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Diagnostic value of contrast sensitivity test and conjunctival impression cytology for the detection of sub-clinical vitamin-a deficiency

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Abstract
Purpose: To determine whether or not contrast sensitivity test and conjunctival impression cytology can be used as diagnostic tools to detect sub-clinical vitamin-A deficiency; and if there is a correlation within the two diagnostic methods.

Methods: A series of diagnostic studies comprising of contrast sensitivity test, conjunctival impression examination and serum retinol concentration were performed on literate children, six to ten years old, at West Java Elementary School. All subjects underwent a basic eye examination. Sample size, receiver operator curve, negative and positive predictive values, sensitivity and specificity were calculated.

Results: A total of 109 subjects out of 154 elementary school children corresponding to the inclusion criteria were included in this study. Forty-four (41.9%) children were detected to have sub-clinical vitamin-A deficiency. The contrast sensitivity test had a sensitivity value of 45.5% and a specificity value of 50.8% with a positive predictive value of 40%. The area under the receiver operator characteristic (ROC) curve was 49.5% (95% CI 38.1%-60.9%), whereas the conjunctival impression cytology test had a sensitivity value of 90.9%, and a specificity value of 16.4% with a positive predictive value of 43.9%. The ROC area was 56.1% (95% CI 45.0% - 67.1%). Pearson’s analysis showed that there is no correlation between the two diagnostic tools (p 0.538).

Conclusion: The results of this study indicated that neither the contrast sensitivity test nor the conjunctival impression cytology was found to be a favorable screening tool to detect sub-clinical vitamin-A deficiency. Moreover, there is no correlation between the two methods.

Key words: Sub-clinical vitamin-A deficiency, contrast sensitivity, impression cytology

Introduction
Vitamin-A deficiency is a state or condition resulting from a lack of vitamin-A concentration level in the body tissue, causing either sub-clinical or clinical disorders, including the eyes.1 Vitamin-A deficiency currently remains a public health problem worldwide, especially in developing countries, such as Africa and South-East Asia, affecting predominantly the young children during their period of growth.2

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Starting as a silent threat, then later pursuing a pathological course, Vitamin-A deficiency is purported to be the cause of vision loss, especially in children, if not properly and timely managed and treated. In its early stages, it will cause difficulties in seeing in low-light settings, producing a condition known as night blindness. Treated inadequately, the condition will further progress to form abnormalities on the conjunctiva, and later on the cornea, generating a condition known as xerophthalmia, causing permanent damage in the cornea as well as loss of vision.2,3

The predicament of vitamin-A deficiency is a perfect example of an iceberg phenomenon where, to date, only a few xerophthalmia cases have been established despite extensive sub-clinical vitamin-A deficiency found in the community. The ratio of sub-clinical vitamin-A deficiency to xerophthalmic patients is ten to one.3-5 Sub-clinical vitamin-A deficiency is defined as serum retinol concentration below 20 µg/dl (biochemical indicator), which serves as the gold standard, however, this measurement is an invasive, highly-skilled and costly procedure.

Early detection of sub-clinical vitamin-A deficiency is made possible by performing conjunctival impression cytology. This is an objective, less invasive, simple test performed using bio-pore membrane paper to identify the metaplastic changes of conjunctival surface epithelial found in sub-clinical vitamin-A deficiency.6-10 A contrast sensitivity test may be performed in a setting of regular lighting to detect any changes of vision quality despite normal visual acuity.12,13 This test is considered simple and reliable, making it a practical tool for early diagnostics.

In this study, we postulated that the contrast sensitivity test as well as the conjunctival impression cytology test prove to be an effective screening tool. The purpose of this study was to acknowledge the diagnostic value of the contrast sensitivity test and conjunctival impression cytology test in the detection of sub-clinical vitamin-A deficiency. We also aimed to compare the value of both tests as a potential tool for screening.

Methods
This diagnostic study was performed in the Elementary School of Plered Sub-district, Purwakarta, West Java, Indonesia by the School Health Unit – Community Eye Health Service in Cikampek. Thirty elementary schools were randomized and only one school was chosen. Another school was later selected, due to the insufficient number of subjects. The authors obtained prior approval for the study protocol by the Ethics Committee of the Faculty of Medicine University of Indonesia. Written informed consent was obtained from all subjects before recruitment.

