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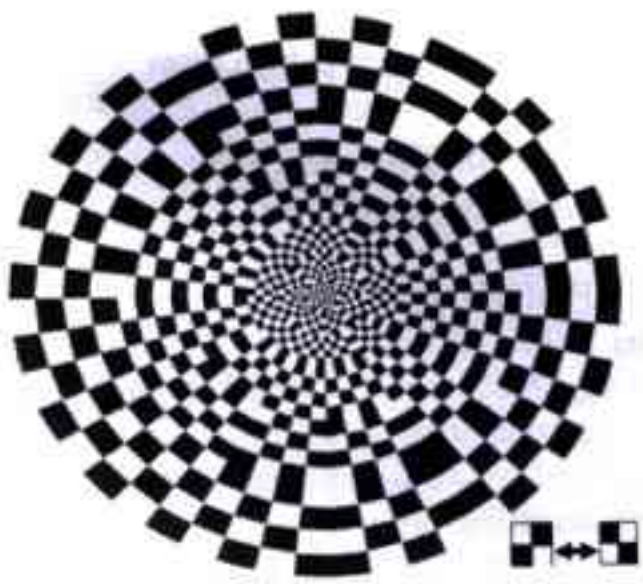

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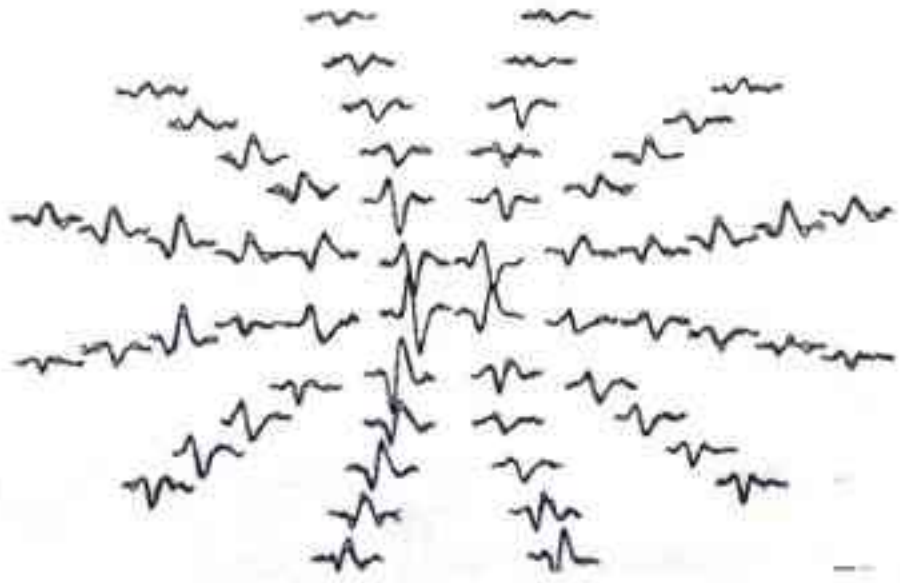

mfVEP responses from 60 locations

Editorial

This issue not only has a new look, but also has articles from all disciplines of ophthalmology and vision science. The second part of the article on ocular manifestations in HIV is presented in this issue. Compounding prisms to alleviate diplopia is elaborated in the article from the Elite School of Optometry. A detailed study on polymerase chain reaction and its ophthalmic applications is finely elucidated in the article from Department of Microbiology. Biostatistics series has another interesting chapter in this issue and finally the multifocal VEP is covered in the technology update in a lucid manner.

27th May 2009
S. Meenakshi
Editor

Perspective

Acquired immunodeficiency syndrome and its ophthalmic complications, Part II

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BACTERIAL DISEASES

Bacterial keratitis

Ocular flora in HIV-infected individuals is not very different from that in the general population, but the risk of infection with this "normal" flora may be greater for severely immunosuppressed individuals. *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* are most frequently implicated. HIV-infected hosts may be predisposed towards these spontaneous bacterial keratitides because of pre-existing keratoconjunctivitis sicca (KCS) and viral keratitis, which create corneal epithelial erosions allowing subsequent bacterial entry.

The clinical presentation in HIV-infected individuals is usually bilateral, involves multiple pathogens and carries a higher risk of perforation. Paucity of inflammation in immunosuppressed patients contributes to delay in diagnosis and treatment.

