Pupillary threshold as an index of population vitamin A status among children in India¹⁻³

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ABSTRACT Two hundred seven vitamin A-deficient southern Indian children aged 1–7 y (mean age: 56.9 mo) underwent testing of dark-adapted visual and pupillary thresholds in their village setting according to a previously reported protocol. One hundred thirty (62.8%) of the children also underwent serum retinol testing, and 178 (86.0%) participated in a randomized, placebo-controlled vitamin A dosing trial with pre- and postdose testing of dark-adaptation threshold. Most subjects (184 of 207, 88.9%) were able to complete pupillary testing, an objective sign requiring minimal cooperation, including a high proportion of the youngest children (72.2% of subjects aged 2 y). The proportion of children completing visual threshold testing, which requires greater understanding and cooperation, was significantly smaller than that able to complete pupillary testing (131 of 207, 63.3%; P < 0.0001, chi square). At baseline (predosing), the mean serum retinol concentration declined in linear fashion with a higher pupillary threshold (0.73 μmol/L with a score ≤ 4; 0.47 μmol/L with a score ≥ 8; P < 0.01). The mean pupillary threshold for these highly vitamin A-deficient Indian children (−0.622 log cd/m²) was significantly higher than that for 136 more moderately deficient Indonesian children (−0.985 log cd/m²; P < 0.001, two-sample t test) and 56 normal American children (−1.335 log cd/m²; P < 0.0001, two-sample t test). The improvement in pupillary dark-adaptation testing was not significant for children receiving vitamin A or placebo, though there was a nonsignificant trend toward greater improvement in children receiving vitamin A (P = 0.2, two-sample t test). Pupillary threshold testing represents a new, noninvasive, practical, and seemingly valid approach to assessing the vitamin A status of a moderately to severely deficient preschool population. Am J Clin Nutr 1997;65:61–6.

KEY WORDS Vitamin A, night vision, dark adaptation, pupil, children

INTRODUCTION

Vitamin A deficiency is both the leading cause of pediatric blindness in the world today (1, 2) and a significant source of childhood mortality in the developing world (3–5). Our ability to combat vitamin A deficiency has been limited in part by the lack of a simple, practical field technique for identifying communities in need of intervention.

We recently reported encouraging results from a field trial in Indonesia of a portable, inexpensive, hand-held device designed to measure the pupillary and visual light threshold as an index of vitamin A status (6). The afferent pupillary reflex (tendency of the pupil to constrict under illumination) has been shown to correlate well with a subject’s ability to detect a light stimulus near the visual threshold (7). Dark vision is known to be impaired in vitamin A deficiency, often before the appearance of other clinical signs such as Bitot’s spots (8). We found that both visual and pupillary thresholds of a group of mildly vitamin A-deficient Indonesian preschool-aged children were significantly correlated with other measures of vitamin A status, including serum retinol. Most importantly, visual and pupillary thresholds rose significantly (their retinas became less light sensitive) the longer the time since a single high-dose (105 μmol) supplement of vitamin A. Thresholds subsequently fell significantly among children supplemented in randomized, double-blind fashion with vitamin A, but not among control children receiving placebo (6).

The current report presents results of pupillary and visual threshold testing on a group of children in southern India who were clinically (therefore more severely) vitamin A-deficient or the siblings of clinically deficient children, in whom deficiency is more common than in children at large (8), in contrast with the more mildly deficient, nonxerophthalmic Indonesian subjects. None of the Indian subjects had recently received vitamin A. Additionally, we tested the technique under less favorable field conditions. Although subjects in Indonesia had been tested at a single, central site, in the current study children from 40 different villages were examined.

SUBJECTS AND METHODS

Subjects and study design

A total of 213 southern Indian children aged 1–7 y (x ± SD: 56.9 ± 15.2 mo, 55.4% male) were identified as eligible for the study. They were recruited from 21 villages in three different districts of southern India: Madurai (122), Rameshwaram (45), and Pudukkotai (46). The majority of children were identified with the assistance of the local government health departments, although some were also identified by local community leaders at their homes. The children were from a population of approximately 2.5 million people, of whom 32% are Christian and 30% are castes and tribes (6). All children had been identified as vitamin A-deficient, based on the greater than two standard deviations below the mean serum retinol level of 207 healthy preschool children recruited from the study villages (6). Additionally, we recruited additional children (18) with known vitamin A deficiency (retinol < 0.2 μmol/L for children < 1 y and < 0.4 μmol/L for children 1–7 y) who were receiving placebo in the original placebo-controlled trial (6).