Included in this study were literate subjects, six to ten years old, presenting with a visual acuity of 6/6 with maximal spectacle correction of 1.0 Dioptri and showing no abnormalities in the anterior and posterior segments, and who were willing to accept all research procedures.

All subjects underwent the contrast sensitivity examination using Pelli-Robson card with a range illumination of 60-120 cd/m².14 This was followed by a conjunctival impression cytology which was done after administration of anesthesia eye drops in both eyes. A conjunctival swab was taken with acetate cellulose paper (HA,
HA WP 04700, Millipore Corp, Bedford, MA, USA) and manipulated with an objective glass to extract the epithelial cell. The cellulose paper was then placed into a Petri dish containing fixating solution; alcohol 70%, formaldehyde 37% and acetate glacial acid (20:1:1). The fixated conjunctival samples for the impression cytology evaluation were transferred immediately after collection. The abnormal goblet cells were assessed using PAS and Papanicolaou staining. The staining procedure was conducted by a pathology expert, who was oblivious of the two previous assessment results. Density metaplasia goblet cell/mm² was calculated using Tseng criteria.¹⁰

The venous blood was drawn to quantification of serum retinol concentration using high performance liquid chromatography (HPLC) which was performed in the SEAMO-TROPMED (South East Asia Malnutrition Tropical Medicine) Laboratory, Jakarta.

**Operational definition**

The contrast sensitivity test is an assessment of the subject’s ability to see and read letters on various gradations of contrast between characters and background under constant illumination. When the contrast sensitivity test was found to be less than 1.75 log unit, it was termed as abnormal. The ability to accurately read more than 1.75 log unit was defined as normal.

Sub-clinical vitamin-A deficiency was defined when serum retinol concentration was within the range of 0.35-0.70 µmol/l or 10-20 µg, whereas serum retinol concentration of higher than 0.70 µmol/l was defined as normal.

The interpretation of conjunctival impression cytology as grades 2, 3, 4, 5 was classified as abnormal, whereas grades 0 and 1 were considered a normal result.

Nutritional status was determined using the Growth Health Card for Elementary School Children issued by the Indonesian Ministry of Health. This chart is adopted from the Growth Reference Data for children aged between five and 19 years, World Health Organization 2007, which determines the nutritional status of elementary school children, based on gender, age and body-mass index. This chart categorizes the subjects into obese, normal and thin.

**Statistical analysis**

The collected data was analyzed using computerized SPSS 16 program. Sample size was calculated by expected sensitivity of both tests of 90% with an expected false value of 15%. Eighty-eight children altogether were enrolled. We calculated point estimates of predictive value, sensitivity and specificity, as well as the receiver operating curve of contrast sensitivity test and conjunctival impression cytology in detecting sub-clinical vitamin-A deficiency; this was compared to the gold standard, serum retinol concentration. Correlation between the two tests was assessed using Pearson’s test.
Results
A total of 109 (70.8%) literate children out of 154 elementary school children were included in the study and underwent contrast sensitivity test, conjunctival impression cytology and blood test over a period from March to April 2009. One subject was excluded due to unsuccessful attempts to obtain venous blood. Three subjects were considered as excluded for inconclusive results of the conjunctival impression cytology; thus 105 subjects were considered favorable candidates for this study.

There were 46 (43.8%) female subjects participating in this study, the average age of subjects being eight years old. Ninety-one (86.7%) subjects were classified to have normal nutritional status. The prevalence of sub-clinical vitamin-A deficiency was found to be 41.9% (44/105), suffered equally by male and female subjects. Thirty-four (77.27%) subjects detected with decreased level of serum retinol concentration were determined as having a normal nutritional status (Table 1).

