PROGRESS IN THE DIAGNOSIS OF HYPOVITAMINOSIS A: CLINICAL AND BIOCHEMICAL CORRELATIONS

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SUMMARY

Hypovitaminosis A is a widespread problem, especially among preschool children in many parts of the world.

According to the World Health Organization (WHO) estimates, about 100,000 children become blind every year, and many are dying due to vitamin A deficiency while millions of others are suffering from other consequences of vitamin A deficiency such as growth retardation and increased susceptibility to infection. It is, therefore, very important that not only the severe cases of hypovitaminosis A be diagnosed for immediate treatment, but also the marginal cases of vitamin A deficiency in vulnerable populations be diagnosed as early as possible so that appropriate preventive measures be implemented. Available methods for the diagnosis of vitamin A deficiency can be classified into four catgegories: clinical, biochemical, functional, and dietary. Clinical diagnosis is based on examining ocular and extraocular signs of hypovitaminosis A, and is only useful for the detection of severe cases. Biochemical methods are based on the plasma concentrations of retinol and retinol-binding protein (RBP), and liver reserve of vitamin A whenever possible. Two other commonly used

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diagnostic tests are: functional testing for nightblindness using dark adaptation time, and pathological testing for ocular signs of conjunctival xerosis, with or without the use of Rose Bengal or lissamine green dye. Dietary method for the diagnosis of vitamin A deficiency is based on the estimation of dietary intake of vitamin A and carotenoids. In addition, there are newer methods such as isotope dilution and relative dose response (RDR) techniques which have been recently proposed for the diagnosis of vitamin A deficiency. RDR appears to be a reliable and sensitive indicator of marginal vitamin A. However, the most desirable approach to diagnosis may be one based on multiple indicators for the accurate assessment of vitamin A status in the community.

The purpose of this paper is to draw attention to the problems of diagnosis, to recent advances and to our Brazilian experience in this important area of vitamin A research with significant global implications.

INTRODUCTION

Hypovitaminosis A is a clinical condition resulting from inadequate intake or utilization of vitamin A. Vitamin A deficiency is usually primary from prolonged dietary deprivation. The underprivileged, the preschool and school age children are especially vulnerable. High nutrient requirement, early weaning, parental ignorance of correct post-weaning nutrition and hygiene, and poverty combine to make the first years of life the most susceptible period for hypovitaminosis A.

Less commonly, vitamin A deficiency is secondary to interference with absorption, metabolism, storage and excretion of vitamin A. Interference with absorption or storage is likely to occur in parasitic infestation, celiac disease, sprue, gastrointestinal operations and liver diseases. In protein-energy malnutrition (marasmus and kwashiorkor) vitamin A deficiency is common, not only because the diet is deficient but also because vitamin A storage and transport are defective. Retinol decreases or disappears from the blood during infections such as pneumonia or scarlet fever. In deficiency, liver stores are depleted before plasma levels begin to fall, followed later by retinol dysfunction and finally by the structural changes in the epithelial cells.

Hypovitaminosis A results whenever the dietary intake of this vitamin is inadequate and/or the supply of this vitamin from the body store is inadequate for the normal functioning of the tissues. When the intake is inadequate we have a primary or dietary vitamin A deficiency. This deficiency may also result from various bodily states that interfere with absorption or utilization of vitamin A or from factors that increase its requirement, such as lactation, pregnancy or fast growth. This type of deficiency is known as secondary or conditioned deficiency.

Between the occurrence of a vitamin A inadequacy, primary or secondary, and the onset of the clinical signs of the deficiency, time elapses. The time may be short or long, depending on the previous amount of retinol reserves and the degree of nutritional imbalance. But when the primary or secondary factor persists, tissue depletion occurs. Tissue depletion is followed in succession by biochemical "lesions", functional changes and finally, anatomical lesions. No step in this chain of events is necessarily complete before the next one begins (Figure 1).

