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Different methods of occlusion



EDITORIAL

Amblyopia, yes that age-old problem of the young, has received a lot of attention recently. The perspective on amblyopia treatment study summarizes and gives the essence. Na K ATPase, an important part of many physiological processes gets a closer look in the Laboratory sciences article. Vitamin A analysis for Vitamin A deficiency is looked into by a novel method, in a pilot study. An interesting case report on Frosted Branch angiitis with review of literature is presented. Occupational optometry, an essential and field of the future is introduced in a lucid fashion. A muscle puzzle that sets you thinking completes the issue.

Dr S Meenakshi

Editor



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Perspective:

Amblyopia Treatment Study: Where do we go from here?

S. Meenakshi, Sankara Nethralaya ORBIS Pediatric Ophthalmology Learning and Training Centre

Introduction:

Amblyopia has traditionally been defined as a decrease in visual acuity caused by pattern vision deprivation or abnormal binocular interaction for which no causes can be detected by the physical examination of the eye and which in appropriate cases is reversible by therapeutic measures. Amblyopia is said to afflict **1-4%** of population, this number based on large population studies. The rate is even higher in medically underserved populations.

Amblyopia has traditionally been treated by occlusion and penalization with a few studies suggesting medical treatment options. While the effectiveness of both occlusion and penalization had been proven by several studies, individual practice variability in the dose of occlusion, or even the definition of part time and full time occlusion prompted the Pediatric Eye Disease Investigator group to study amblyopia as one of the areas of interest and research.

What is PEDIG?

The **Pediatric Eye Disease Investigator Group (PEDIG)** is a collaborative network dedicated to facilitating multicenter clinical research in strabismus, amblyopia and other eye disorders that affect children. The network, which was formed in 1997, is funded by the **National Eye Institute (NEI)**. The NEI is a part of the National Institutes of Health, which is the branch of government that funds medical research. There are currently over 60 participating sites (offices) with over 120 pediatric ophthalmologists and pediatric optometrists in the United States and Canada participating in the network.

The multi center randomized controlled studies to answer several questions on amblyopia are collectively and popularly known as the **Amblyopia Treatment Study (ATS)**.

What is the Amblyopia Treatment Study (ATS) ?

The ATS addressed questions regarding both moderate and severe amblyopia. The therapeutic regimens compared either atropine and patching or different amounts of patching. The studies addressed children in the 3 to 7 years age group. However a separate study addressed amblyopia in children in 7 to <17 year olds as well.

Like other multicenter trials with visual acuity as an important treatment outcome, the ATS established and published a new protocol for visual acuity testing during the study.

The protocol used isolated surrounded **HOTV optotypes** (Figure 1). The protocol was evaluated using the **Baylor Video Acuity tester (BVAT)**. The protocol had a high level of testability in 3 to 7 year olds and excellent test-retest reliability.

20/200



Figure 1

Answers to questions:

The following are several questions addressed and answered by the ATS:

1. To compare 6 hours versus full time daily patching for severe amblyopia defined as visual acuity in the 20/100 to 20/400 range in less than 7 year olds. 175 children were studied. The patching was combined with one hour of near visual tasks.

Visual acuity in the amblyopic eye improved a similar amount in both groups. The improvement in the amblyopic eye acuity from baseline to four months averaged 4.8 lines in the six-hour group and 4.7 lines in the full-time group ($P=0.45$). The mean difference between groups in **log MAR acuity** adjusted for baseline acuity = -0.03 , 95% confidence interval, -0.11 to 0.05 .

The study concluded that for severe amblyopia, prescribing six hours of daily patching produces an improvement in visual acuity that is of similar magnitude to the improvement produced by prescribing full-time daily patching in children 3 to less than 7 years of age.

2. To compare moderate amblyopes with visual acuity in the 20/40 to 20/80 range, also in the less than 7 years age group who receive 2 hours versus 6 hours, both treatments combined with one hour of near visual tasks. 189 children were enrolled of who 181 completed 4 months follow up. Visual acuity in the amblyopic eye improved a similar amount in both groups. The improvement in the amblyopic eye acuity from baseline to 4 months averaged 2.40 lines in each group ($P=0.98$). The mean difference in log MAR acuity between groups = 0.001 , 95% confidence interval, -0.040 to 0.042 .

The study concluded that for moderate amblyopia in the studied age group two hours of patching was as effective as six hours.

3. To compare atropine and patching treatments for moderate amblyopia with visual acuity in the amblyopic eye $\leq 20/40$ to $\geq 20/100$, intereye difference ≥ 3 log MAR lines. The patients were required to have worn their refractive correction for 4 weeks prior to enrollment. Four hundred and nineteen patients were enrolled.

The initial number of patching hours was prescribed based on an investigator's usual practice with minimum of 6 hours per day prescribed. After 5 weeks of treatment patients prescribed 10 or more hours of patching per day had greater improvement in visual acuity compared with atropine or patients with 6 to 8 hours of treatment.

At six months, visual acuity was improved from baseline by about 3 lines of vision in both the atropine and patching groups. Improvement initially was faster in the patching group, but after six months, the difference in acuity between treatment groups was small. The mean visual acuity (Snellen approximation) at six months was 20/32 in the patching group and 20/32⁻² in the atropine group. This small difference between groups was considered clinically inconsequential.

Both treatments were well tolerated, although atropine had a slightly higher degree of acceptability on a parental questionnaire. More patients in the atropine group than in the patching group had reduced acuity in the sound eye at six months but this did not persist with further follow up.

The conclusion is that both atropine and patching are effective treatments for moderate amblyopia in children in the age range of 3 to less than 7 years old. Patching has the potential advantage of a more rapid improvement in visual acuity and possibly a slightly better acuity outcome, whereas atropine has the potential advantage of easier administration and lower cost. There appeared to be a transient decrease in vision in the sound eye in the atropine treated patients but this was found to resolve.

4. The next question answered was on **amblyopia recurrence after cessation of treatment**. The study enrolled 156 children with successfully treated anisometropic or strabismic amblyopia who were younger than 8 years of age and who had received continuous amblyopia treatment for previous 3 months and who had improved at least 3 log MAR levels during the period of continuous treatment.

The study found that approximately one fourth of successfully treated amblyopic children experience a recurrence within the first year off treatment. For patients treated with six or more hours of daily patching a weaning to two hours prior to cessation reduced recurrence.

5. Evaluation of **amblyopia treatment in the 7 to <17 year olds** was the next issue. The aims were to study the effectiveness of amblyopia therapy in the older amblyopes and the rate of recurrence after cessation of therapy. The study concluded that for patients 7 to <13 years old, prescribing 2 to 6 hours per day of patching with near activities and atropine can improve visual acuity even if the amblyopia has been previously treated. For patients 13 to <18 years old, prescribing patching 2 to 6 hours per day with near activities may improve visual acuity when amblyopia has not been previously treated but appears to be of little benefit if amblyopia was previously treated with patching.

The studies also found that atropine was psychologically better tolerated.

Unanswered questions:

There are however several unanswered questions concerning amblyopia, which the ATS did not clearly address or have indicated that they need further study. Some of theirs and some of ours are as follows:

1. The nature of near activity proposed as part of the treatment regimen is unclear. The impact of the same on the outcome is

also unclear. This was also not studied by the ATS.

2. The regimen for tapering of occlusion is an area in need of further study.
3. Weaning off atropine to prevent recurrence of amblyopia is an area that needs further looking into.
4. Will there be ethnic variation in the response to treatment? Can these results be generalized to other countries?
5. Is atropine safe in tropical countries with more sunshine throughout the day where children may not be able to afford protective sunglasses?
6. **Is there a longer period of refractive adaptation in many children, which need to be studied separately before instituting any amblyopia therapy?**
7. **In other causes of amblyopia such as deprivational, can these results be extrapolated?**

Further studies are needed both by the PEDIG and other similar groups of pediatric ophthalmologists in other parts of the world.