Table 1 Characteristic of subjects

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>59</td>
<td>56.2%</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>43.8%</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1.9%</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>14.3%</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>41.9%</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>28.6%</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>13.3%</td>
</tr>
<tr>
<td><strong>Nutritional Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>2</td>
<td>1.9%</td>
</tr>
<tr>
<td>Normal</td>
<td>91</td>
<td>86.7%</td>
</tr>
<tr>
<td>Thin</td>
<td>12</td>
<td>11.4%</td>
</tr>
<tr>
<td>Sub-clinical vitamin A deficiency</td>
<td>44</td>
<td>41.9%</td>
</tr>
</tbody>
</table>
Contrast Sensitivity test

There were 50 (47.6%) subjects who demonstrated an abnormal ability during the contrast sensitivity test, with an average serum vitamin-A level of 0.83 ± 0.034 µmol/l (mean ± SD). However, the normal contrast sensitivity group showed a similar level of serum vitamin A. In order to prove whether contrast sensitivity test can be used as a diagnostic tool to detect sub-clinical vitamin-A deficiency in school children, it was necessary to conduct several statistical tests.

The sensitivity value is described as the ability of a diagnostic tool to detect a disease. Data showed that the contrast sensitivity test could only detect 45.5% of the subjects who presented with sub-clinical vitamin-A deficiency, whereas the specificity of the contrast sensitivity test to rule out subjects with sub-clinical vitamin-A deficiency is 50.8% (Table 2).

The positive predictive value (PPV) of the contrast sensitivity test was 40%, showing that there was a 40% probability of a subject indicating a positive result to actually have sub-clinical vitamin-A deficiency. Whereas the probability of not having the disease was derived from the negative predictive value (NPV), which is 56.4%.

Furthermore, receiver operator characteristic (ROC) analysis revealed that the ROC curve seems to be too close to the diagonal reference line - the area under curve 49.5% (95% CI 38.1% - 60.9%). If the cut-off point of the contrast sensitivity test is decreased into ≤ 1.57 log unit, the specificity of the test will be raised to 100%, however, its sensitivity will be down to 4.5%. These results demonstrate that the contrast sensitivity test is not an accurate tool in detecting sub-clinical vitamin-A deficiency.

Table 2 Diagnostic value of Contrast Sensitivity test

<table>
<thead>
<tr>
<th>Contrast Sensitivity</th>
<th>Serum Retinol Level</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0.35-0.70 µmol/l)</td>
<td>(&gt;0.70 µmol/l)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>20 (a)</td>
<td>30 (b)</td>
</tr>
<tr>
<td>Normal</td>
<td>24 (c)</td>
<td>31 (d)</td>
</tr>
<tr>
<td>Total</td>
<td>44 (a + c)</td>
<td>61 (b+d)</td>
</tr>
</tbody>
</table>

The sensitivity value is a/(a+c) = 20/44 = 45.5%; whereas the specificity yield from d/(b+d) = 31/61 = 50.8%. The PPV was derived from a/(a+b) = 20/50 = 40%, whereas the NPV is d/(c+d) = 31/55 = 56.4%.

Conjunctival impression cytology

Conjunctival impression cytology is a minimally invasive pathological assessment to detect any pathological changes within the conjunctival surface. There was only 1 (0.9%) subject who showed grade-2 abnormality and a total of 90 (85.7%) subjects presented with grade-4 and grade-5 abnormality of conjunctival impression cytology. Fourteen (13.3%) subjects were classified to have normal pattern (grade 1). The mean serum retinol concentration of subjects with abnormal conjunctival impression
Sub-clinical vitamin-a deficiency detection

cytology was $0.82 \pm 0.029 \mu\text{mol/l}$. Subjects with normal conjunctival impression cytology showed a mean serum retinol concentration of $0.98 \pm 0.035 \mu\text{mol/l}$. The difference of serum retinol concentration between subjects with abnormal and normal conjunctival impression cytology was statistically significant ($p: 0.036$).

The sensitivity value for conjunctival impression cytology was 90.9%, indicating therefore that this diagnostic tool demonstrated a 90.9% chance of detecting sub-clinical vitamin-A deficiency. The specificity value was 16.4%, which meant the probability of this test showing a negative result on a healthy subject was 16.4%. The positive predictive value (PPV) for conjunctival impression cytology was 43.9%, showing that there was 43.9% probability of a subject with a positive test result to actually have sub-clinical vitamin-A deficiency. The probability of a subject with negative result (normal conjunctival impression cytology) for not having the disease was 71.4% (negative predictive value/NPV)(Table 3).