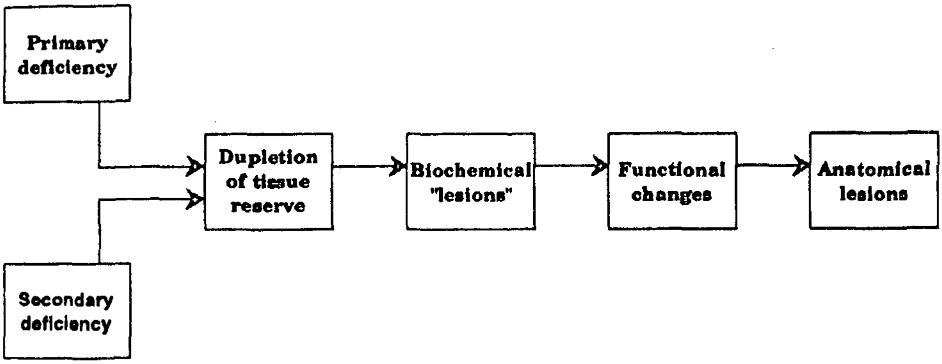

FIGURE 1

Pathogenesis of hypovitaminosis A

The presence of anatomical lesions is characteristic of the clinical stage of vitamin A deficiency. Before its appearance we have a short or longer period of protection according to the previous vitamin A reserve. During this period tissue depletion, biochemical disturbances and functional changes occur, but no physical signs are detected. This is the preclinical stage of vitamin A deficiency.

The knowledge and understanding of this pathogenesis is important because it explains the simultaneous presence of clinical and laboratory signs in advanced stages of vitamin A deficiency, and only biochemical and functional impairements in the marginal or preclinical phase of the hypovitaminosis A.

DIAGNOSIS

Various classical methods are available and some newer methods have recently been proposed for the diagnosis of vitamin A deficiency. Each of these methods will be briefly described, and their usefulness and limitations will also be discussed with special reference to studies carried out in Brazil.

A. *Clinical Aspects*

Several clinical criteria related to the deficiency of vitamin A in humans have been used. The ocular lesions that are quite specific to vitamin A deficiency are xerophthalmia and keratomalacia.

1. *Diagnosis of ocular signs*

The most widely used clinical diagnosis of vitamin A deficiency is based on WHO classification (1), as shown in Table 1. The lesions are descriptive rather than diagnostic. This classification is useful for the diagnosis of severe cases in clinical and field surveys. Nevertheless, some signs are nonspecific and difficult to measure or standardize. Information regarding clinical ocular signs of vitamin A deficiency in Brazil is inadequate. It appears, from the limited data available that severe cases of xerophthalmia and vitamin A deficiency do not exist in most regions of Brazil except in the Northeastern State of Paraiba (2) and in Jequitinhonha valley of Minas Gerais (3). There may exist, however, many undetected subclinical cases of vitamin A deficiency with overt possibility of developing ocular lesions, since low dietary intake of vitamin A and carotenoids, and low plasma retinol levels have been found to be a common public health problem in various regions of Brazil (2-9).

2. *Diagnosis of early ocular signs using vital stains*

Conjuctival xerosis is one of the earliest ocular signs of vitamin A deficiency and xerophthalmia, but is not easy to detect. It was proposed that the use of vital stains such as Rose Bengal and

TABLE 1

CLINICAL OCULAR DIAGNOSIS OF VITAMIN A DEFICIENCY*

Clinical signs	Classification
Night blindness	XN
Conjunctival xerosis	X1A
Bitot's spot	X1B
Corneal xerosis	X2
Corneal ulceration/keratomalacia < 1/3 surface corneal	X3A
Corneal ulceration/keratomalacia ≥ 1/3 surface corneal	X3B
Corneal scars	XS
Xerophthalmia fundus	XF

* According to WHO Report (1).

lissamine green be used to detect early signs of conjuctival xerosis as an indicator of vitamin A status of vulnerable populations (10). It is claimed to be a simple, reliable and objective test for field surveys and it has been used in Africa and Asia, with reasonable success. A main drawback of this procedure is that it gives certain percentage of false positive and false negative results which many be due to factors other than true vitamin A deficiency. Another problem is that the method is not yet properly standardized to obtain uniform and comparable results. It requires instilling one drop of 1% solution of either Rose Bengal or lissamine green stain and looking for a positive or negative staining of conjuctival tissue from a distance of one meter. The difficulty lies in defining a positive or a negative response, which is quite subjective and liable to investigator's bias. In Brazil some investigators have used Rose Bengal staining tests as a useful criteria for the detection of early signs of conjuctival xerosis in Southern Brazil (11, 12). Vital staining with Rose Bengal dye as 1% solution or as strips impregnated with 10 or 15% solution was found to be equally effective in the early detection of conjuctival xerosis, which is around 14.8% in the state of Rio Grande do Sul (11).

3. *Diagnosis of extra ocular signs*

Although the extra ocular manisfestations of vitamin A deficiency are well understood, they are not yet well explored for the diagnosis of hypovitaminosis A in human. Many extra ocular symptoms of vitamin A deficiency have been observed in experimental animals. A few of these are also observed in humans but not all are easy to measure for the purpose of objective diagnosis of hypovitaminosis A. Loss of appetite, growth failure and lowered resistance to infection are few of these extra ocular signs which may be explored in the future for the early diagnosis of hypovitaminosis A.