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SODIUM POTASSIUM ATPase

Sodium Potassium Pump; (Na⁺ - K⁺) pump

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It was in 1957, Jens Skou found that plasma membranes of higher eukaryotes contained the enzyme sodium potassium ATPase (Na⁺ - K⁺) - ATPase. It is a protein of molecular weight 330^x kD with two α and two β sub units as (αβ)₂ tetramer. The two large α subunits are identical, each with a molecular weight of 110[@] kD and not glycosylated. They traverse the plasma membrane with eight transmembrane α helical segments and two large cytoplasmic domains. The α subunits have the catalytic activity and ion-binding sites. The two β subunits are also identical, each with a molecular weight of 55[^] kDa. Each β subunit has a single transmembrane helix and a large extracellular domain with carbohydrate moieties on their extra cellular domain. The function of β subunits is not known.

Cross links are possible between α and α or α and β but not between β and β, suggesting that the α units are close to one another while the β units are far apart (figure 1).

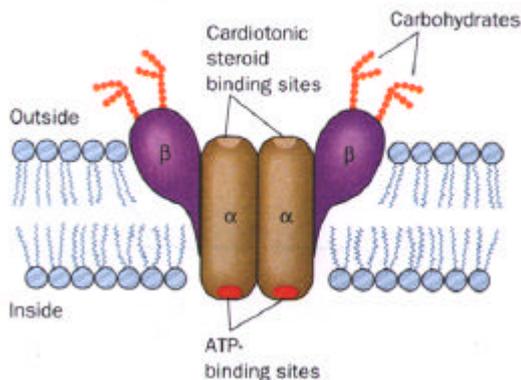


Figure 1:
Na⁺ and K⁺ ATPase - position and composition (Courtesy: Voet et al Fundamentals of Biochemistry)

Each large α subunit contains ATP binding site for the hydrolysis of ATP and a site each for the binding of cardiotonic steroid inhibitors like digitogenin and ouabain. ATP binding site is on the cytosol side of the membrane while the steroid - inhibitor site is on the extracellular space.

The enzyme complex as a whole has one binding site for phosphorylation, three sites for Na⁺ located possibly at the interfaces of the α subunits and one binding site for steroid inhibitor.

x 270 kD
@ 95 kD Earlier report
Δ 40 kD

The enzyme has sulphhydryl groups, which could undergo oxidation to disulphide bridges even under physiological conditions. Glutathione brings it back to its thiol-containing structure.



Function of (Na⁺ - K⁺) ATPase; the (Na⁺ - K⁺) Pump:

The enzyme is omnipresent in the body in biomembranes and is a component of a specific transport system called sodium - potassium pump (Na⁺ - K⁺) pump, which helps in the maintenance of ionic gradients of Na⁺ - K⁺ occurring between the inside and the outside of the cells. While the inside of the cells has a concentration of K⁺ as high as 140 mM, the extra cellular fluid (ECF) has only 4 mM. Thus K⁺ is mostly intracellular. On the other hand, intracellular

why there is (Na⁺ - K⁺) pump only in the epithelium of the lens which section alone possesses mitochondria and produces significant amounts of ATP needed for the operation of the pump. Rest of the lens has no mitochondria and there is no TCA cycle except in lens epithelial cells.

Likewise corneal deturgescence necessary for the passage of light through its stroma will be disturbed by the accumulation of Na⁺ followed by water in the stroma. This is prevented by the corneal endothelial (Na⁺ - K⁺) ATPase. Infact, there are five endothelial pumps including (Na⁺ - K⁺) pump in the cornea. Sodium and through it, water are evicted and corneal transparency is maintained.

During action potential of cells, there is an influx of Na⁺ into the cells and thereby reversal of polarization (usually expressed in physiology as depolarization). There will ensue reversal of electrical changes. Convulsions are a result of a large influx of Na⁺. Acetyl choline, Glutamate, etc., which are neuro transmitters facilitate the entry of Na⁺ in the neuronal cells and help in the transmission of nerve impulses along the axon through a wave of electronegativity. But after the action proper physiological or pathological, it is the (Na⁺ - K⁺) pump which sets right the disturbance in the ionic distribution and rehabilitates the cells to their normal status.

Just as in the brain, the excitability of the muscle cells also disturbs the ionic distribution. These cells also owe to (Na⁺ - K⁺) pump for their revival to normalcy.

The resting cells are reported to spend 25 to 33% of ATP synthesized by them for the operation of (Na⁺ - K⁺) pump. Nerve cells go to the extent of spending 70% of ATP for the purpose.

A doubt may arise on the necessity of operation of the pump under normal circumstances. It is necessary because, due to the concentration gradient, Na⁺ ions from higher concentration in ECF would diffuse passively into the cells with a lower

concentration of Na⁺. This would increase the intracellular Na⁺ which has to be corrected. This is done by the (Na⁺ - K⁺) pump.

In the Rod outer segment (ROS) of the retina, there are channels for Na⁺. Only if the channels are open and Na⁺ enters the ROS, there will be depolarization and neurons will sense the visual message and transmit to the brain. Cyclic GMP binds to the channels and opens them up. So they are called "gated" channels (figure 4). Thus, there are many situations in the body when Na⁺ continuously enters the cells and threatens the normal distribution of ions. It is in such cases Na⁺ - K⁺ ATPase has to come to the rescue.

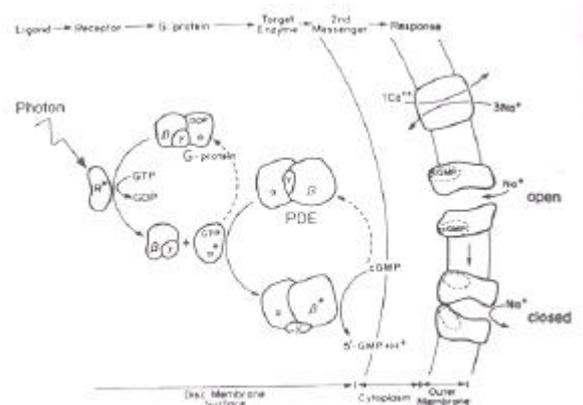


Figure 4: cGMP-gated Na⁺ channel
(Courtesy: Berman's Biochemistry of the eye)

MECHANISM OF ACTION OF (Na⁺ - K⁺) ATP ase

In understanding the mechanism of action of (Na⁺ - K⁺) ATP ase, the following questions come to our mind.

1. How does the enzyme carry Na⁺ ions outwards from inside the cells ?
2. How does the enzyme release Na⁺ ions in ECF ?
3. How does the same enzyme carry K⁺ ions inwards into the cells?
4. How does it release the K⁺ ions inside the cells?

5. How is the enzyme assisted by ATP in donating the energy required for the active transport of ions wherever it is against concentration gradient?

The answers to the above questions are:

1. Conformational changes of the enzyme (E) to E_1 and E_2 .
2. Orientation of high affinity sites of the large sub unit α of E_1 in the intra cellular domain i.e. high affinity sites face inside of the cell.
3. Orientation of high affinity sites of the large sub unit α of E_2 in the extra cellular portion i.e. high affinity sites face the outside of the cell.
4. Phosphorylation at ATP - binding site after binding of 3 Na^+ and in the presence of Mg^{2+} .

Dephosphorylation by hydrolysis after binding of K^+ .

E_1 is stabilized by phosphorylation.

