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Original articles

Screening tests for vitamin A deficiency

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Objective tests for detection of xerophthalmia can solve the diagnostic problems of vitamin A deficiency under field conditions. Reliability of Rose Bengal dye test and Kajal test were tested in rural field practice area of B.J. Medical College, Pune, by finding out their correlation with prevalence of clinically detectable signs of vitamin A deficiency and serum vitamin A levels in 392 primary school children. The combination of these objective tests with clinical diagnosis may improve the detection rate of xerophthalmia.

Key words : Vitamin A; Screening tests; Xerophthalmia; Rose Bengal dye test; Kajal test.

Blindness due to vitamin A deficiency is one of the major public health problems in India. As a prophylactic measure six monthly dose of vitamin A is administered to all preschool children irrespective of their vitamin A nutritional status.[1] Theoretically these massive doses can produce hypervitaminosis A if administered to children with good vitamin A nutritional status. On the other hand, more frequent administration of massive doses to children with detectable deficiency signs may be needed. Diagnosis of vitamin A deficiency would be facilitated if objective tests are available under field conditions which can be used easily by less trained workers.

Material and Methods

Primary school children from villages Karda and Jawala served by rural training centre Sirur were selected to study vitamin A deficiency after undertaking a pilot project to judge the extent of the problem. Three hundred and ninety two children in the age range of 5 to 13 years were included in the study, 248 (63·3%) being boys and 144 (36·7%) girls (Table I). Most of these children were from lower socio-economic group and consumed jowar as a staple food. Green leafy vegetables were occasionally included in their diet and milk and milk products were scarcely consumed beyond infancy.

Information about night blindness was collected from each child. Clinical diagnosis of xerophthalmia was based on guidelines given by WHO.[2] Presence of pallor of conjunctiva, tongue and nails was taken as anemia. Protein energy malnutrition was detected by weight for age method using 80% as dermarcating line using standard of reference of ICMR.[3] Angular stomatitis, chelosis, atrophy of papillae of tongue, glossitis and pellagrous

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Table 1. Age and sex distribution of the study group

Age in years	Male		Female		Total	
	No.	%	No.	%	No.	%
5	1	·40	—	—	1	·26
6	41	16·53	33	22·92	74	18·88
7	35	14·11	30	20·83	65	16·58
8	46	18·55	20	13·89	66	16·84
9	39	15·73	11	7·64	50	12·76
10	48	19·35	25	17·36	73	18·62
11	34	13·71	24	16·67	58	14·80
12	2	·81	1	·69	3	·77
13	2	·81	0	—	2	·51
Total	248	100·0	144	100·0	392	100·0

dematosis either alone or in combination was diagnosed as vitamin B deficiency.

Vitamin C deficiency was detected by screening the children for spongy bleeding gums, scorbutic rosary, patechial hemorrhages or subperiosteal hematoma.

One drop of freshly prepared 1% Rose Bengal dye was instilled in both the eyes and observation was made one minute later for clear cut and distinct patches visible from a distance of about one meter. All children showing such pink patches were considered positive for conjunctival xerosis.[4]

Observation after application of Kajal was made for Kajal test. Detection of black spots in conjunctiva was taken as positive test for presence of Bitot's spots.

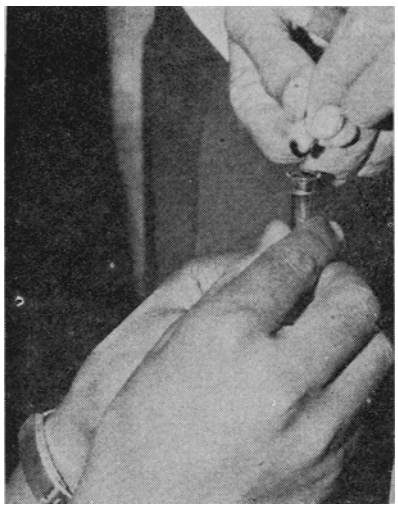
Opthalmological examination was carried out by the ophthalmologist and clinical assessment of nutritional deficiency signs was done by trained interns.

A bold prick was given with a sterile, sharp aluminium lancet and one ml of blood was thus collected by finger prick method in a sterile Drayer's tube (Fig. 1a & b). Serum was separated and vitamin A levels were estimated by microfluorimetric method.[5] All the samples were protected from sunlight by covering each tube by black paper and frozen in a deep freezer. The estimation was carried out within a week of collection of samples as per recommendations of WHO expert group.[6] As serum vitamin A levels in individual cases do not always correlate with clinical diagnosis,[2] comparison of results of RBD test and Kajal test was made with both clinical diagnosis and serum vitamin A levels.

Results

Signs and symptoms of vitamin A deficiency were observed in 219 (55·9%) of the total 392 children. Conjunctival xerosis was the commonest observed sign either alone or in combination with Bitot's spots (Table II). It was observed that Rose Bengal dye test was positive in 211 (53·8%) children and Kajal test was positive in 45 (11·5%) children.