B. *Biochemical Aspects*

Biochemical diagnosis of hypovitaminosis A has special importance since biochemical changes precede the clinical changes and, therefore, are more useful for the detection of early subclinical signs of vitamin A deficiency. Two most commonly used biochemical tests are plasma and liver indicators of vitamin A status.

1. *Plasma retinol and carotenoid levels*

Vitamin A in plasma exists as preformed vitamin A, mainly retinol and as provitamin A, mainly beta-carotene. Although plasma concentrations of vitamin A and carotenoids have been extensively used for the assessment of vitamin A status, it is not a true indicator for two reasons. Firstly, it changes significantly only when the liver stores are severely depleted; and secondly, many exogenous and endogenous factors influence plasma retinol level and thereby complicate the interpretation of plasma retinol and carotenoid values. Figure 2 illustrates various exogenous and endogenous factors, directly or indirectly related to the true vitamin A status of the individual. In spite of these limitations, plasma vitamin A index is commonly used in surveys for the assessment of vitamin A status, since it is easy to measure and may give useful information along with clinical and dietary data for the diagnosis of hypovitaminosis A. The commonly used criteria for the interpretation of plasma retinol value are those of ICNND (13), and are shown in Table 2. The deficient level (< 10 µg/100 ml) tends to be associated with both low liver reserves of vitamin A and an increased prevalence of clinical signs of deficiency. The low level (10 - 20µg/100 ml) is one commonly found in populations with marginal deficiency of vitamin A, and may not necessarily indicate low liver reserves. Plasma carotene level is not a very useful index in the diagnosis of hypovitaminosis A but reflects only the recent intake of dietary carotenoids.

2. *Liver vitamin A level*

Liver has long been known as the major storage organ for vitamin A in mammals, and can be a useful tissue to detect hypovi-

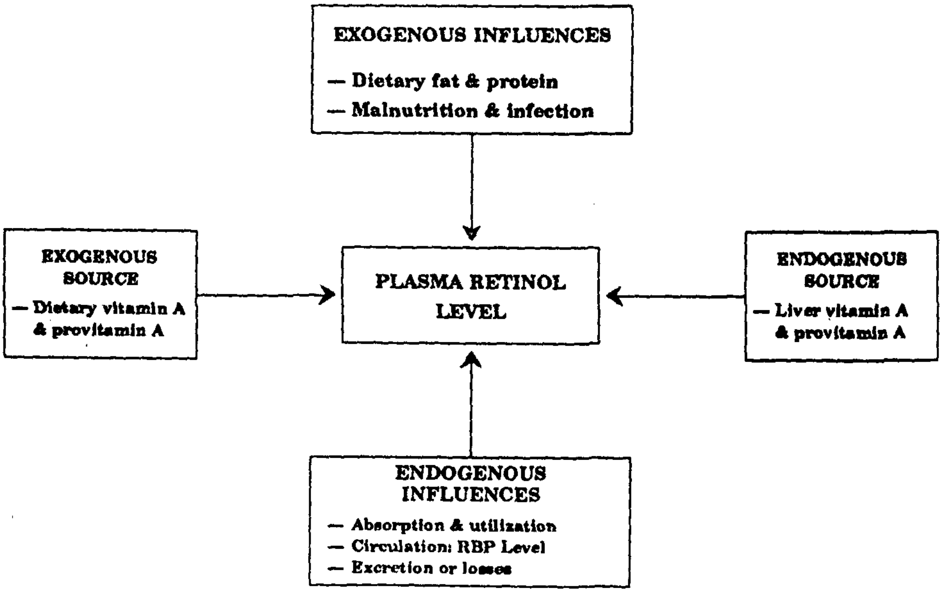

FIGURE 2

Factors influencing plasma retinol level

TABLE 2

BIOCHEMICAL DIAGNOSIS OF VITAMIN A DEFICIENCY*

Vitamin A status	Plasma retinol level (μg/100 ml)
High	Over 50
Normal	20 - 50
Low	10 - 20
Deficient	Below 10

* According to ICNND Report (13).

taminosis A in human populations. Liver biopsy specimens, however, are difficult to get and impractical for routine use in population surveys. This method, therefore, requires access to autopsy samples which should be representative of the population to be diagnosed for hypovitaminosis A. Most of the recent information regarding inadequate body reserves of vitamin A in Canada and the United States have been derived from liver autopsy samples analyzed for vitamin A concentration. It is a more direct indicator of body reserves of vitamin A than the plasma retinol concentration. A tentative guideline has been proposed for the evaluation of liver vitamin A value (14) as shown in Table 3. A liver store of 20 μg/g vitamin A is considered as acceptable level and is expected to provide protection for a period over 100 days; values between

TABLE 3

LIVER STORAGE CRITERIA FOR THE DIAGNOSIS OF HYPOVITAMINOSIS A AS A PUBLIC HEALTH PROBLEM IN CHILDREN*

Vitamin A status	Liver vitamin A concentration (μg/g)
Acceptable	> 20
Marginal	10 - 20
Poor	5 - 10
Very poor	< 5

* According to Olson (14).