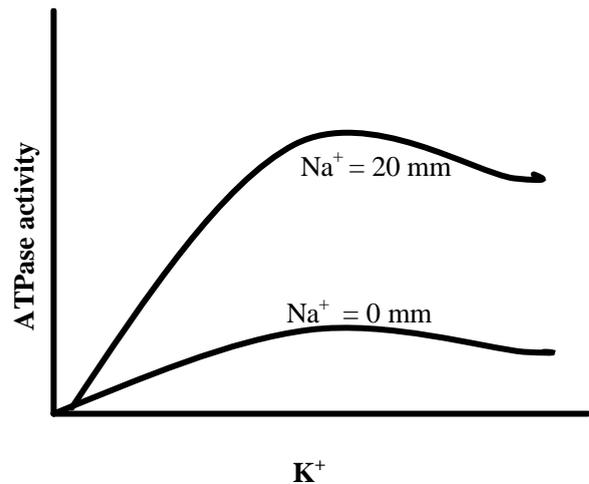
E_2 is stabilized by dephosphorylation

Na^+ triggers phosphorylation

K^+ triggers dephosphorylation

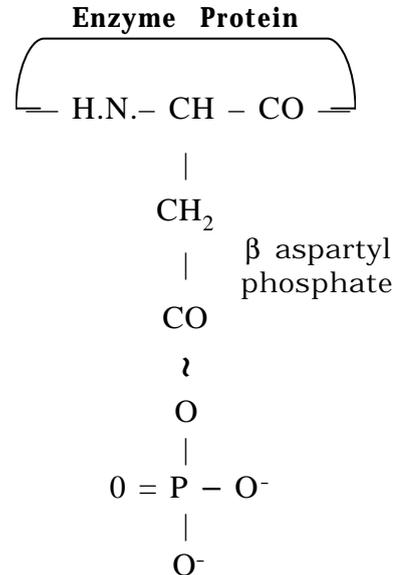
E_1 to E_2 and E_2 to E_1 are by eversion. Ion binding cavity in E_1 faces inside the cell. Ion binding cavity in E_2 faces outside the cell. Before release of bound Na^+ and that of bound K^+ , there are conformational changes of enzyme i.e. E_1 to E_2 and E_2 back to E_1 which are brought about by eversion. Conformational differences need not be large. Shift of a few atoms by a distance of as low as 2\AA might suffice to alter relative affinity of the cavity for Na^+ and K^+ and change its orientation. Covalent modification through phosphorylation can readily produce changes of this magnitude.

It has to be remembered that in the sequence of events described below, hydrolysis of ATP takes place only if Na^+ and K^+ are present.



The sequence of events are as follows.

1. Transporter in the enzyme in its E_1 state of conformation binds three Na^+ ions inside the cell and then binds ATP to yield an $E_1 \cdot ATP \cdot 3 Na^+$ complex.
2. ATP hydrolysis produces ADP with a high-energy aspartyl phosphate intermediate.
 $E_1 \sim P \cdot 3Na^+$



3. This high energy intermediate relaxes to its low energy conformation of the enzyme (E_2) forming $E_2 \sim P \cdot 3Na^+$ and releases the three bound Na^+ ions outside the cell.
4. $E_2 \sim P$ binds to K^+ ions from outside the cell to form $E_2 \sim P \cdot 2K^+$ complex.

- The phosphate is hydrolysed to yield $E_2 \cdot 2K^+$.
- $E_2 \cdot 2K^+$ changes its conformation to E_1 back and releases its two K^+ ions inside the cell.

E_1 can now take up three Na^+ ions to continue the transport cycle. (Figures 5, 6, 7)

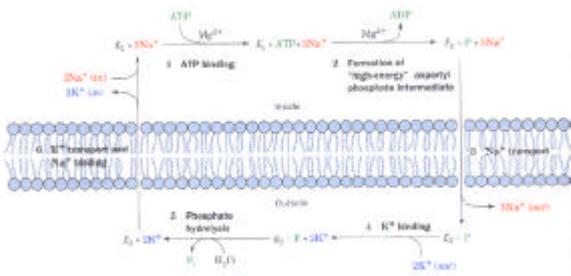


Figure 5:

$Na^+ - K^+$ pump - sequence of events

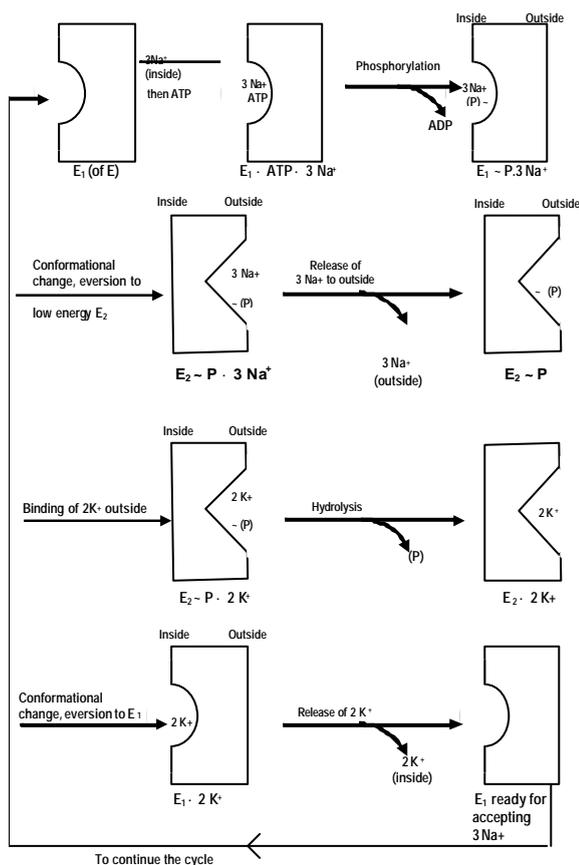


Figure 6: Schematic Diagram Conformations and functions of $Na^+ - K^+$ ATPase

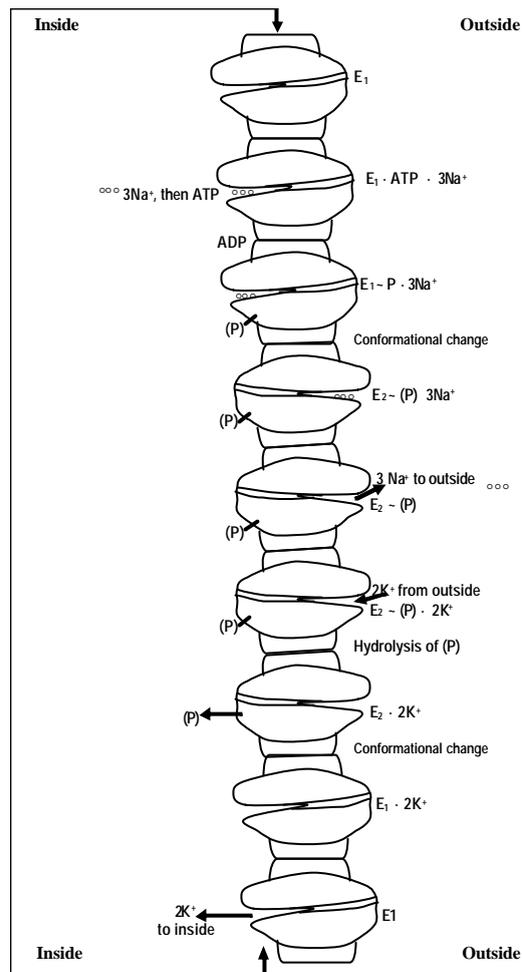


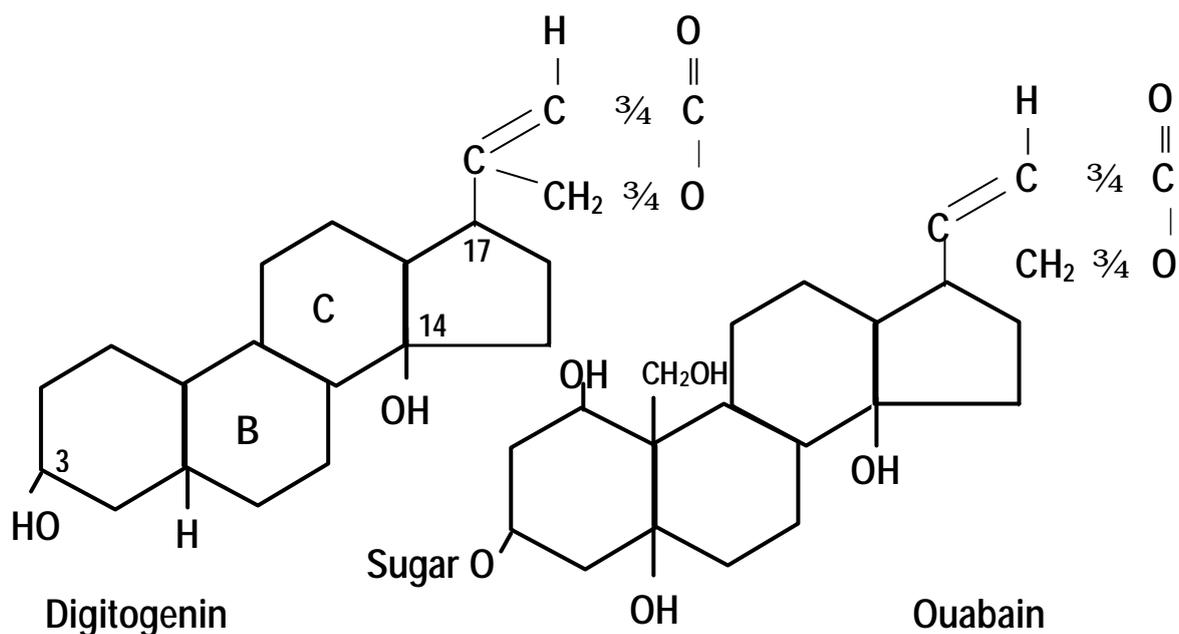
Figure 7: Conformations and functions of $Na^+ - K^+$ ATPase