The results of clinically detected conjunctival xerosis and Rose Bengal dye



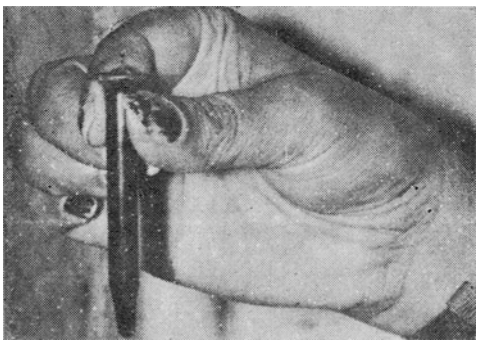


Fig 1 a & b. Collection of blood from finger prick

Table II. Distribution of various types of nutritional deficiencies

Type	No.	%
PEM	242	61·73
Vitamin A deficiency	219	55·87
B complex deficiency	69	17·60
Anaemia	44	11·22
Vitamin C deficiency	5	1·28

Total number of children examined were 392

test (Table III) were in agreement in 226 (57·7%) cases. Relation of conjunctival xerosis was found out with serum vitamin A levels (Table IV). It was observed that group showing positive test by Rose Bengal dye had slightly lower mean serum vitamin A level than those diagnosed clinically. Both these groups had lower mean serum vitamin A level than the group of students in whom conjunctival xerosis was not detected by both the methods. However, none of these differences were statistically significant.

Sensitivity and specificity of clinical diagnosis of conjunctival xerosis and Rose Bengal dye test as well as Bitot's spots were found out by taking serum vitamin A levels of <10 μgm% as representing deficiency state.[2]

Out of 392 children studied, serum samples of 318 could be analysed. One hundred forty eight were below 10 μg% showing biochemical vitamin A deficiency. Out of these 85 had clinically detectable conjunctival xerosis and 99 had RBD test positive. Sensitivity*[7] therefore was 85/148=57·5% for clinically detected conjunctival xerosis and 99/148=66·9% for RBD test.

Specificity**[7] was 77/170=45·3% and 78/170=45·8% respectively for clinical detection of Bitot's spots and RBD test.

Bitot's spots were present in 22 and Kajal test was positive in 26 out of 148 children with serum vitamin A levels less than 10 μgm%. The sensitivity for clinical detection of Bitot's spots was 22/148=

$$*\text{Sensitivity} = \frac{\text{Diseased persons with +ve test}}{\text{All persons in the population with the disease}}$$

$$**\text{Specificity} = \frac{\text{Non diseased persons with —ve test}}{\text{All persons in the population without disease}}$$

14·9% and for Kajal test was 26/148=17·6%. Specificity for clinical detection was 165/170=97·1% and for Kajal test was 155=170=91·2%.

Clinical detection of Bitot's spots and Kajal test were in agreement when both positive and negative results were considered in 360 (91·8%) cases (Table IV).

The results of clinical examination, Kajal test were correlated with serum vitamin A levels (Table V).

Discussion

Of all the signs of vitamin A deficiency conjunctival xerosis is an early manifestation of deficiency state. Detection of conjunctival xerosis therefore is valuable for early diagnosis of vitamin A deficiency. On one hand over diagnosis of conjunctival xerosis, based on presence of a few and minimal changes is a real problem and on the other hand the condition may be easily missed by an inexpreienced investigator. To overcome these diagnostic difficulties possibility of an objective test is considered.[8] Vital dyes like Rose Bengal and Lissamine green selectively stain and degenerate dead cells but cannot penetrate the normal living cells.[4]

Vital staining of conjunctiva and cornea has been introduced by Norn in 1972.[4] Applicability of the techniques has been claimed by Sauter[9] and K. Vijayraghavan.[10] They found the test useful for detection of conjunctival xerosis.

Doubts about the sensitivity and specificity of vital stains has been expressed by Whitfield and Dekker[11] and also by Kusin.[12] In the present study mean serum vitamin A levels of children with RBD test positive are lower than those with presence of xerosis on clinical grounds as well as those without xerosis by both methods. These differences however are

Table III. Clinical diagnosis of conjunctival xerosis and Rose Bengal dye test

Rose Bengal dye test	Clinically detected conjunctival xerosis		Total
	+ve	—ve	
+ve	139	94	233
—ve	72	87	159
Total	211	181	392

Table IV. Mean serum vitamin A levels and conjunctival xerosis

Category	No.	Serum samples analysed	X serum vitamin A level in μgm %	S.D.
Clinically diagnosed conjunctival xerosis	211	179	11·2	4·8
RBD test +ve	233	191	10·8	4·6
Conjunctival xerosis absent by both methods	87	63	11·9	4·8
Conjunctival xerosis positive by both methods	139	115	10·6	4·3

Table V. Clinical diagnosis of Bitot's spot and kajal test

Kajal test	Bitot's spot		Total
	+ve	—ve	
+ve	21	24	45
—ve	8	339	347
Total	29	363	392

not statistically significant, indicating that in addition to vitamin A deficiency state some other factors might be responsible for development of conjunctival dryness. For an experienced observer thus RBD test does not appear to have any additional benefit, though it may be of value for less trained workers under field conditions where clinical diagnosis of conjunctival xerosis may not be possible but positive RBD test could be read by training and practice.