The children were subdivided into three age groups: 1–2 y (n = 70), 3–5 y (n = 55), and 6–7 y (n = 88). The study was approved by the Johns Hopkins University School of Medicine Institutional Review Board and the National Institutes of Health Office of Human Subjects Research and the Office of Research at the Madurai Medical College. Written informed consent was obtained from the parents/guardians of all children. The study was part of a larger trial of vitamin A supplementation, the results of which have been previously reported (6). An additional request for reprints should be addressed to N G Congdon, Dana Center for Preventive Ophthalmology, 120 Wilmer Building, Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, MD 21205.

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study on the basis of a history of nightblindness, the presence of Bitot’s spots on clinical examination, or being the sibling of a child with nightblindness or Bitot’s spots. Subjects were from a total of 40 villages in the vicinity of Madurai in the state of Tamil Nadu. We attempted both pupillary and visual testing in 207 (97.2%) of the eligible children, after informed written consent had been obtained from their parents in the local language. Blood for serum retinol analysis was obtained from 130 (62.8%) of the participating subjects (mean age: 57.1 ± 13.6 mo). Among 184 subjects (88.9%) completing pupillary testing, 178 (96.7%) participated in a placebo-controlled dosing trial in which 89 children were selected at random to receive 105 μmol vitamin A and 89 to receive placebo in double-blind fashion. All 178 subjects (100%) presented for follow-up pupillary and visual testing 2 wk after dosing (Figure 1). All children initially assigned to the placebo group received 105 μmol vitamin A at follow-up.

The protocols followed in testing subjects were approved by the Johns Hopkins Joint Committee on Clinical Investigation.

Apparatus and testing procedure

The hand-held illuminators used to assess pupillary and visual threshold were described previously (6). Briefly, the battery-powered machines had a yellow-green, light-emitting diode (LED) light source with 12 intensity settings at ~0.4 log unit intervals, the range of the pupillary machine being ~2 log units higher than the device used for visual testing. The device fit entirely over one eye, with an obliquely mounted red LED light source providing illumination of the contralateral eye. Before testing, subjects underwent a binocular partial bleach (ie, exposure to bright light to counteract previous partial dark adaptation) followed by 10 min of dark adaptation in a standard \(1.5 \times 2.0 \times 2.0\) m black-cloth tent assembled on a portable metal frame in the testing area in each village, usually a central site such as a school or clinic. The tent was set up indoors and away from windows, and its interior darkened to the degree that a dark-adapted observer was unable to read black, hand-sized letters a finger’s-breadth in width on a white background. The tent was cooled internally by a battery-powered air cooler.

The visual threshold was measured first, according to a forced-choice protocol, where the threshold stimulus was defined as that level at which a subject could correctly distinguish stimulus from nonstimulus on three successive trials. The red pupillary visualization light remained off during this phase of testing to avoid confusing the subject. Next, the pupillary threshold was measured with the machine over the subject’s right eye, the left eye being observed by means of a 2.5× loupe. With the subject’s attention directed to a nonaccommodative target at a distance of 2 m (ie, a target at a sufficient distance to prevent accommodation or close-up focusing of the eye, which causes the pupil to constrict), the stimulus intensity was increased until a pupillary response in the contralateral eye was clearly visible to the observer on two successive trials.

All examinations were carried out in the child’s village of residence by one of two workers at the Aravind Eye Hospital after 1 wk of training by one of the authors (NGC). Note that the unit of measurement used throughout this paper to denote light intensity is log \(cd/m^2\), the metric unit for luminance, denoting the light reflected or emitted by a surface per unit area and per unit solid angle.