Though each of the reaction steps is individually reversible, the cycle circulates only in the clockwise direction under normal physiological conditions. This is because, ATP hydrolysis and ion transport are coupled vectorial (unidirectional) processes. The vectorial nature of the reaction cycle results from the alteration of some of the steps of exergonic ATP hydrolysis with some of the steps of the endergonic ion transport process.

Maximal turn-over number of ATPase molecules is about 100 sec^{-1}

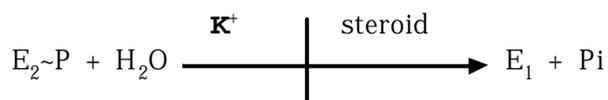
Inhibition of ($Na^+ - K^+$) ATPase

Cardiotonic steroids are specific inhibitors of ($Na^+ - K^+$) ATPase and thereby ($Na^+ - K^+$) pump. Digitogenin and ouabain



are called cardiotonic steroids as they have profound effect on the heart. There is structural similarity of the two in that both of them have 5 or 6 membered unsaturated lactone ring with b configuration at C-17, a hydroxyl group in C-4 and C is fusion of rings C and D. Ouabain has sugar residues at C 3 but these do not contribute to inhibition.

The reaction of inhibition is the hydrolysis of E ~ P



i.e. inhibition at the level of dephosphorylation. An important condition is that the steroids should be available at the outside face of the membrane to enable them to bind to the specific sites in the A subunits of the enzyme.

Digitalis, the extract of the leaves of Foxglove plant contains digitogenin and ouabain.

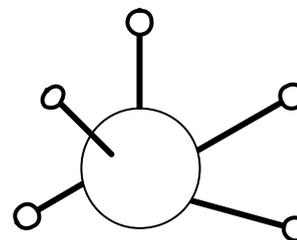
These steroids increase the force of contraction of heart muscle and are the choice drugs in the treatment of congestive heart failure. Inhibition of (Na⁺ - K⁺) pump leads to a higher levels of Na⁺ inside the cell which, in its turn, increases the level of intracellular calcium. Such an increase of intracellular calcium enhances the

contractility of the cardiac muscle.

It is interesting to note that even before the enzyme was found out, William Withering used Foxglove plant extract to treat congestive heart failure as early as 1785.

Ouabain which was once thought to be produced only by plants has recently been discovered to be an animal hormone that is secreted by the adrenal cortex and functions to regulate cellular Na⁺ and overall body salt and water balance.

The enzyme is also inhibited by nm levels of vanadate V⁵⁺ ions. Pentavalent ion locks the E₂ of E. Vanadate structure is analogous to the bipyramidal structure of phosphoryl group.



Structure of V⁵⁺

Biphiamidal array of ligands around central vanadium ion is like that around Phosphorus atom during hydrolysis of phosphoryl group.

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AN APPEAL

A lot of things in this world depend on money - security, shelter, education and even health. But at Nethralaya, money has ceased to be a pre-requisite for sight.

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COME, GIVE THE GIFT OF SIGHT

Frosted branch angiitis in renal transplantation: a case report

Sudha K Ganesh, Jyotirmay Biswas and Swapnil M Dongaonkar

Ito and co-workers first reported a rare type of retinal vasculitis in a 6-year-old boy from Japan.¹ Though the initial reports were in the younger age groups around 9 yrs, the disease has also been reported in older age groups between 20-29 years in otherwise healthy individuals. These patients have been found to have acute bilateral or unilateral visual disturbance with signs of anterior and posterior segment inflammation. The fundus appearance is dramatic with swelling of retina and severe sheathing of the retinal vessels creating an appearance of frosted branches of a tree. There have been reports of frosted branch angiitis in Rubella⁴ and in AIDS.^{5,6} We report a case of FBA and CMV retinitis following immunosuppression for renal transplant Uveitis seen at a referral Uveitis clinic at Sankara Nethralaya Chennai, India.

CMV retinitis is the most common opportunistic infection in immune deficiency states, that occurs due to AIDS or due to a pharmacological immunosuppression to support organ transplantation or to treat malignancies.

CMV retinitis is the most common ocular opportunistic infection in organ transplant patients. CMV retinitis occurs in 1-5% of renal transplant recipients.¹

CASE REPORT

A 29 year old, male reported to our institute with complaints of floaters and defective vision in the right eye for the past 6 weeks. The left eye was asymptomatic. The past medical history revealed that he had undergone a renal transplant 6 months back. He was on immunosuppressive medication, with Cyclosporine 400mg per day and Azathioprine 150mg per day and oral steroids 30mg per day for the past 6 months to support the renal transplant.

On presentation his BCVA was 6/9 N6 in both eyes. Slit lamp examination of right eye revealed a mild nongranulomatous anterior uveitis, aqueous flare 2+ aqueous cells 2+, and vitreous cells in the retroretinal space. Slit lamp examination of left eye was normal. The intraocular pressure by applanation tonometry was 14 mm of Hg in both eyes.

Fundus examination with indirect ophthalmoscopy of the right eye revealed frosted branch angiitis along the superotemporal vessels and features of CMV



Figure 1 - Right eye showing CMV retinitis and frosted branch angiitis

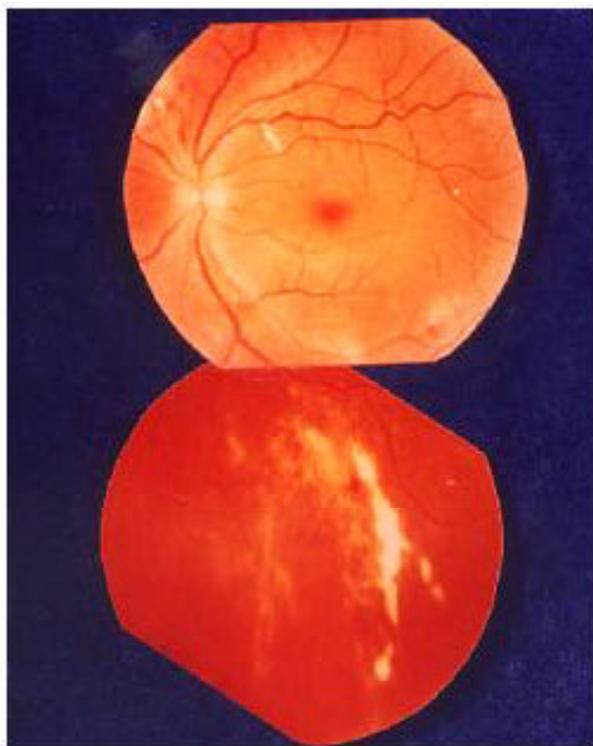


Figure 2 - Left eye showing frosted branch angiitis

retinitis in the inferotemporal quadrant (Fig. 1). Fundus of left eye showed early features of FBA along the inferotemporal arcade. (Fig. 2)

Preliminary investigations revealed that the leukocyte count was 4,400 cells/cmm, the differential count was normal, and the liver function tests and renal function tests were within normal limits. The ELISA for HIV was negative, ELISA for CMV IgM was +ve, and CD4 cell counts were 363 cells per cmm.

Patient was started on IV Ganciclovir 5 mg/kg body weight for 3 weeks and simultaneously his immunosuppressives were gradually tapered to 200mg of cyclosporine and 75mg of azathioprine per day under the supervision of a nephrologist.