10-20 μg/g, as poor, and values below 5 μg/g, as very poor. In Brazil, the incidence of hypovitaminosis A in Salvador, Recife and Brasilia were estimated using autopsy samples of neonates, preschool children and adults for the determination of liver vitamin A reserves (15-17).

C. *New Approaches*

In recent years several new aproaches have been initiated towards better diagnosis of hypovitaminosis A. First of these is a dark adaptation test which is being developed as a rapid functional test for the diagnosis of nightblindness in field surveys. The second new approach has been to develop objective diagnosis of liver reserve and body pool of vitamin A, using the relative dose response test and isotope dilution technique. The third of these new approaches uses conjunctival impression cytology for the detection of early xerophthalmia.

1. *Rapid dark adaptation test*

The classical test for dark adaptation is a useful test for the diagnosis of nightblindness as an early indicator of the functional deficiency of vitamin A. This classical test is nonetheless little used in field studies, due to the fragility and high cost of the equipment. In 1977, a rapid method for dark adaptation was proposed (18). Subsequently, this method was suggested for the field assessment of hypovitaminosis A in the community (19). It has been shown that the method is applicable to children studies (20, 21). The basis for the rapid test is the measurement of time of occurrence of the so called Purkinje shift, a phenomenon whereby the peak wave length sensitivity of the retina shifts from the red toward the blue end of the visual spectrum during the transition from photopic or cone-mediated day vision to scotopic or rod-mediated night vision. This test has the advantages of being carried out in a relative short period of time with simple and inexpensive equipment.

2 *Relative dose response test*

Relative dose response test is a newer approach to the diagnosis of hypovitaminosis A. It was originally developed in rats and is now being applied to humans for the assessment of liver reserves in individual patients (22, 23) and in population surveys (24) using only plasma samples for the analysis of retinol. Relative dose response (RDR) is defined as the percentage increase in plasma retinol level relative to the plasma retinol level five hours after the oral administration of a standard dose of 450 retinol equivalents. The formula can be expressed as follows:

$$\mathrm{RDR}\ (\%) = \frac{A_5 - A_0}{A_5} \times 100$$

In principle, the short-term response of plasma retinol levels to a dose of vitamin A could provide a measurement of the amount by which plasma retinol levels are below normal levels due to inadequate hepatic store. Comparison of dose response could also provide a means for distinguishing between individuals whose predose plasma vitamin A levels are low because of vitamin A deficiency, and those whose plasma vitamin A levels are low for other reason. This dose response relative to the normal plasma level of the individual under conditions of vitamin A sufficiency, could serve as an indicator of poor vitamin A status and depleted hepatic reserves. Mild or moderate protein energy malnutrition do not seem to invalidate the use of the RDR test to assess inadequate reserves. Tentatively, $< 20\%$ RDR is proposed as a negative response, and $> 20\%$ RDR as a positive response, indicating inadequate liver vitamin A reserve (23). In a field study carried out with marginally malnourished Brazilian children in Recife, Pernambuco, the RDR test was found to be a more reliable indicator of inadequate vitamin A status, especially when the serum retinol values were above 20 µg/dl, than the serum value alone (24). It appears from the limited data available that RDR is a reliable and sensitive indicator of marginal vitamin A status in populations with no obvious clinical signs of hypovitaminosis A, as is the case in Brazil and in many other parts of the world.

3. *Isotope dilution technique*

Another alternative approach of interest is the application of isotope dilution technique for the indirect estimation of hepatic vitamin A store. A method developed for studies in rats (25) is based on the principle of isotope dilution, whereby a dose of labelled vitamin A is administered and allowed to equilibrate with body stores, and the size of the body pool is calculated from the following equation:

$$\text{Body pool } (\mu g) = \frac{\text{Total liver radioactivity (cpm)}}{\text{Specific activity of plasma vitamin A (cpm/}\mu g)}$$

Excellent correlations were obtained between analyzed and calculated values for liver vitamin A in rats with low hepatic reserves of the vitamin (25). Same method was assessed over a wide range of values for liver vitamin A, and showed significant correlations between analyzed and calculated values at low and high levels but not at intermediate levels (26). Results of these studies suggest that the variability of incorporation of the label into the liver is a factor that might limit the usefulness of the isotope dilution method, especially if it is to be applied to human beings on different diets and under varying physiological conditions. Further research is needed in this new approach to the diagnosis of hypovitaminosis A.

