Improved Methods for Determining Vitamin A Status

Vitamin A status is the combined term used to reflect the dynamic processes of intake, storage, utilization and excretion of the vitamin over time. Deficiency of vitamin A is a result of poor dietary intakes of vitamin A or its precursor carotenoids causing depletion of liver reserves expressing ultimately as clinical signs or symptoms. The diagnosis of clear cut cases of deficiency is rather simple but measures of marginal vitamin A status are urgently needed in view of the functional implications in morbidity and mortality. Methods available at present for assessing vitamin A nutriture are four types: (i) Dietary, (ii) clinical, (iii) Physiological and (iv) Biochemical.

In general dietary methodology is applicable on a broad basis to groups or population and may not yield very reliable data because of inherent variations in methodology and fluctuations due to seasons. A semi quantitative approach based on frequency of intake of vitamin A rich foods to characterise groups of population 'at risk' for nutritional education has been recently introduced. Appropriate guidelines have been issued by International Vitamin A Consultative Group (IVACG). The clinical diagnosis which is commonly used focuses on ocular signs like night blindness, conjunctival xerosis, bitot spots and corneal lesions. Although conjunctival xerosis is an early manifestation, its diagnosis is prone to subjective error. Bitot spots are readily detectable but may not always indicate vitamin A deficiency, especially in higher age groups. Physiological indicators like impaired dark adaptation and abnormal retinograms can be more readily used in a clinical setting for adults but are not suitable for young children, especially in a field situation.

History of night blindness is also difficult to determine in very young children. In certain situations lack of specific word characterizing night blindness, leads to failure of this approach. Biochemical analyses have been mainly limited to serum/plasma vitamin A concentration although there are a few studies on analysis of liver stores, which forms the best measure of vitamin A status. The need of obtaining a biopsy and the appreciable variation in distribution of vitamin A in liver limit the application of this approach. The difficulty of obtaining appropriate stable isotopes and sophisticated instrumentation restrict isotope dilution methods.

Functional indicators are an alternative and more appropriate means of identifying marginal vitamin A status in individuals or populations. Three functional parameters have been recently suggested: the relative dose response (RDR) test, conjunctival impression cytology (CIC) and urinary ammonium N/Creatinine ratio (Am N/Cr.) Ocular surface abnormalities due to vitamin A deficiency are detected histologically by the CIC. While keratinization may be hallmark of vitamin A depletion in skin, in other epithelial tissues, depletion in or absence of goblet cells forms the typical feature. In CIC, a strip of cellulose ester filter paper (0.48 μ pore size) is gently applied to the outer (temporal) portion of the conjunctiva for a few seconds and then removed with a peeling motion. The cells are either transferred on to a slide or the filter paper is fixed in an airtight bottle until stained. Usually periodic acid schiff and hematoxylin stains are used for light micro-
scopic examination. Impressions showing sheets of small epithelial cells with the presence of goblet cells are considered normal, while those showing enlarged epithelial cells and absence of goblet cells are indicative of vitamin A deficiency. The method has been validated in some recent studies. 

A clinical trial was conducted in India to compare the results of CIC with two other indicators of vitamin A status, ocular signs and serum vitamin A concentration. A total of 248 children aged 1-10 years were investigated. About 25% of the children with normal eyes showed abnormal cytology with lower plasma vitamin A levels, suggesting that abnormal cytology reflects subclinical deficiency. In the same way 65% of the children with normal cytology also had low plasma vitamin A levels pointing out fall in serum vitamin A precedes the cytological changes. Children with conjunctival xerosis had significantly lower plasma vitamin A, whereas only 25% of them had normal cytology, the discrepancy arising mainly with Bitot spots (higher age) children. Thus impression cytology could distinguish between acute cases of Bitot spots (generally deficient) and those without vitamin A deficiency. In areas where vitamin A estimation is difficult, impression cytology can be used. However, it is difficult to carry out the test in very young children who may not cooperate well. Also the data may be influenced by conjunctival infections and malnutrition.

Measurement of plasma or serum vitamin A remains the most practical method available for biochemical analysis. A plasma concentration < 0.35 μmol/L (10 μg/100 ml) indicates low body stores and correlates with clinical manifestation. A concentration > 1.05 μmol/L (30 μg/100 ml) reflects adequate stores. In the intermediate range plasma vitamin A does not correlate with body stores and leads to problems of interpretation. To overcome this, a serum response to supplementary vitamin A to indicate whether the individual is below his homeostatic level, is designed which is termed as RDR.

In conditions of adversity or under dietary deprivation, plasma retinol levels are maintained as a result of mobilization from liver. Only when the liver stores are depleted below a threshold value, the rate of vitamin release from the reserve is diminished but the synthesis of its carrier protein, retinol binding protein (RBP), continues and leads to accumulation of apo RBP in liver. An exogenous bolus of retinol or retinyl esters would cause the release of accumulated RBP as holo RBP into circulation in a time dependent manner. This brings about an increase in plasma vitamin A subsequent to the dose.

After drawing a fasting blood sample, an oral dose of about 450-1000 μg (1.6-3.5 μmol) retinyl palmitate is administered to the subject. The dose is followed by a small meal containing minimal vitamin A (and some fat). A repeat blood sample is collected 5 hrs after the dose.

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RDR(\%) = \frac{\text{Vitamin A at 5 hr}}{\text{Vitamin A at 0 hr}} \times 100
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An RDR > 20 % is considered to be positive and indicates inadequate hepatic stores; i.e. marginal status. This method had been validated by Amedee-Mancsme et al. However, in children with chronic undernutrition or in those with liver disease, there was no good correlation between the RDR test and the vitamin A nutritional status.

RDR was tested in a group of under-