![Grade 4 and 5 conjunctival impression cytology abnormality, showing pycnotic nucleus and un-nucleated basophilic cells on sample no. 13 and 16.](image)

Table 3 Diagnostic value of conjunctival impression cytology

<table>
<thead>
<tr>
<th>Impression Cytology</th>
<th>Serum Retinol Level</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0.35-0.70 µmol/l)</td>
<td>(&gt;0.70 µmol/l)</td>
</tr>
<tr>
<td>Abnormal (&gt; grade 2)</td>
<td>40 (a)</td>
<td>51 (b)</td>
</tr>
<tr>
<td>Normal</td>
<td>4 (c)</td>
<td>10 (d)</td>
</tr>
<tr>
<td>Total</td>
<td>44 (a + c)</td>
<td>61 (b+d)</td>
</tr>
</tbody>
</table>

The sensitivity and specificity were 90.9% and 16.4%, respectively; whereas the PPV and the NPV were 43.9%, and 71.4%, respectively.

Receiver operator characteristic (ROC) curve showed the correlation between sensitivity and specificity of conjunctival impression cytology to be a favorable diagnostic tool. The aim was to find the cut-off point of a diagnostic study. Area below ROC was 56.1% (95% CI 45.0% - 67.1%). Further analysis revealed that the most appropriate trade-off balance was 56.8% vs 52.5%, for sensitivity and sensitivity values, respectively, and 46.3% vs 62.7% for PPV and NPV, respectively, was achieved by using the grade-5 pathology of the conjunctival surface as the cut-off point. However, these results still indicate that conjunctival impression cytology is not sufficient enough to be used as an effective diagnostic tool.
Discussion
This study revealed that 44 (41.9%) out of 105 subjects were found to have sub-clinical vitamin-A deficiency. Surprisingly, 34 (77.27%) of them were actually grouped in the ‘normal’ nutritional status. This finding was in agreement with the fact that sub-clinical vitamin-A deficiency has no clinical manifestations other than the finding of serum retinol concentration is less than 20 gr/dl.\textsuperscript{15,16} This condition is also known as micronutrient deficiency or hidden hunger. People with this condition are usually unaware of the deficiency experienced by their body. These micronutrients are needed in small amounts only but are truly essential for general health, especially for the eyes.\textsuperscript{17,18}

Subjects aged six to ten years were selected to participate in this study, since these particular age groups are not covered by the Indonesian Ministry of Health’s vitamin A supplementation program, employed in February and August annually. This program only covers pre-school children under five years old.\textsuperscript{19} This study showed that the majority of sub-clinical vitamin-A deficiency subjects are eight years old, without gender disparities. These results correspond with those of our previous studies.\textsuperscript{20,21}

The serum retinol concentration of 0.35-0.70 µmol/l, used as an indicator for diagnosing sub-clinical vitamin-A deficiency, does not provide an exact reflection of the total amount of body retinol stored in the liver. It represents only a borderline level between vitamin-A deficiency and normal serum level.\textsuperscript{22,23} A stable serum retinol level can be achieved when the liver storage level is very low.\textsuperscript{23}

High prevalence of sub-clinical vitamin-A deficiency in children in this study indicated the dietary intake situation in the area. As the nutritional status might hold a role in this finding, considering the high number of children who are undernourished in Plered sub-district, West Java.\textsuperscript{24} Dietary habits of the children in this area constitute one of the factors, since this study was conducted in the mountain and plantation areas, where it is difficult for the people to find animal sources of vitamin A, such as meat, milk, fish, liver and eggs. Low social economy status might also contribute to this predicament. The level of vitamin A in the body is also influenced by respiratory tract infection, persistent diarrhea, anemia, and middle ear infection, frequent afflictions suffered by these children.\textsuperscript{25,26}