4. *Conjunctival impression cytology*

Conjunctival impression cytology is a technique which involves collection of conjunctival surface cells by touching a 5 x 5 mm piece of cellulose acetate filter paper to the conjunctiva, pressing it gently and then peeling back the paper (27). The adherent epithelial cells on filter paper are fixed in a solution containing 70% ethyl alcohol, 37% formaldehyde, and glacial acetic in the proportion of 20:1:1 by volume. Specimens fix in ten minutes but could be left in for several days for storage and transport to the laboratory. The cell samples are then stained with periodic acid-Schiff and hematoxylin or periodic acid-Schiff and modified Papanicolau's staining process (27, 28). The stained specimens are then examined microscopically for the loss of globlet cells and the appearance of enlarged, partially keratinized epithellial cells.

The technique of conjunctival impression cytology is a simple, noninvasive and objective method for the early diagnosis of subclinical signs of vitamin A deficiency and xerophthalmia in developing countries. A recent field trial in Guatemala found the method to be of use because a large number of children could be sampled without the need for phebotomizing, and specimens could be easily transported and processed. Conjunctival impression cytology can be done quickly and inexpensively as compared to measuring serum levels of vitamin A using biochemical methods such as high performance liquid chromatography (HPLC). After appropriate standardization of interpretation criteria, this method would be a promising field test for the early diagnosis of vitamin A deficiency in developing countries. Limited field trials have recently been carried out to test the validity and usefulness of this method in India, Indonesia and Guatemala (27, 29). To our knowledge, this method has not yet been tested in Brazil and other countries of South America.

CLINICAL AND BIOCHEMICAL CORRELATIONS

In general there seems to be a good correlation between the clinical and biochemical parameters of vitamin A deficiency. Biochemical observations are useful to confirm the specificity of clinical observations which otherwise may have been influenced by factors other than vitamin A deficiency.

In experimentally-induced vitamin A deficiency in volunteer subjects, it has been shown that clinical and biochemical changes are usually associated with decreased body pools of vitamin A, reduced utilization rates, and lowered plasma levels of the vitamin (30). WHO (1) has used the established correlations between clinical and biochemical criteria to diagnose the public health significance of vitamin A deficiency in vulnerable populations around the world. For example, vitamin A deficiency in a group of at-risk children six months to six years of age, is of serious public health significance if the minimum prevalence rate of clinical and biochemical indicators is: Bitot's spots > 0.50%, corneal xerosis/corneal ulceration/keratomalacia > 0.01%, corneal scar > 0.05% and plasma vitamin A level below 10 µg/dl > 5.00%. Whether such clinical/biochemical correlation exists between the early signs of vitamin A deficiency and plasma vitamin A levels remains to be established.

DISCUSSION

A. *General*

It seems obvious that no one approach, whether it is clinical, biochemical, physiophathological or dietary, is adequate for the accurate assessment of hypovitaminosis A, especially in preschool children considered to be the most vulnerable group for vitamin A deficiency. The marginal vitamin A deficiency is even more difficult to assess than the clear-cut cases of hypovitaminosis A. It is, therefore, recommended by the International Vitamin A Consultative Group (IVACG) that emphasis be placed on the use of multiple indicators including dietary, biochemical (liver and plasma levels) and clinical signs to assess the vitamin A status of a given population, when determining the risk of hypovitaminosis A (31). A summary of the suggested guidelines of IVACG (31) for the assessment of relative levels of vitamin A nutrition in young children is presented in Table 4. Emphasis should be given to the assessment of overall nutritional status rather than just the biochemical and/or clinical assessments. Dietary, anthropometric (especially growth measurements) and other public health informations provide supportive evidences to biochemical and clinical observations for the accurate diagnosis of the problems of vitamin A deficiency in a given community.

B. *Experience in Southern Brazil*

We have recently carried out a comprehensive study to assess the vitamin A status of marginally malnourished preschool children of socioeconomically deprived families living in the periurban communities of Ribeirão Preto, a typical agricultural town in the sugar cane and coffee region of the State of São Paulo in Southern Brazil (9). The intake of vitamin A and carotenoids from the rice and bean-based diet of these children was found to be low, and appears to influence blood concentrations and liver reserves of this vitamin. With respect to plasma vitamin A, 1.8% of the children had deficient levels (< 10 µg%), whereas 47.2% of the children had low levels (< 20 µg%). Most of the children with low-plasma vitamin A responded positively to a massive dose of 200,000 IU, suggesting thereby that they may be at risk of having low liver stores of the vitamin. Rose Bengal staining test and rapid dark