**FIGURE 1.** Flow chart showing the progress of 213 preschool-aged children throughout the study.
PUPILLARY THRESHOLD AS INDEX OF VITAMIN A STATUS

Statistical methods

SAS programs (SAS, Inc, Cary, NC) were used to measure the association between pupillary and visual threshold and other indicators of vitamin A status, and between the change in postdosing threshold and a subject’s assignment in the placebo-controlled dosing trial. The two-tailed Student’s t test was used to compare means between two groups, whereas the chi-square test was used to compare proportions. The chi-square test of trend was used to assess the significance of association between the two dark-adaptation tests and serum retinol. Analysis of variance (ANOVA) was calculated for a model comparing the population mean dark-adapted thresholds for American, Indonesian, and Indian children. The minimum number of Indian and Indonesian subjects that would have to be tested to establish a significant difference in mean threshold at the 0.05 level between these groups and normal American children with a power of 99% was calculated with a standard sample-size formula. A two-tailed probability < 0.05 was considered significant.

RESULTS
Subject compliance

Most subjects (184 of 207, 88.9%) were able to complete pupillary testing, including a high proportion of the youngest children (13 of 18, 72.2% of subjects aged 2 y). The proportion of children completing pupillary testing was higher than that for visual testing at all ages, with the overall difference in compliance between the two tests being highly significant (P < 0.0001, chi-square; Figure 2). The difference in level of compliance between the two tests was most marked among the youngest children, who generally complied poorly with visual testing (Figure 2).

Correlation between threshold testing and vitamin A status

Mean serum retinol declined linearly with increasing pupillary threshold scores (lower retinal sensitivity) (Figure 3; P < 0.01, chi-square test of trend). A similar monotonic trend was observed for mean serum retinol and visual threshold, although this relation was not significant (Figure 3; P < 0.3, chi-square test of trend). Age, sex, breast-feeding status, and a recent (previous 2 mo) episode of mumps, measles, cough, or diarrhea were not significantly correlated with serum retinol or dark-adaptation score on linear modeling (data not shown).

Children reported by mothers to be nightblind did not have significantly lower serum retinol concentrations (0.57 ± 0.27 compared with 0.61 ± 0.25 μmol/L; P = 0.4, Student’s t test) or higher (less sensitive) pupillary threshold scores (0.55 compared with −0.67 log cd/m²; P = 0.25, Student’s t test), although the relations were in the expected direction. All children either had evidence suggesting clinical vitamin A deficiency (a history of nightblindness or current Bitot’s spots) or were the siblings of deficient children, who are themselves more likely to be deficient (8).

Results of a randomized, placebo-controlled dosing trial

For 89 children receiving high-dose vitamin A supplements, pupillary dark-adaptation scores improved by an average of 0.159 ± 0.60 log cd/m², whereas for children receiving placebo, scores improved by only 0.016 ± 0.50 log cd/m². Neither the change in score for children receiving vitamin A (P = 0.15, paired t test), nor that for subjects receiving placebo (P = 0.7, paired t test), nor the difference in improvement between the two groups (P = 0.20, two sample t test) was significant. There was no significant difference in the change in visual scores between vitamin A and placebo recipients (0.020 ± 0.59 and −0.013 ± 0.54 log cd/m² respectively; P = 0.50, two-sample t test) (Figure 4). Resistance to multiple blood draws among children and their parents made postdose serum testing impractical.

Comparisons between different populations

The children tested in India were more vitamin A-deficient than the children of similar age tested in Indonesia: the mean serum retinol concentration was significantly lower (P < 0.0001, two-sample t test), and the proportion of children with a serum retinol concentration < 0.70 μmol/L (P < 0.0001, chi-square test) and reported to be nightblind (P < 0.0001, chi-square test) were significantly higher for the Indian children (Table 1).

FIGURE 2. Compliance with pupillary and visual testing by age (n = number of subjects attempting testing). The difference between the two curves was significant, (P < 0.0001, chi-square test).