Detecting the manifestations of sub-clinical vitamin-A deficiency is a challenging task, considering night blindness is the early subjective symptom and more often than not unnoticed by patient. Decreased ability to see in low light intensity is caused by disturbance in the rhodopsin regeneration cycle, subsequently resulting in lower level of contrast sensitivity, which, clinically, can be assessed by contrast sensitivity test. Patients might have visual acuity of 6/6 while experiencing difficulties to see at night.\textsuperscript{27}

This study found that the average value of the 50 (47.6%) subjects with abnormal contrast sensitivity is 1.64 log unit, whereas the normal value is 1.75 log unit. Moreover, the mean serum retinol concentration is 0.83 ± 0.034 µmol/l in both normal and abnormal contrast sensitivity subjects, resulting in the low sensitivity and specificity values. However, this finding was quite different from that of
Sekarsari who found the sensitivity of 100% and specificity of 80.5% based on the school children population with a mean of abnormal contrast sensitivity value of 1.52 log unit, and an average of serum retinol level of 0.63 µmol/l. Similar results were also found by Handayani. These facts might suggest that the contrast sensitivity test appears to be successful as a diagnostic tool only in the more advance serum retinol level depletion.

Since our finding of low sensitivity values together with the low PPV for contrast sensitivity test indicated that this test is unfavorable as a screening tool in the detection of deficiency of vitamin A, since it revealed only a 40% probability of a subject with positive test result to actually have sub-clinical vitamin-A deficiency. Several factors contribute to this result; background lighting, light reflection, distance, and personal factors pertaining to the subject. The drawback of this study was that no measurement was taken on room illumination during the test. Moreover, the contrast sensitivity test is a subjective test and might contribute to the variation of its diagnostic values found in our last studies. Fear and shyness in our subjects might also have affected the test results. Measurement bias was avoided by taking a repeated test up to two to three times.

This study showed a total of 91 subjects (86.7%) with abnormal conjunctival impression cytology, which is higher than the previous study by Rostami et al. who only revealed 23.6% prevalence of abnormal conjunctival impression cytology among two to five-year-old children in Teheran. Reddy reported that 70.5% out of 246 children aged six to ten years, had serum retinol concentration which is lower than 0.7 µmol/l, whereas 85% of them were found to have an abnormal profile. The prevalence was raised to 97% in the pre-school children (one to five years old). Moreover, 65% amongst those who presented with normal conjunctival impression cytology were found to have low serum retinol concentration; which was in contrast to our finding, incorporating only 4 (28.6%) subjects.

Incongruence found in our study between conjunctival impression cytology and serum retinol concentration was most likely due to a poor cytology sample collection process, inadequate amount of serum, poor reagent quality or other unknown contributing factors. The disadvantage of conjunctival impression cytology as a diagnostic tool was the necessity of optimal cooperation between researcher and the subjects. Uncooperative subjects, especially the children, served to hamper sample collection process, thus affecting the end result. Conjunctival impression cytology also presented its own extent of subjectivity.

The contrast sensitivity test is a functional clinical indicator, whereas the conjunctival impression cytology is a tissue pathological indicator. Further comparability analysis using Pearson’s test was not in total accordance and no correlation (p 0.538) was found between the contrast sensitivity test and the conjunctival impression cytology (Table 4). This finding may indicate that there are different kinds of vitamin-A metabolism within the two tissues.
In conclusion, the results obtained did not support our postulation that either the contrast sensitivity test or the conjunctival impression cytology can be used as a favorable diagnostic screening tool to detect sub-clinical vitamin-A deficiency.

Table 4 Comparability between conjunctival impression cytology and contrast sensitivity test.

<table>
<thead>
<tr>
<th>Contrast Sensitivity</th>
<th>Impression Cytology</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abnormal</td>
<td>Normal</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>44 (a)</td>
<td>6 (b)</td>
<td>50 (a+b)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>47 (c)</td>
<td>8 (d)</td>
<td>55 (c+d)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>91 (a + c)</td>
<td>14 (b+d)</td>
<td>105</td>
<td></td>
</tr>
</tbody>
</table>

Pearson's analysis showed that there was no correlation (p 0.538) between the conjunctival impression cytology and the contrast sensitivity test.

References

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