He was reviewed on 10th day, at end of 3 weeks, and at end of 6 weeks. His vision improved to 6/6 N6 in both eyes with dramatic resolution of both anterior and posterior segment inflammation (Fig.3). His CD4 counts on last review were 883 cells/cmm. Subsequently his immunosuppressive therapy was monitored with CD4 counts, and periodic ophthalmic reviews.

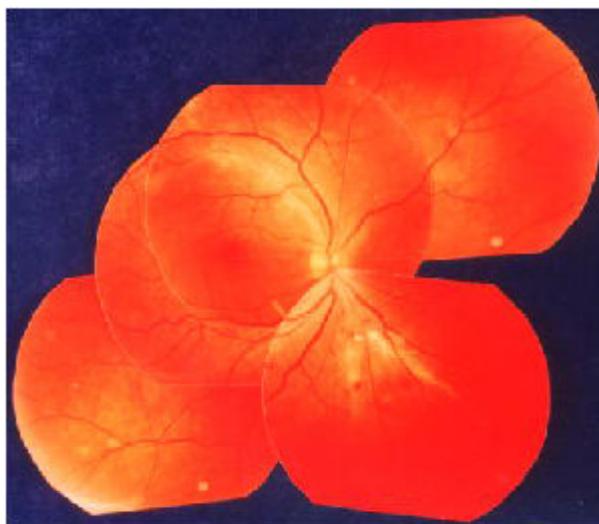


Figure 3 - Montage showing resolution in the right eye

Discussion:

Frosted branch angiitis (FBA) is an uncommon syndrome previously described as a primary immunologic response in immunocompetent patients that responded well to systemic steroid therapy.^{1,3}

Subsequently FBA was reported as a secondary phenomenon in immunocompromised AIDS patients in association with CMV retinitis where it was thought to be a direct infection of the retinal vessels and the adjacent retina by the CMV virus. This type of FBA responded to anti CMV therapy^{3, 4}.

CMV retinitis is the most common opportunistic infection in immune deficiency states, which may be due to AIDS or due to a pharmacological immunosuppression to support organ transplantation or to treat malignancies.

CMV retinitis is the most common ocular opportunistic infection in organ transplant patients. CMV retinitis occurs in 1-5% of renal transplant recipients.¹

There are two studies on CMV retinitis in non- HIV patients with chemotherapeutic immunosuppression and all these patients have responded to anti CMV therapy.^{6,7}

Wagle⁷ et al have reported frosted branch angiitis with CMV retinitis in 6 out of the 15 patients with CMV retinitis following organ

transplant in their study. However our patient had features of frosted branch angiitis and CMV retinitis in the right eye and features of frosted branch angiitis in the left eye.

Our patient was pharmacologically immunosuppressed to support a renal transplantation. He presented with the secondary form of FBA and required anti CMV therapy. We also tapered his immunosuppressive medications in order to improve his immune status. This resulted in rapid resolution of CMV retinitis, which had presented as FBA.

In patients with AIDS the irreversibility of the immunosuppressed status allows the CMV retinitis to progress. However with the advent of HAART therapy that improves the immune status of the patient CMV retinitis is becoming a rarity but is still at large in the developing countries where HAART therapy is unaffordable.

Most studies accept that the CMV retinitis in organ transplant is due to direct spread from other patients or transfusion of donated blood containing CMV virus.². Porter et al in their study of 39 patients of allogenic renal transplant found 2 patients (5%) developed CMV retinitis and 35 patients (87%) had detectable complement fixing antibodies to CMV.

Since the CMV virus is present in the sera of patients having renal transplant, patients at risk are those who have had high doses of immunosuppressives for prolonged periods of time.

The systemic risk factor associated with CMV retinitis especially in AIDS is a low CD4 count less than 50 cells per microlitre. In our patient the CD4 count was more than 300 cells per microlitre. Probably the poor qualitative function of the lymphocytes in this chronically immunosuppressed patient resulted in an opportunistic infection with CMV.

Conclusion: FBA is a descriptive fundus finding and is not a disease entity. We need to accurately identify the cause of FBA to effectively treat these patients. It would be wise not only to monitor the CD4 counts of

organ transplant patients but also periodically screen the eye for evidence of CMV retinitis or FBA. In our case that had CMV retinitis and FBA following a renal transplantation and immunosuppression, when the immunosuppressives were tapered, without jeopardizing the transplant, it resulted in improving the immune status and increased the CD4 counts and this in conjunction with ganciclovir therapy hastened the resolution of CMV retinitis or FBA.

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Occupational Vision testing – An Introduction

R. Krishna Kumar, Elite School of Optometry

Introduction:

World Health Organization committee defining Occupational health says it should aim at the promotion and maintenance of the highest degree of physical, mental and social well being of workers in all occupations; the prevention among workers of departures from health caused by their working conditions; the protection of workers in their employment from risks resulting from factors adverse to health; the placing and maintenance of the worker in an occupational environment adapted to his physiological and psychological ability and to summarize the adaptation of work to man and of each man to his job **(1)**.

There are various types of occupations with varied complexity specific to individual job. Eye care professionals like optometrists and ophthalmologists involved in vision screening, would need, in the first instance, to know about the visual requirements of the job the individual is engaged in, then plan the battery of vision tests based on visual requirements of the job and finally suggest the treatment to match the visual ability with visual requirements /demand of the job. The goal is to minimize stress on the visual system, which will result in an efficient and safe visual performance.

Occupational Vision Testing approach:

There are four important steps involved in occupational vision testing. They are as follows:

- 1) Occupational Analysis
- 2) Modified Clinical technique - using conventional testing methods or using vision testing instruments
- 3) Recommendation to match visual ability to visual demand of the job
- 4) Follow-up - every year

1) Occupational analysis:

This is the first step in occupational vision testing. There must be an analysis of the visual tasks involved in the occupation concerned. The analysis requires the examiner/optometrist visiting the occupation site/job site and note down the factors such as distance and size of the critical details of the task, color of the reference object, need for depth perception, body, head and eye movements, contrast and illumination of the work area. The potential hazards in the work place also can be noted. This analysis will help in the appropriate selection of visual function to be evaluated in the next step. The logical method for determining the visual factors required for a particular task has been proposed by Grundy **(2)** and is given as the guide below:

Based on the job analysis, the visual demand of the task can be planned.

2) Modified Clinical Technique: The evaluation can be done by qualified optometrists using standard instrumentations who can assess all the visual functions based on the input from job analysis and compare with the visual demand of the job. This technique includes history taking, refraction procedure, ocular motility examination, anterior segment and posterior segment evaluation, amplitude of accommodation, binocular vision assessment, visual field, color vision, contrast sensitivity testing and so on. Based on the evaluation, referrals or recommendations can be made. There are other techniques using Instrument screeners and computer programs which do not require optometrists. The screeners usually have internal lightning and the targets are mounted either on a rotary drum or separately on cards, which change manually or by remote control. There are

Occupational analysis

S. No	Name	General description of the task
1	Working distances	(a) Far (beyond 200 cm) (b) Intermediate (200-55cm) (c) Near (55-30cm) (d) Very near (less than 30 cm)
2	Size of detail	a) Large (angular size of critical detail over 10') (b) Medium (5-10') (c) Small (3-5') (d) Very small (2-3') (e) Extremely small (1-2') (f) Minute (< 1')
3	Main working positions	a) Sitting b) standing c) Moving d) Mixture
4	Size of working areas (in which critical vision is required)	a) Large b) medium c) Small
5	Head movements	a) Side to side b) up and down c) Mixture
6	Direction of gaze	a) Ahead b) Up c) down d) side e) Mixture
7	Changes in direction of gaze	a) Frequent b) Occasional c) Seldom
8	Movement of the task	a) Stationary b) Slow movement c) Fast
9	Potential danger	a) High risk b) Medium risk c) Low risk
10	Special accuracy or care	a) Required b) Limited requirements c) not required
11	Binocular vision and stereoscope	a) required b) not important c) Monocular vision adequate
12	Color vision requirement	a) Good color discrimination required b) limited requirements acceptable c) Not required
13	Visual fields	a) good field required b) Fair field acceptable c) Not important
14	Visibility (the relationship between the size of the detail, working distances, contrast and time available for viewing the task)	a) Good b) Fair c) Poor
15	Type of lightning in use, its adequacy and suitability	
16	Eye protection requirements	a) Required b) Not required
17	Hazards	Basic, impact, Molten metal, dust, gas, chemicals, radiation laser, other

many different vision screeners in the market and all are modified stereoscopes. The types of tests commonly included in the instrument screeners are: Visual acuity - distance and near, heterophoria - horizontal and vertical at distance and near, stereopsis, fusion, color vision, and

visual field. There are few screeners to measure contrast sensitivity function also. Keystone and TNO Titmus II vision tester are the commonly available instrument vision screeners available in the market. Computer programs are available to evaluate visual acuity, search tasks,

oculomotor balance, fixation disparity and central visual fields (3,4).