TABLE 4

SUGGESTED GUIDELINES FOR ASSESSMENT OF RELATIVE LEVELS OF VITAMIN NUTRITION IN YOUNG CHILDREN*

Vitamin A status	Dietary intake (retinol equivalent μg/day)	Biochemical indicators		Clinical signs
		Liver (μg/g)	Plasma (μg/dl)	
Adequate	> 400	> 20	> 20	None
Marginal	200-400	10 - 20	10 - 20	Decreased growth Decreased appetite Decreased resistence to infection
Critical	< 200	< 20	< 10	Xerophthalmia
Clinical deficiency				Night blindness Conjunctival xerosis Corneal xerosis Corneal ulceration Keratomalacia

* According to IVACG Report (31).

adaptation time did not indicate definite signs of conjunctival xerosis and night blindness. Not a single case of xerophthalmia was observed among them. The results of our study clearly indicate that although there are no severe cases of hypovitaminosis A in this population, vitamin A deficiency could be a public health problem among young children in this region of Southern Brazil.

With respect to the application of presently available methods for the rapid field diagnosis of hypovitaminosis A in marginally malnourished preschool children of socioeconomically deprived families in Southern Brazil, we have avaluated Rose Bengal staining test and rapid dark adaptation test (20). The results of our study indicate that neither the Rose Bengal staining test nor the rapid dark adaptation test were found more useful than the known conventional methods based on the estimation of dietary intake of vitamin A equivalent, and plasma levels of retinol. These tests were difficult to interpret, since no significant correlations were obtained between the results of these tests and either the dietary intake of retinol equivalent or plasma levels of retinol (Figure 3). Rose Bengal staining test gave 6 to 7% of the false positive and equal

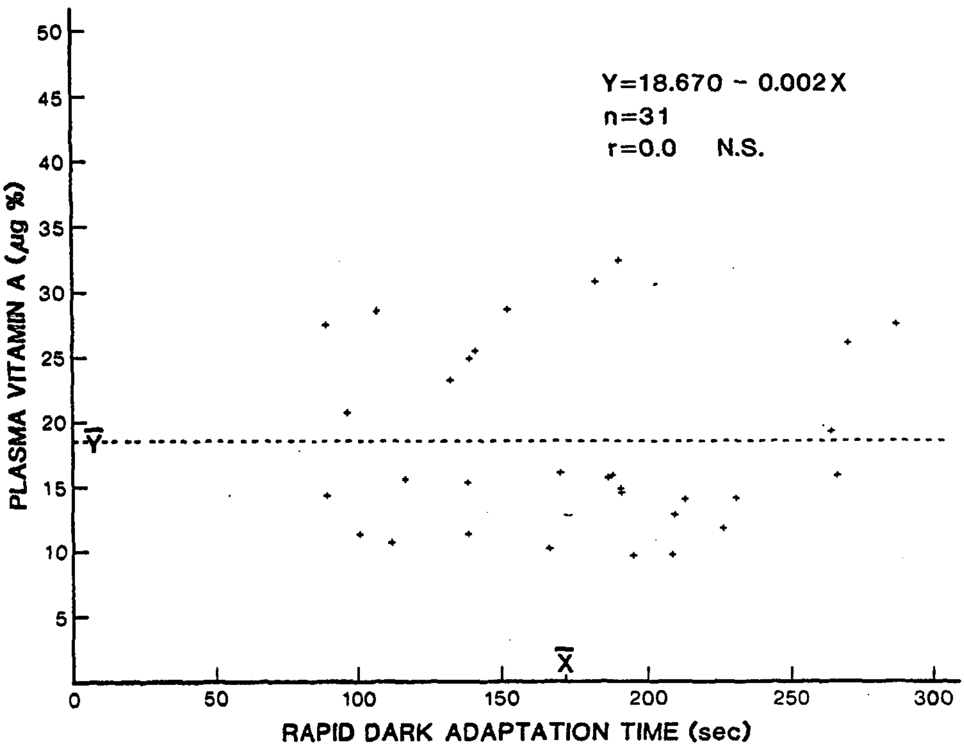

FIGURE 3

Relationship between plasma vitamin A and dark adaptation test of preschool children in Ribeirao Preto, Brazil

number of false negative results. It appears to us that further research is needed to make these tests more reliable and useful for the early detection of vitamin A deficiency. At present we are conducting trials to test the usefulness of the relative dose response test (RDR), and future trials are in planning to test the conjunctival impression cytology as a field method for the early diagnosis of vitamin A deficiency in marginally malnourished preschool children of Southern Brazil.