Detection of Bitot's spot when fully developed does not pose much of a problem, but at an early stage could be missed very easily. Use of another objective test using an oil bound preparation, commonly known as 'Kajal', is therefore considered.

It is stated that Bitot's spots take up black stain following application of maskera.[3] It was thought that application of Kajal to the eyes of children, which is a traditionally accepted custom in Indian community, can be made use of in early detection of Bitot's spots.

Though the Bitot's spot does not take up the aquaous Rose Bengal dye, it does take up Kajal making the spot look black against white conjunctiva which does not take up the black stain. This contrasting black spot can be easily detected even by ordinary field workers. It was interesting to observe high sensitivity of Kajal test for early lesions and a low sensitivity for late lesions. Detection of Bitot's spot was possible in 24 children by using Kajal test in present study which were missed clinically. This group of 24 students had a slightly higher value of serum vitamin A level of 11·8 rg% as compared to apparently normal group of 11·6 rg %. This difference in their mean serum vitamin A level was not statistically significant. Both these groups however had a markedly low level of mean serum vitamin A as against 20 to 25 mgm% as stated for normal children.[3] It thus appears that both these groups represented biochemical vitamin A deficiency with early Bitot's spots in 24 and no eye lesions at all in 339 children (Table VI).

Eight cases clinically showing clearcut Bitot's spots were missed by Kajal test. The silver gray, foamy patch of early Bitot's spots is superficial and can be readily removed by manipulation of lids or direct wiping revealing rough conjunctival surface.[3] On the other hand thick plaques of debris of more extensive and long standing Bitot's spot are adherent. The debris from less marked lesions might have been removed easily while application of Kajal was made. Penetration of Kajal might have been a problem in severe adherent lesions. No attempt was made in the present study to forcefully remove the plaques. This could explain the negative results of Kajal test in these eight cases.

Though this is a limitation of Kajal test, it does not appear to be a serious problem. The extensive clear cut spots are so well marked that they can be diagnosed very easily with some training even without contrasting black colour.

Table VI. Correlation of serum vitamin 'A' levels (μgm%) with diagnosis of Bitot's spot by clinical method and by Kajal test

	No.	Samples analysed	X gm%	S.D. gm%	Value*	Statistical significance
Group I Clinically Bitot's spot present Kajal test positive	21	20	8·75	2·8	$t_{288}=2·51$	<0·05
Group II Clinically Bitot's spot present Kajal test negative	8	7	7·5	2·9	$t_{275}=2·2$	<0·05
Group III Clinically Bitot's spot absent Kajal test positive	24	21	11·8	5·5	$t_{289}=0·18$	<0·05
Group IV Clinically -ve negative Kajal test negative	339	270	11·6	5·0		Reference category

*Unpaired 't' test.

This test therefore when used in combination with clinical detection appears to be a very useful diagnostic tool in the hand of less trained workers.

Clinically diagnosed cases of Bitot's spots with negative Kajal test show lowest mean serum vitamin A level. This finding is in support of the hypothesis put forward earlier that these cases represent more severe and extensive Bitot's spots.

Clinical diagnosis of xerophthalmia though simple, involves observer error and hence requires considerable training. Biochemical assessment though more reliable is difficult requiring sophisticated elaborate technique in addition to resistance of the population for collection of blood sample. Screening test may bridge the gap between these two by obviating observer error and being simple at the same time.

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ACUPUNCTURE AND ASTHMA

Exercise-induced asthma (EIA) is a simple and reproducible model of asthma, and therefore is valuable in examining anti-asthma medication. In 1983, Chow et al found that auricular acupuncture had no effect on basal bronchomotor tone, and did not significantly reduce EIA, although the fall in FEV_1 was slightly lessened. Now Dr. Fung and his colleagues, in a controlled, single, blind, prospective study of traditional acupuncture, reach the opposite conclusion. They studied 19 children with a history of EIA and at least a 20% improvement in FEV_1 after salbutamol aerosol. Acupuncture had no effect on resting bronchomotor tone as judged by spirometry. However, a 49·5% fall in the control peak expiratory flow rate was reduced to 25·9% after real acupuncture, and to 34·3% following the sham treatment. Comparable changes occurred in FEV. Acupuncture is a poor bronchodilator but may reduce EIA. This effect is small in comparison with beta-2 agonists, making its role in asthma therapy limited.

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