- 3) Recommendation: Based on the examination findings, the recommendation can be either referral to appropriate specialists or optical management in the form of appropriate lenses - correcting refractive error with safety lens materials (polycarbonate, CR 39), useable color tint/ coating and / or side shield, and orthoptic exercises. In addition, appropriate magnifiers and appropriate illumination specific for working needs will be advised. In rare situation, recommendation can be given to the employee to switch over to another visually less demanding job appropriate to his visual capability.
- 4) Follow up: Every year evaluation of the visual function has to be done to make sure that visual demand of the job and individual visual capability continues to match.

In conclusion, for maximum visual performance, the task and the worker should be assessed so as to match the requirements

of the task with the visual capabilities of the worker.

Acknowledgement:

Dr P. P. Santanam, Faculty, Occupational Optometry of Elite School of optometry for introducing me to this wonderful field of optometry.

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Dried Blood Spot (DBS) for Vitamin A analysis – a field friendly method

N. Angayarkanni, A. V. Sai Jyothi, K. Coral, R. Punitham, K. Krishnakumar, Madhava Rao and S. Ramakrishnan

The knowledge of Vitamin A – the chemistry, metabolism and deficiency consequences occurred rapidly in the first eight decades of the 20th century. Identification of the intestinal beta-carotene cleavage enzyme in the laboratory of James Allen Olson paved way for understanding the mechanism of formation of vitamin A from ingested carotenoids and studied dose dependent methodology clarifying the sequence of appearance of deficiency signs and symptoms. The synthesis of vitamin A around 1930 permitted fortification of foods and supplements virtually eliminating the ocular forms of vitamin A deficiency (VAD) as a public health problem in the developed countries¹. But it is still a fact that this has not happened even today in many developing countries including India as VAD continues to take quite a heavy toll on the sight and life of children.

Vitamin A deficiency (VAD) is the leading cause of preventable blindness in children and raises the risk of disease and death from severe infections, especially in Africa, South and East Asia. WHO sponsored the first systematic attempt to quantify the magnitude of the prevalence in the 1960s. In 1995, WHO redefined VAD to include tissue depletion below which functional impairment was likely. Serum level of less than 0.7µmol/L of vitamin A is used as the population based indicator of health risks. Nearly 75-140 million children of preschool age are vitamin A deficient and nearly one third of them become blind every year. Roughly 45% of VAD and xerophthalmic children and pregnant women with low-to deficient VAD status live in South and Southeast Asia. These regions harbor 60% of all cases of maternal Xerophthalmia i.e. Nightblindness,

three fourths of whom seem to live in India².

Lack of vitamin A is particularly severe for preschool aged children once they have been weaned and are no longer dependent on breast milk as the primary source of micronutrients such as vitamin A, causing severe visual impairment as well as significantly increasing the morbidity and mortality from common childhood infections as diarrhoeal disease and measles³. For pregnant women in high-risk areas, vitamin A deficiency occurs especially during the last trimester when demand by both the unborn child and the mother is highest. VAD may also be associated with mother-to-child HIV transmission⁴.

Clinical VAD Surveys – UNICEF (Year, 2000)

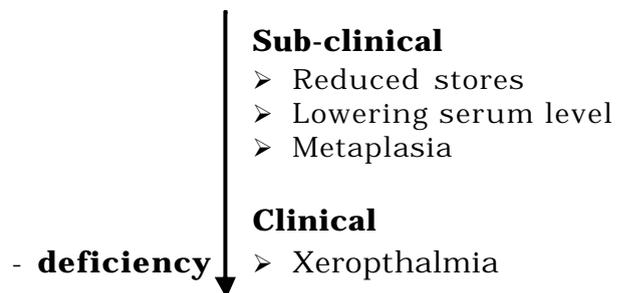
Area	Sample Size	Indicator/ Prevalence (%)
India MICS 2000 prevalence of Night Blindness 0-4 yrs	65,741	0.6%
ICMR ,District Nutrition Project Survey 2001 XN	6633	2.8%
NINWHO/UNICEF Orissa & AP study using serum retinol <0.70umg/l as a cutoff 12-36 months base line and post VA	2,134 Orissa AP 2,120	Orissa 63.8% AP 52. 3%
ICMR 16 district study Night blindness pregnant women in India (2001)	6,633 113,202	2.7% 1.03 %
Night blindness among children 24-71 months		

Vitamin A and carotenoids⁵: Out of the hundreds of carotenoids in nature only a small number are present in our food and these are of two kinds. One is beta-carotene, the most important and the one that can be converted in the body to retinol. It is split by an enzyme in the intestine to produce 2 molecules of retinol. The other kind of carotenoid including lutein and lycopene etc. cannot form vitamin A, though they have other important functions. Laboratory methods are now available for measuring the amount of different forms of retinol collectively known as **retinoids**. The HPLC (high performance liquid chromatography) has replaced most other methods of vitamin A estimation.

Mechanism of action of vitamin A⁵: Like lipids, retinol through the small intestine becomes part of chylomicrons that enter the blood circulation reaching liver and is stored in special cells called stellate cells as retinyl esters. The transport to the other parts of the body is as retinol through the **retinol binding protein (RBP)** as well as **transthyretin (TTR)**. At the cell membrane retinol is taken up by receptors (RBP receptors) and within the cell retinol binds to the **cellular retinoid binding proteins (CRBP)** synthesized independently. Most importantly it is also directed to the nucleus of the cell. The nuclear receptors (RAR and RXRs) are activated by the acid form of the retinol called the retinoic acid, which is the active form in the tissues except the eye. Many genes are activated by these receptors and they function like hormones such as the steroid with which they are closely linked. The functions of vitamin A are brought about by this mechanism except for the mechanism of vision. This is because the functional form of vitamin A in the rods and the cone cells is the 11-cis retinaldehyde (retinal) and not retinoic acid. Thus the vitamin A functions are mediated through the two oxidized metabolites of retinol namely retinaldehyde and retinoic acid.

Test for vitamin A deficiency⁵: While it is encouraging to note that the rate of

blindness due to Xerophthalmia is steadily decreasing, only in recent years has it become evident how widespread sub-clinical deficiency is. The majority is from the south and south east Asia (WHO, 2000). Of great importance is the fact that only carrying out biochemical or other laboratory tests can reveal the deficiency. This sub-clinical deficiency is much more common and widespread than clinical deficiency like xerophthalmia affecting young children and mothers who are either pregnant or lactating⁶.



The state of vitamin A nutrition in the body is called vitamin A status. The biochemical test measuring serum retinol is the test that has been most widely used. With HPLC accurate estimates can be made. However, the level of retinol in serum does not truly reflect changes in vitamin A status because it is in equilibrium with the stores in the liver and some other organs. Serum retinol does not fall appreciably until body stores have been virtually exhausted. Even then serum retinol values for large population groups have been found to be reliable of their overall vitamin A status. Presently measurement of serum retinol binding protein (RBP) is gaining importance. The vitamin A status is sometimes assessed also by conjunctival impression cytology. Both the World Health Organization and United Nations Children Fund have proposed the use of serum retinol as a key indicator of vitamin A deficiency.

Table:

Ranges of prevalence of serum retinol to define a public health problem of sub-clinical vitamin A deficiency in young children⁵.