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RESUMEN

La hipovitaminosis A es un problema que se presenta en gran escala en el mundo entero, especialmente entre los niños de edad preescolar.

De acuerdo a predicciones de la Organización Mundial de la Salud (OMS), alrededor de 100,000 niños pierden la vista anualmente mientras que un número considerable muere debido a deficiencia de vitamina A, y millones sufren serias consecuencias relacionadas con la deficiencia de vitamina A, tales como retardo en crecimiento y creciente susceptibilidad a las infecciones. Consecuentemente, no sólo es importante que los casos de deficiencia severa de vitamina A sean diagnosticados, sino también los casos marginales. Sobre todo, es crítico que la población susceptible sea diagnosticada lo antes posible, a fin de aplicar las medidas de prevención del caso. Los métodos disponibles para diagnosticar la deficiencia de vitamina A pueden ser clasificados en cuatro categorías: clínico, bioquímico, funcional y dietético. El diagnóstico clínico se basa en el examen ocular y extraocular para determinar los síntomas de hipovitaminosis A, pero sólo se utiliza para detectar casos severos. Otras dos pruebas de diagnóstico usadas comúnmente son: el test funcional de ceguera nocturna, y las pruebas patológicas, utilizando o no "Rosa de Bengala" o "dissamine green dye".

Los métodos dietéticos para diagnosticar la deficiencia de vitamina A están fundados en la estimación de absorbancia de vitamina A y carotenoides. Además, existen otros métodos tales como dilución isotópica y reacción a dosis relativa (RDR), técnicas éstas que han sido recientemente propuestas para diagnosticar la deficiencia de dicha vitamina. La RDR parece ser un indicador sensitivo de vitamina A entre grupos sin signos clínicos de hipovitaminosis A. No obstante, el mejor enfoque puede ser el basado en indicadores múltiples para el asesoramiento acertado de la función de vitamina A en la comunidad.

El objetivo del trabajo que nos ocupa es llamar la atención a los problemas de diagnóstico, a los recientes adelantos, y a nuestra experiencia en Brasil en este importante campo de investigación de la vitamina A, investigación de implicaciones globales significativas.

BIBLIOGRAPHY

1. WHO. Serie de Rapports Techniques Nº 672, Geneve, 1982.
2. Santos, L.M.P., J.M. Dricot, L.S. Asciutti, M.J.C.N. Benigna, & C. Dricot d'Ans. Estudo de Paraiba. **Rev. Bras. Med.,** 40: 419-425, 1983.
3. Araujo, R.L., M. Beatriz, D.G. Araujo, R.O. Sleino, R.D.P. Machado & B.V. Leite. Diagnostico da situação da hipovitaminose A e da anemia nutricional na população do vale de Jequitinhonha, Minas Gerais, Brasil. **Arch. Latinoamer. Nutr.,** 36: 643-653, 1986.
4. Araujo, R.L., E.L. Borges, A.M. Santos, D.J.B. Silva & L.M. Oliveira. Situação nu-

tricional da população da cidade de Belo Horizonte. **Cien. Cul.**, **29** (Suppl. 7): 768, 1977.