FIGURE 3. Mean (± SE) serum retinol concentrations by pupillary and visual dark-adaptation scores (number of subjects in brackets). The apparent decline in retinol with higher (less sensitive) scores was significant for pupillary score (P = 0.01, chi-square test of trend) but not for visual score (P = 0.21, chi-square test of trend).
As anticipated, the apparently poorer vitamin A status of the Indian children was associated with a higher mean pupillary threshold (lower retinal sensitivity) compared with the Indonesian children (−0.622 compared with −0.985 log cd/m²; \( P < 0.001, \) two sample \( t \) test), who in turn showed significantly poorer dark adaptation than a group of 56 apparently well-nourished American children aged 54 ± 18 mo with dark irides (two-sample \( t \) test: −1.335 log cd/m², \( P < 0.0001 \); comparison of the three populations by ANOVA: \( F = 55.95, \) \( P < 0.0001 \) (Figure 5). Although serum retinol was not measured in the American subjects, the extremely low prevalence of vitamin A deficiency among well-nourished American children is well-documented (9). American children with dark irides were chosen because we found previously (unpublished data) that American children with light irides showed a slightly more sensitive response to pupillary threshold testing.

Although pupillary data differed significantly between the Indian and Indonesian populations, visual threshold data did not (−4.00 compared with −4.09 log cd/m²; \( P = 0.5, \) two sample \( t \) test). Comparable visual threshold data were not recorded for the American subjects.

**DISCUSSION**

This study is a sequel to our previous clinic-based assessment of this new test of dark adaptation, with measurements made under actual village-based conditions. All testing was conducted in a portable testing site, constructed of locally available materials and reassembled in each of the 40 villages, which were within a 3-h drive of our base at the Aravind Eye Hospital. The inability of a dark-adapted observer to read standard-sized test letters inside the test site guaranteed a reproducible, minimum level of darkness. Our results indicate that this technique is sufficiently robust to allow valid testing under these conditions.

As had been the case in Indonesia (6), a very high proportion of children completed pupillary dark-adaptation testing, significantly greater than the percentage able to comply with visual testing. Pupillary testing, in which the response was objectively graded by an observer, was more useful among young children, especially children 4 y of age and younger, the group at greatest risk for vitamin A deficiency.

Our data suggest that pupillary testing is not only more acceptable to young children, but may also provide a more accurate indication of impaired dark adaptation: pupillary threshold was significantly correlated with serum retinol whereas visual threshold was not. If pupillary testing does indeed prove to be a more accurate indicator of vitamin A status in this age group, it may well be due to practical issues of testing rather than to any fundamental difference between the two tests. Both pupillary and visual testing assess the function of the dark-adapted retina, known to be impaired in vitamin A deficiency. It is possible that further experience will show that the two tests provide complementary information, but we are unaware of any data to suggest that either offers a theoretical advantage in the assessment of vitamin A status.

The degree of success in pupillary threshold testing is notable, given that children in this part of India are quite unused to sitting in darkened conditions, and that testing was carried out during the hottest part of the year. These problems were in part overcome by designing a portable testing area that was large enough to accommodate the subjects’ mothers, and that could be made comfortable by means of an electric air cooler weighing < 10 kg and 70 cm in height.

As expected, mean serum retinol declined in linear fashion with higher (less sensitive) scores on both pupillary and visual dark-adaptation testing, although only the pupillary scores

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**TABLE 1**

| Vitamin A status of 130 Indian children compared with 136 Indonesian children |
|---|---|---|
| Indicator | Madurai, India | Bandung, Indonesia |
| Age (mo) | 56.9 ± 15.2 \( ^2 \) | 58.5 ± 7.0 |
| Serum retinol (μmol/L) | 0.59 ± 0.26 | 0.85 ± 0.34 \( ^2 \) |
| Proportion with retinol | 47/122 (38.5) | 92/130 (70.8) |
| Proportion reported as nightblind (%) | 0/136 (0) \( ^4 \) | 93/207 (44.9) |

\( ^1 \) Six months after receiving a baseline vitamin A dose of 105 μmol.
\( ^2 \) \( x \) ± SD.
\( ^3, ^4 \) Significantly different from Madurai, India: \( ^3 \) \( P < 0.0001 \) (Student’s \( t \) test), \( ^4 \) \( P < 0.0001 \) (chi-square test).
were significantly correlated with retinol. The relation between dark-adaptation threshold tests and serum retinol concentrations was much stronger than that observed in Indonesia (6), where vitamin A deficiency was less severe, the degree of depression in dark-adaptation threshold less marked, and the range of disturbance narrower (Table 1). Nonetheless, this relation persisted even at the upper end of both the pupillary and visual curves among children who would not be expected to be vitamin A-deficient. These observations support our findings among the less-deficient Indonesian children, further suggesting that this technique for assessing dark-adaptation status may be useful in identifying populations at risk of relatively mild (subclinical) vitamin A deficiency.

