamin A (SVA) concentrations are both commonly used in the assessment and diagnosis of vitamin A deficiency among preschool children.⁵ However, both have practical limitations under field conditions. Biochemical assessment of SVA status requires careful storage and handling and is expensive. Besides, the extraction of blood samples from children may be culturally unacceptable in many communities. However, clinical signs and symptoms of vitamin A deficiency, such as night-blindness and xerophthalmia, can occur late, and are at times rapidly progressive and irreversible.⁶ Therefore, there is a need to develop an alternative diagnostic method which is as sensitive as, and more practical than, the clinical and biochemical assessments.

Conjunctival impression cytology (CIC) has been suggested as a method for the early detection of vitamin A status.^{7,8} In line with this, a clinical nutrition survey was conducted on the prevalence of xerophthalmia, using CIC among pre-schoolers. Comparisons were made between

anthropometric measurement, ocular signs and SVA levels with results of conjunctival impression cytology. This was followed by an evaluation of the sensitivity and specificity of CIC as a screening test for children identified as suffering from vitamin A deficiency, using ocular signs and serum concentrations of vitamin A.

Subjects and methods

A total of 427 randomly selected subjects aged 6 months to 6 years were included in the study. This population, drawn from members of the statistically selected households covered by the Third National Nutrition Survey in 1987,⁹ was studied by the Food and Nutrition Research Institute in collaboration with the Institute of Ophthalmology, University of the Philippines–Philippines General Hospital (UP–PGH). Clinical manifestations such as xerophthalmia, night-blindness, Bitot's spot and other signs and symptoms of vitamin A deficiency were looked for by medical nutritionists who had been previously trained by experts from the Institute of Ophthalmology to clinically assess vitamin A nutriture. Blood

Correspondence address: Celeste C Tanchoco, Scientist I, Food and Nutrition Research Institute, Gen. Santos Ave, Bicutan, Taguig, Metro Manila, Philippines.

Tel: +63 2 837 2071, Fax: +63 2 837 3164

samples were collected to estimate the SVA, using the trifluoroacetic acid (TFA) method of Neeld and Pearson.¹⁰ This was done by members of the Nutritional Standard and Requirements Division.

Conjunctival impressions were taken by medical nutritionists using a standardized strip of filter paper cut with both ends pointed. The centre of one side of the filter paper was marked with an indelible pencil dot for easier orientation during the imprinting. After parental consent was obtained, the strip was applied to the temporal bulbar conjunctiva of each eye and then pressed using a glass rod. The paper was cut at the dot after the imprinting and was immediately placed in a pre-numbered vial containing fixative. The specimens were submitted to the Institute of Ophthalmology for analysis. Fixing and staining of all imprints was performed at the same time (January 1988), following the International Centre for Epidemiologic and Preventive Ophthalmology (ICEPO) procedure, which uses periodic acid-Schiff (PAS) and modified Papanicolaou's stain.¹¹

Results of SVA analysis were interpreted based on the cut-off points set by WHO/IVACG/HKI 1982.⁴

The CIC slides were read by a trained ophthalmologist and each imprint was categorized by at least two readers based on the flow-chart devised by the ICEPO. Conjunctival impressions showing mucin spots, sheets of small epithelial cells with the presence of goblet cells, were indicative of normal vitamin A status. Abnormal specimens had enlarged epithelial cells and very few or absent goblet cells and mucin spots.

Descriptive statistics were also derived to characterize the subjects and describe the quality of imprints *vis à vis* clinical and biochemical results.

Chi-square analysis was undertaken to determine whether significant differences existed between SVA levels in relation to quality of imprints.

Sensitivity and specificity analyses were performed comparing CIC assessment to SVA levels.