Indicator	Mild	Moderate	Severe
Serum retinol ($\leq 0.70 \mu\text{mol/L}$)	$\geq 2 - \leq 10\%$	$\geq 10 - \leq 20\%$	$\geq 20\%$

Presently a more appropriate cut off of $0.50 \mu\text{mol/L}$ instead of $0.70 \mu\text{mol/L}$ is proposed for marginal deficiency⁵.

Use of Dried blood spots – a field method of collecting blood samples

Currently we have switched over to the most frequently used method for measuring vitamin A status using HPLC from the spectrophotometric method. The method is to take venous blood samples, centrifuge them (and after freezing and transporting to the lab if necessary) to measure the retinol content in serum by HPLC. This is quite tedious as well as expensive in terms of collection and transport for field collections. A less expensive and more efficient way is to measure Retinol Binding protein (RBP), which correlates well with the retinol content is the ELISA technique for measuring RBP along with C-Reactive protein (CRP) and alpha-1 glycoprotein (AGP) as indicators of acute and chronic infections⁷.

In developing countries collecting venous blood in field studies particularly in children is not very easy. The cultural beliefs, local taboos, use of needles as well as the need for trained phlebotomist and the risk of transmitting blood-borne viral diseases in such field studies complicates the study. Moreover isolation of the serum component, storage and transport are the other obstacles.

But recently Craft Technologies developed a procedure to measure blood retinol concentration in venous or capillary blood from dried blood spots (DBS). They also validated the method by comparing it with venous and capillary serum retinol and concluded that it is possible to compare blood retinol concentration from DBS^{8,9,10}.

The objective of this study was to adopt the same method, suitably modified, in order to compare it with the existing method of vitamin A (retinol) estimation in venous blood

by HPLC with that of the DBS specimen and to arrive at the recovery if retinol in the DBS method, so that it can be adopted in the future field studies.

Subjects and Methods:

Two blood specimens were collected, for dried blood spots as well as serum separation. 4 samples for retinol analysis based on the method of Craft et.al⁸ were generated. The subjects were either in the fasting or the postprandial state. 1-2 drops of the venous blood ($40 \mu\text{L}$) was placed using a micropipette, onto each of the blood collection spot on the card. The remaining whole blood was allowed to coagulate at room temperature (25°C) and centrifuged at $1500 \times g$ and the serum transferred to a vial. Care was taken to protect the samples from light during all procedures. The DBS cards were allowed to dry in the dark (in a cupboard) for 1 hr. The cards were then stored in ziplog bags at -20°C for a maximum of 2 weeks.

Materials: Retinol, Retinyl acetate, Hydroquinone, Butylated hydroxytoluene, (sigma) ascorbic acid, diethylene triamine penta acetic acid, (Merck) methanol, ethanol hexane, acetonitrile (Merck, HPLC grade) and DBS cards (Schelicher and Schuell 903 specimen collection paper, Germany)

Extraction of retinol: The total 13.0 mm diameter circle on the card was cut from the DBS card (Fig 1) and extracted in 1.0 ml of 0.15 M phosphate buffer, containing 60 mM ascorbic acid as an antioxidant and 2 mM DTPA, sonicated for 5 minutes and cyclomixed for 10 minutes. Subsequently 0.9 ml of ethanol containing 100 mM BHT was added. Retinyl acetate ($10 \mu\text{g}$) was used as the internal standard and vortexed for 20 seconds. One ml of hexane was added and cyclomixed for 1 minute for extraction and centrifuged for 1 minute at $1500 \times g$. The hexane layer was removed and the extraction

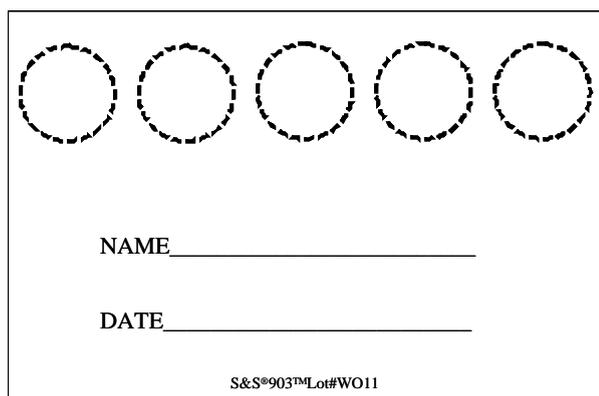


Fig 1: DBS Card

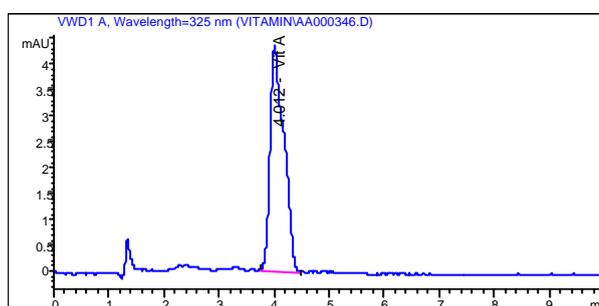


Fig 2: Retinol Standard (20ng)

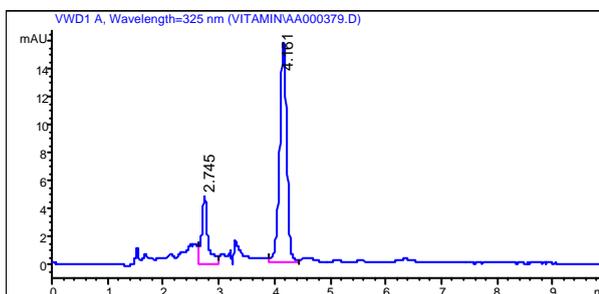


Fig 3: Serum retinol

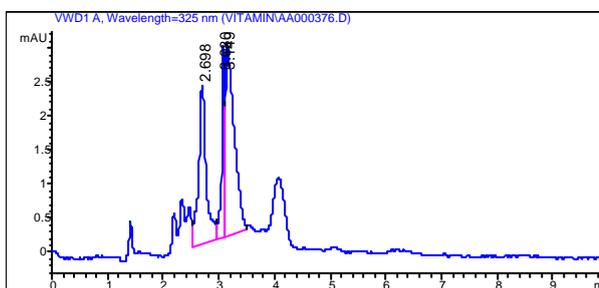


Fig 4: DBS retinol

process repeated. The hexane layers were pooled, and dried under a stream of nitrogen and the residue obtained was dissolved in 200µl mobile phase (methanol, acetonitrile

and water, 65:34:1), cyclomixed for 1 minute and 50µl of the sample was injected into the HPLC (HP-HPLC). The HPLC separation of retinol was performed isocratically using acetonitrile and methanol and water (65:34:1) at a flow rate of 0.5ml/min in a hyper-siloctadecyl silane column (C18, 5µ). Retinol was monitored at 325 nm using the variable wavelength UV detector.

Results:

Linear calibrations were prepared using 4 concentrations of retinol as standard (20,40,60 and 80ng.concentrations) for the estimation of retinol in the serum and DBS samples.

S.No.	Name	Serum retinol (venous) (mg/dl)	DBS retinol (mg/dl)
1	Control 1	32.8	28.8
2	Control 2	30.2	25.2

➤ Plasma volume adjustment factor = Serum retinol / DBS retinol = 1.2

➤ mean of ± 11% variation

Fig 2 shows the chromatogram of standard retinol(RT=4.01min). Fig 3 shows the serum retinol with a retention time of 4.1min. Fig 4 shows the DBS extracted retinol (RT=4.07min) while Fig 5 shows DBS spiked with external (10µg retinol) and the internal standard (10µg retinyl acetate). Retinyl acetate had a retention time of 5.1 min.