In the absence of a gold standard for the assessment of vitamin A status, the randomized dosing trial provided an unequivocal means of improving vitamin A status in one group of deficient children compared with others (placebo recipients). Our previous trial in Indonesia had shown a significant improvement in the pupillary and visual dark-adaptation scores of children randomly assigned to receive vitamin A, but not of those receiving placebo. Although children randomly assigned to receive vitamin A in the current study tended to improve their pupillary scores compared with children receiving placebo, the difference was not significant. Several explanations might account for this discrepancy. We decided on a period of 2 wk for retesting postdosing on the basis of animal (10) and human (11, 12) studies that suggested that this would be adequate for return to baseline dark adaptation, a period that appeared to have been sufficient in Indonesia. In view of the more severe degree of vitamin A deficiency among the Indian subjects, a longer postdosing interval may have been needed. Whereas some sources indicate a shorter time for resolution of nightblindness after dosing in less-severe cases of deficiency (8), other studies have suggested that multiple doses of vitamin A may be needed to reverse the effects of xerophthalmia in highly deficient subjects (13, 14). Finally, deficiencies of other nutrients known to be involved in vitamin A release and utilization (eg, zinc) might have been present. In view of the potential effect of vitamin A supplementation on mortality in this age group, it was not felt ethical to withhold dosing from children assigned to the placebo group for &gt; 2 wk. Longer follow-up of both groups might have been revealing, however.

The ability to identify populations in need of vitamin A intervention requires a simple, inexpensive, and practical field technique for assessing vitamin A status in preschool children (the group most likely to be severely deficient). Pupillary dark-adaptation testing provides distinctly different results in populations with presumably normal (eg, United States), moderately deficient (Indonesia), and severely deficient (southern India) vitamin A nutriture. This mode of testing offers obvious advantages over currently available techniques for the assessment of a population’s vitamin A status, given that results are immediate, it is noninvasive, and that blood samples do not require delicate transport, storage, and sophisticated analysis. Future testing might be made even more efficient by eliminating the flash, which calculations have shown to bleach &lt; 10% of available rhodopsin, and by allowing children in groups of a dozen or so to dark-adapt simultaneously and maximally (eg, for 30 min). In this way, the time for full evaluation of each child would be reduced to the ≦ 5 min required for the testing itself, without separate, sequential dark-adaptation periods for each subject.

The smaller the sample size required to determine whether a stated level of vitamin A deficiency exists, the more practical the test. Among the various factors that determine sample size, the prevalence of the condition being assessed and the validity of the test itself (the level of "background noise") are both important. Detection of impaired dark adaptation should, theoretically, have advantages in this regard. It represents a demonstrable physiologic consequence of deficiency, and, because it occurs at relatively mild levels of deficiency, is more prevalent than more severe clinical manifestations. For example, examination of the mean (± SD) dark-adaptation thresholds for Indian, Indonesian, and American children allowed us to calculate the minimum number of children required to show a difference between populations at, eg, the 0.01 level of significance. A sample of 27 Indonesian children would have been adequate to show a difference between the Indonesian and American populations, and only 7 Indian children would be needed to show a difference between the American population and the much more deficient Indian population. In comparison, identifying deficiency with the use of more severe clinical indicators requires the examination of hundreds to thousands of children (15). In interpreting these numbers, it is necessary to remember that the populations tested in both Indonesia and India are not representative of average pre-school-aged children in those countries, but were rather chosen to be deficient in vitamin A. Among more borderline populations, larger numbers of subjects would presumably need to be tested to establish deficiency.

Obviously, testing a larger and more representative group is necessary to determine the extent and severity of deficiency and to provide an adequate baseline for assessing the impact of interventions. Only by testing additional populations of varying vitamin A status will it be possible to determine appropriate mean dark-adaptation thresholds to serve as valid criteria for differing degrees of deficiency, and as the basis for determining when an intervention program is warranted and whether or not it is having its intended effect.

REFERENCES