Results

Comparison of the biochemical determination of SVA with CIC results as shown in Table 1, revealed that irrespective of SVA levels, more than 50% of the subjects were noted to

Table 1. Percentage distribution of 6 month- to 6-year-old children by their combined clinical and biochemical vitamin A status using conjunctival impression cytology for screening

Group	Total subjects with clinical and biochemical Vitamin A status results	
	No. children	Per cent
1	—	—
2	—	—
3	3	1.2
4	8	3.2
5	124	50.2
6	112	45.3

Criteria: Group 1, imprint cytology abnormal; (+) XN and X1B; SVA < 20; Group 2, imprint cytology abnormal; (+) X1B; SVA < 20; Group 3, imprint cytology abnormal; (+) XN; SVA < 20; Group 4, imprint cytology abnormal; (+) XN; SVA > 20; Group 5, imprint cytology abnormal; clinical assessment of vitamin A is normal; SVA > 20; Group 6, imprint cytology normal; clinical assessment of vitamin A is normal; SVA > 20.

Table 2. Conjunctival impression cytology results among randomly selected 6 month- to 6-year-old children, Philippines, 1987

Age group	No. subjects	Imprint cytology		
		Normal	Abnormal	Unreadable
6 mo-< 1 year	4	0.2	0.7	—
1	18	1.4	2.3	0.5
2	24	2.3	3.3	—
3	60	4.7	7.3	2.1
4	83	6.8	11.5	1.2
5	113	11.7	13.8	0.9
6	125	13.3	15.4	0.5
Total	427	40.4	54.3	5.2

Table 3. Sensitivity and specificity of conjunctival impression cytology with biochemical method of vitamin A deficiency assessment

Imprint cytology results	Serum vitamin A levels		
	Abnormal	Normal	Total
Abnormal	92	138	230
Normal	60	114	174
Total	152	252	404

have abnormal CIC results. The highest proportion of abnormal imprints were seen among those with low SVA levels. The relationship, however, was found not to be significant.

Table 2 shows that out of the 247 subjects examined, 95.5% had normal clinical and biochemical assessments of vitamin A. Of this figure, 50.2% had abnormal cytology imprints while 45.3% did not.

Comparing CIC and SVA results, sensitivity and specificity tests were conducted on the extreme groups, taken as approximations of the true deficiency (abnormal SVA and CIC), and normality groups (normal SVA and CIC). The sensitivity for detecting vitamin A deficiency was 40% while the specificity was at least 65.5% (Table 3).

Discussion

The objectives of this study were to compare the results of SVA levels with the results of CIC and to evaluate the sensitivity and specificity of CIC as a screening test for children previously identified as having xerophthalmia, using clinical and biochemical assessments. Ocular examination as a diagnostic method for vitamin A deficiency in a community is well accepted.⁴ However, the clinical signs of xerophthalmia manifest late and so when they are noted, irreversible damage may already have been inflicted. Besides, clinical examination is not specific and consequently, a large sample size is needed to determine the prevalence. However, a biochemical assessment of vitamin A status of populations is fraught with technical, analytical and cultural problems as blood needs to be extracted and analysed, careful storage and proper handling of blood samples in the field is challenging, serum analysis is expensive, and the invasive nature of blood extraction may be culturally unacceptable.⁷ Thus, diagnosing vitamin A deficiency in a population is complicated by issues of feasibility.

Previous studies suggest that CIC may turn out to be a simple, reliable and objective method of detecting vitamin A deficiency in people.^{7,8,11-14} However, there is a scarcity of documented and controlled field trials that assess the validity of CIC use in populations whose nutritional status has been determined by other established means of assessment.

The results of this study further showed that a greater proportion of subjects had abnormal CIC regardless of vitamin A levels. Thus, even those with high SVA had a greater percentage of abnormal CIC. This result contradicts the findings of Amedee-Manesme *et al.* which indicated that all children with normal vitamin A status had normal CIC, while all children with abnormal vitamin A status had abnormal CIC in spite of their seemingly normal clinical ocular examination.¹⁴ Probable factors that explain this inconsistency are: poor handling of the specimen, insufficient amount of serum, and poor quality of reagents, among other things. Also, the TFA method may not be as sensitive as the high performance liquid chromatography (HPLC) method. In the study of Rider *et al.*, HPLC showed the highest sensitivity and least interference from other serum components in comparison to TFA and UV methods. Correlation coefficients for values obtained by HPLC vs TFA and vs UV were 0.92 and 0.93, respectively.¹⁵