5. Rocada, M.J., D. Wilson, A.L. Netto, O.B. Netto, A.C. Kalil, M.F. Nunes & E.T. Okani. Hipovitaminose A em filhos de migrantes de São Paulo, Brasil. Estudo clinico-bioquimico. **Rev. Saude-publ. São Paulo, 12**: 345-350, 1978.
6. Rocada, M.J., D. Wilson, R.N. Mazzilli & Y.R. Gandra. Hipovitaminose A em communidades do estado de São Paulo, Brasil. **Rev. Saude-publ. São Paulo, 15**: 338-349, 1981.
7. Rocada, M.J. Hypovitaminosis A: A public health problem. **Internat. J. Vitamin. Nutr. Res.**, (Suppl. 24): 17-22, 1983.
8. Rocada, M.J., D. Wilson, E.T. Okani, & S. Amino. Prevalencia de hipovitaminose A em pre-escolares de municipio da area metropolitana de São Paulo, Brasil. **Rev. Saude-publ. São Paulo, 18**: 218-224, 1984.
9. Favaro, R.M.D., N.V. Souza, S.A.M. Batista, M.G.C. Ferriani, I.D. Desai & J.E. Dutra de Oliveira. Vitamin A status of young children in Southern Brazil. **Am. J. Clin. Nutr., 43**: 852-858, 1986.
10. IVAGG Report: **The Symptoms and Signs of Vitamin A Deficiency and Their Relationship to Applied Nutrition.** New York, N.Y., The Nutrition Foundation, 1981.
11. Wilson, D. & M.E. Silva Nery. Hypovitaminosis A in Rio Grande do Sul, Brazil. Preliminary study. Internat. **J. Vitamin Nutr. Res.**, (Suppl. 24): 35-43, 1983.
12. Wilson, D., M.J. Rocada, R.N. Mazzilli, M.L.F. Cavalvanti & D.B.G. Pattoli. Nutritional status of children, inmates of a small institution for homeless children in the capital of the State of São Paulo, Brazil. **Rev. Saude-publ. São Paulo, 14**: 300-309, 1980.
13. ICNND Report. **Manual for Nutrition Surveys.** Washington, D.C., U.S. Government Printing Office, 1983.
14. Olson, J.A. Evaluation of vitamin A status in children. **Wld. Rev. Nutr. Diet, 31**: 130-134, 1978.
15. Olson, J.A. Liver vitamin A reserves of neonates, preschool children and adults dying of various causes in Salvador, Brazil. **Arch. Latinoamer. Nutr., 29**: 521-545, 1979.
16. Flores, H., C.R.C. Araujo, F.A.C.S. Campos & B.A. Underwood. Importance of the early diagnosis of vitamin A deficiency at the epidemiological level. **Int. J. Vitamin. Nutr. Res.**, (Suppl. 24): 23-24, 1983.
17. Dorea, J.G., J.A. Souza, M.O. Galvao & M.A.F. Iunes. Concentration of vitamin A in the liver of foetuses and infants dying of various causes in Brasilia, Brazil. **Internat. J. Vitamin. Nutr. Res., 54**: 119-123, 1984.
18. Thornton, S.P. A rapid test for dark adaptation. **Ann. Opthalmol., 9**: 731-735, 1977.
19. Vinton, N.E. & R.M. Russell. Evaluation of a rapid test of dark adaptation. **Am. J. Clin. Nutr., 34**: 1961-1966, 1981.
20. Favaro, R.M.D., N.V. Souza, H. Vannucchi, I.D. Desai & J.E. Dutra de Oliveira. Evaluation of rose bengal staining test and rapid dark adaptation test for the field assessment of vitamin A status of preschool children in Southern Brazil. **Am. J. Clin. Nutr., 43**: 940-945, 1986.
21. Solomons, N.W., R.M. Russell, E. Vinton, A.M. Guerrero & L. Mejía. Application of a rapid dark-dadaptation test in children. **J. Pediatr. Gastroenterol. Nutr., 1**, 571-574, 1982.
22. Mobarhan, S., R.M. Russell, B. Underwood, J. Wallingford, R.D. Mathieson & H. Al-Midani. Evaluation of the relative dose response test for vitamin A nutriture in

cirrhotics. **Am. J. Clin. Nutr., 34**: 2264-2270, 1981.
23. Amedee-Manesme, O., D. Anderson & J.A. Olvon. Relation of the relative dose response to liver concentrations of vitamin A in generally well-nourished surgical patients. **Am. J. Clin. Nutr., 39**: 898-902, 1984.
24. Flores, H., F. Campos, C.R.C. Araujo & B.A. Underwood. Assessment of marginal vitamin A deficiency in Brazilian children using the relative dose response procedure. **Am. J. Clin. Nutr., 40**: 1281-1289, 1984.
25. Rietz, P., O. Wiss & F. Weber. Metabolism of vitamin A and the determination of vitamin A status. **Vitamins Horm., 32**: 237, 1974.
26. Huque, T. Assessment of vitamin A status by an isotope dilution method. **Internat. J. Vit. Nutr. Res., 51**: 119-123, 1981.
27. Whittpen, J.R., M.D. Scheffer, C.G. Tseng, & A. Sommer. Detection of early xerophthalmia by impression cytology. **Arch. Ophthalmol., 104**: 237-239, 1986.
28. Tseng, S.C. Staging of conjunctival squamous metaplasia by impression cytology. **Ophthalmology, 92**: 728-733, 1985.
29. Kjolhede, C.L., A.M. Gadomski, J. Whittpen, J. Bulux, A. Rosas, N.W. Solomons, K.H. Brown & M.R. Forman. Conjunctival impression cytology: Feasibility of a field trial to detect subclinical vitamin A deficiency. **Am. J. Clin. Nutr., 49**: 490-494, 1989.
30. Sauberlich, H.E., R.E. Hodges, D.L. Wallace, H. Kolder, J.R. Canham, J. Hood, N. Raica & L.K. Lowry. Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. **Vitamins Horm., 32**: 251-275, 1974.
31. IVACG Report. **Recent Advances in the Metabolism and Function of Vitamin A and Their Relationship to Applied Nutrition**. New York, N.Y., The Nutrition Foundation, 1979.