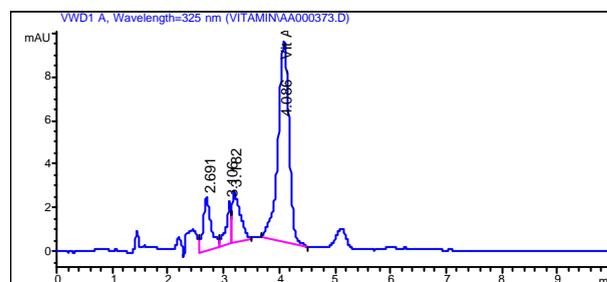


Fig 5: DBS spiked with external (10ug retinol) and Internal standard (10ug retinyl acetate)

Discussion:

Quite often it is impossible to obtain venous blood samples from specific populations. A finger prick method of obtaining the sample needs to be adopted as a smaller volume is involved. The approach of collecting and drying the small blood samples on filter paper for further extraction and analysis off the site of collection becomes more valid in the context of not only in the transport of specimen over larger distances before analysis but also eliminates the need for even small machineries as well as the need for expertise to collect and process the sample at the site of collection.

More number of samples is being analyzed by the DBS method and simultaneously in the venous blood for validation of the methodology. The major advantage of using the entire spot is that higher sensitivity is obtained and uneven distribution of retinol throughout the whole spot is avoided. Using the DBS method we were able to get reproducible results that were comparable to our previous extraction procedure from the serum followed by the reverse-phase HPLC separation, with the same retention time of 4.1 min and a mean variation around 11%.

Retinol being bound to retinol binding protein is greatly protected from degradation and remains stable even at room temperature. Erhardt et.al have reported that DBS is stable for 3 months and longer when samples are stored at about 23°C in the dark. But still it is preferable to maintain the sample at cooler temperature if possible. Of the storage conditions tested as reported by the same group, it is reported that the storage of inadequately dried DBS in a Ziploc bag without desiccant was detrimental to DBS retinol analysis.

Thus this method once validated in our laboratory with more number of samples can be used in larger studies where plasma or serum samples are not readily obtained. But the sensitivity of the method to detect severe deficiency as against the serum retinol is yet to be reported.

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Muscle Puzzle

Anand Vaijwade and **S. Meenakshi**, Sankara Nethralaya ORBIS Pediatric Ophthalmology Learning and Training Centre

35 years old man presented with double vision in up and down gaze, with history of injury with a stick one month back.

On examination visual acuity was 6/6 N6 OU with RAPD 2+ in left eye.

Anterior and posterior segment examination is within normal limit except for sutures of upper lid tear repair with mild edema.

Ocular motility is shown in the photos. What is your diagnosis?

(Answer in next page)



(Answer to Muzzle Puzzle in page 25)

Extra ocular movement shows limitation of up and down gaze in left eye. Cover test shows ortho in primary gaze with 25 PD left hypotropia in up and down gaze, 15 PD exotropia with flick right hypertropia in right gaze and 4 PD esotropia in left gaze.

CT scan showed fracture of anterior, medial wall and floor on left side with entrapment of Inferior rectus.

On Diplopia charting patient had binocular crossed vertical diplopia more in down and up gaze. Hess charting showed under action of Inferior rectus.

Forced duction testing was positive for inferior rectus restriction.

Strabismus after blow out fracture has been estimated to occur in about 58 % of patient. The term "blow out fracture" was coined by Smith and Regan. These are seen after injury with cricket or tennis ball or road side accidents. Immediately following injury it present as black eye and restriction of ocular movement in all gazes which usually subsides by end of first week and presents as specific restrictive strabismus.

Patient develops hypoesthesia along infra orbital nerve, diplopia in up and down gaze due to entrapment of soft tissue in fractured fragment and enophthalmos due to herniation of orbital contents in to the maxillary sinus.

The presence of muscle entrapment can be confirmed on FDT (force duction test). Saccadic movement testing can differentiate between paretic and entrapped muscle. Usually associated severe globe damage is rare since blow out is a protective phenomenon.

Plain X ray (Waters view) and CT scan aid diagnosis.

In initial phase of injury, surgery should be withheld until two weeks till edema subsides. Patient should be followed up closely with serial diplopia and Hess charting .

Surgery should be defered till sufficient diplopia free area is present in primary and down gaze. Surgery can also be performed for significant enophthalmos. Teflon plate can be placed subperiostially. For troublesome diplopia fresnel prisms trial can be considered. For surgical correction first inferior rectus recession can be planned.

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Sankara Nethralaya

Invites you to
Indo Israel Ophthalmic Congress
November 19 & 20, 2005 - Chennai

Dear Colleague,

I have great pleasure in inviting you for the inaugural Indo Israel conference. This meeting will bring together luminaries from India and Israel under one roof.

Over the two days there will be sessions addressed by experts from the premier institutions the highlight being the session on cataract surgery by Prof Blumenthal and team. Other topics to be covered include updates on the latest trends in glaucoma, oculoplasty, diabetic retinopathy and ARMD. The program will feature a plenary session addressed by International leaders in Ophthalmology. Sessions for free posters are also planned.

In addition to high quality scientific discussions an evening of cultural events and a gala dinner will give you the opportunity to interact with the faculty.

So don't miss this opportunity to participate in this unique program.

I hope to see you in Chennai.

Dr Prema Padmanabhan.

Scientific Programme

GLAUCOMA

Current imaging modalities have added an impetus to diagnose and detect glaucoma early. Emphasis to use these in a rational manner is important for high quality glaucoma management. There is a consensus that very little is known about the exact pathophysiology of glaucoma and research for evidence at molecular level is becoming a priority "This possibly will be the way to go". Constant efforts to find the ideal surgical technique have been yielding qualified results. Let's gather and upgrade information on evidence based management of glaucoma

OCULOPLASTY

The board sweep of oculoplastic surgery encompasses the areas of orbital, eyelid, and lacrimal disease and has seen rapid strides and exciting developments in the recent past. There is a surge of interest in this area and this sub-specialty session aims to provide an exciting range of topics to be discussed by experts and of interest both to the oculoplastic surgeon and the general ophthalmologist, including students in training.

VITREO RETINA

The program includes 4 mini-symposiums: VR surgery and diabetic retinopathy update, age - related macular degeneration update, posterior segment complications of anterior segment surgery and anterior segment complications of posterior segment surgery.

Vitreoretinal surgery update would highlight newer innovations such as 25 G instrumentations, role of newer imaging techniques such as OCT in

understanding vitreoretinal interface, newer dyes to stain ILM, pharmacological agents for macular edema, surgical approaches to manage venous occlusions. Diabetic retinopathy update would focus on epidemiology, emerging trends, systemic considerations, and role of diabetic vitrectomy.

Age-related macular degeneration update would give and overview of clinical profile, role of ancillary tests, polypoidal choroidal vasculopathy, role of TTT/PDT, pharmacologic agents and submacular surgery.

Posterior segment complications of anterior segment surgery and vice versa would involve issue as globe perforation, posteriorly dislocated nucleus/IOL, cystoid macular edema. Suprachoroidal hemorrhage, and management issues related to muscle imbalance, cornea, cataract and glaucoma following vitreous surgery.

CATARACT

The cataract sessions will deal with small incision cataract surgery. Instruction courses on the popular small incision techniques are planned. Prof Michael Blumenthal, one of the pioneers of manual small incision cataract surgery, will conduct an instruction course on the Blumenthal technique of cataract surgery. These sessions will deal with all aspects of the techniques including wound construction, instrumentation and nucleus management. Doing cataract surgery in difficult situations with each of these methods will also be highlighted. The meeting has sessions devoted exclusively to the management and avoidance of complications.

Patrons

Prof. M Blumenthal
Dr. S S Badrinath

List of Invited Faculty

Dr. Anat Lowenstein, Israel
Dr. Aung Tin, Singapore
Dr. Ayala Pollack, Israel
Dr. Dov Weinberger, Israel
Dr. Ehud Assia, Israel
Dr. Elish Bartov, Israel
Dr. Issac Hemo, Israel
Dr. Joseph Moisseiev, Israel
Dr. Melamed, Israel
Dr. Nachum Rosen, Israel
Dr. Pe'er J. Israel
and
faculty from host institutions

Registration Details

Fees

Delegate Type	Up to 31.07.05	Regular	Spot
Indian Post Graduate Students,	Rs.1000	Rs.1500	Rs.2000
Indian International Delegates	Rs.750	Rs.750	Rs.1000
	USD 200	USD 200	USD 200

Poster Specification

4 feet height, 3 feet width
(4/3 in vertical)

Maximum No of PPT Slides - 18

Host

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