This study, similar to other studies,^{11,13} found that regardless of CIC classification, the mean SVA and clinical xerophthalmia did not agree well with cytological findings. Of the 247 subjects examined, 95.5% had normal clinical and biochemical assessment of vitamin A, of which 50.2% had abnormal cytology imprints while 45.3% did not. In the study of Reddy *et al.* about 25% of children with normal eyes showed abnormal cytology.¹³ The majority of the children in this study had normal SVA, reflecting adequate vitamin A nutrition. In contrast, Reddy *et al.*'s study on the same number of children revealed that the majority had low SVA. These results therefore suggest that SVA by itself is not the best measure of vitamin A status. Plasma vitamin A concentrations less than 0.35 $\mu\text{mol/L}$ indicate low body stores whereas concentrations above 1.05 $\mu\text{mol/L}$ reflect adequate stores. The problem with interpretation arises with intermediate concentrations, which show poor correlation. Plasma vitamin A-values do not reflect intermediate concentrations of depleted liver reserves.¹⁵ Plasma vitamin A will be stable until liver stores decrease.

This study covered a much younger population group (1-6 years) compared to Reddy *et al.*'s study in which approximately 62% were aged from 6 to 10 years. The greater proportion of abnormal cytology in spite of normal SVA and clinical assessment could be possibly attributed to the difficulty in collecting imprints from younger children. However, in Reddy *et al.*'s study approximately 65% of all children with normal cytology had low serum vitamin A concentrations, closer to the 50% found in this study.

These results suggest that our abnormal cytology may not be reflective of vitamin A deficiency. Wittpenn *et al.* have demonstrated the capacity of CIC to distinguish between a small number of supposedly normal children and those with mild xerophthalmia.⁷ Natadisastra *et al.* investigated a group of 148 Indonesian pre-schoolers by classifying subjects into subgroups based on their combined clinical and biochemical vitamin A status.¹¹ The study revealed that the proportion of subjects with abnormal impression cytology was directly related to the likelihood that they were vitamin A deficient. The sensitivity of CIC for selecting vitamin A deficiency was 93% and its specificity was at least 94%.

Using sensitivity and specificity analyses, CIC was compared with SVA levels in this study. The computed sensitivity, specificity and positive predictive value (PPV) were 60.5, 45.2 and 60%, respectively. These results, except for specificity, were much higher than Godamski's findings (26.0% sensitivity, 81% specificity and 22% PPV). This study, however, had lower sensitivity and specificity compared with the earlier studies done by Natadisastra *et al.* on 148 Indonesian preschoolers, half of whom had mild xerophthalmia and half of whom were age-matched controls where sensitivity of 93% and specificity of 94% were reported. The lack of significant difference in mean serum retinol levels between normal and abnormal CIC groups suggests that there is no significant difference in vitamin A status between these two groups. This may explain the low sensitivity and PPV of the CIC.

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**用結膜印模細胞學 (CIC)，臨床和生化數據
評估學齡前期兒童的維生素 A 營養狀況****摘 要**

作者在全國營養調查中隨機選擇 427 名 6 個月—6 歲兒童為對象，比較了結膜印模細胞學 (CIC)。視覺體征和維生素 A 水平，由醫學營養專家進行視覺體征檢查和細胞印模。所有印模交給眼研究所分析。按照國際眼流行病和預防中心的操作固定、染色和鑒定所有印模。

比較血清維生素 A 和 CIC 的結果不盡符合，未發現有明顯的關係，通過 247 名對象的檢查，有 95% 的臨床體征和生化結果是正常，但有 50.2% 細胞印模不正常，45.3% 正常。用敏銳和特殊方法分析 CIC 和血清維生素 A 水平的關係，得到其敏感性、特殊性和陽性預知值 (PPV) 分別為 60.5%，45.2% 和 60.0%。

在正常和異常 CIC 兒童，血清平均維生素 A 無明顯差異可部份解釋為 CIC 的陽性預知值 (PPV) 和敏感性低。在這一研究人群中，CIC 不能鑒定兒童不明顯的維生素 A 缺乏。

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