PROTOZOAL DISEASES

Toxoplasmosis

In the majority of AIDS cases, toxoplasmosis is a primary infection rather than a reactivation. Ocular toxoplasmosis in AIDS is often bilateral, multifocal, associated with retinal haemorrhages or rhegmatogenous retinal detachment and not associated with chorioretinal scars. Additionally, in AIDS, toxoplasmic retinitis is associated with encephalitis in more than half of the cases and therefore warrants neuroimaging with MRI typically showing "ring-enhancing lesions". It may also cause a variety of other ocular abnormalities including iritis, vitritis, choroiditis, multifocal or diffuse necrotizing retinitis, papillitis or retrobulbar neuritis, or outer retinal toxoplasmosis. Treatment with standard antiparasitic drugs such as pyrimethamine (100 mg loading dose, followed by 25–50 mg daily), clindamycin (300 mg four times a day) and sulphonamides (4–6 g daily) is successful in controlling ocular toxoplasmosis in most cases. Folinic acid is added with pyrimethamine to reduce bone marrow suppression.

Recurrences are common, with some patients requiring an indefinite maintenance therapy. Severely immunosuppressed individuals with AIDS can develop a primary toxoplasmic anterior uveitis, which can occur in the absence of retinal lesions. Iris nodules with tissue destruction and severe anterior segment inflammation have been noted. Inflammation responds well to anti-parasitic therapy but not to corticosteroids alone.

Microsporidial keratitis

Microsporidia are spore-forming, obligate intracellular and protozoan parasites. They occur when the $CD4^+$ counts drop to about 100 cells/mm^3. Thirty-nine to forty-four per cent of HIV-infected individuals with diarrhoea are infected with *microsporidia*.

AIDS-associated microsporidial keratoconjunctivitis is characterized by bilateral superficial punctate epithelial keratitis, white intraepithelial infiltrates, mild anterior chamber reactions and mild conjunctival follicular hypertrophy. Patients may complain of photophobia and grittiness. Vision loss is secondary to keratitis.

Diagnosis is by Gram or Giemsa stain, and spores from conjunctival scrapings or corneal biopsies can be easily seen with Masson trichrome or Giemsa stain. Immunofluorescence and electron microscopy can help confirm the diagnosis. Ocular microsporidiasis should be suspected in all HIV-positive patients with persistently negative cultures for epithelial keratitis. Although highly active antiretroviral therapy (HAART) is useful in resolving microsporidial keratoconjunctivitis in HIV-positive hosts, post-immune recovery-mediated microsporidial keratoconjunctivitis reactivation has also been reported.

Treatment is with Fumagillin 70 mg/mL. Eye drops are used indefinitely in HIV-positive individuals. Albendazole 400 mg twice daily orally should be used as an adjunct for the management of systemic infection.

PNEUMOCYSTIS DISEASE

Ocular manifestations of *P. carinii* are rare and include conjunctivitis, orbital mass, optic neuropathy and choroiditis. It is seen as classically bilateral and multifocal yellowish, well-demarcated, choroidal lesions located in the posterior pole not associated with vitritis, iritis or vasculitis. The lesions are slowly progressive and do not interfere with vision. Ocular lesions respond in most cases to induction and subsequent maintenance treatment with systemic pentamidine, trimethoprim and sulphamethoxazole or dapsone.

MYCOBACTERIAL DISEASE

Tuberculosis (TB) implies the presence of active disease from infection with the acid-fast bacillus. *Mycobacterium avium*, a non-tuberculous mycobacteria, causes endophthalmitis in patients with AIDS. Prominent iris nodules caused by *M. avium* complex (MAC) has also been observed as the initial manifestation of panophthalmitis in an individual with AIDS. Clinical manifestations include ulcers, tubercles, granular masses or pedunculated polypoid tumours. Patients may present with localized nodule in the eyelid simulating a chalazion. Orbital and lacrimal gland involvements by *Mycobacterium tuberculosis* lead to localized granuloma though unusually they can present as a conjunctival mass.

Infection of the iris can present either with or without iris nodules. Presentation of ocular tuberculosis in HIV-infected individuals is also similar.

TB may cause interstitial keratitis with corneal stromal infiltration. Interstitial keratitis secondary to TB may be associated with uveitis. Sclerokeratitis can be seen with peripheral stromal inflammation in a triangular fashion with localized scleritis. Localized lesions are focal elevated nodules of the sclera that may undergo necrosis leading to scleromalacia and scleral perforation if untreated. Diffuse scleritis is less common than localized nodular scleritis.

M. tuberculosis infection of the conjunctiva may involve the palpebral, bulbar or forniceal conjunctiva. In recalcitrant cases of chronic red eye, a definitive diagnosis requires the identification of *M. tuberculosis* organisms in conjunctival biopsy specimens. Keratoconjunctivitis may occur in association with cutaneous TB.

Tuberculosis can also present as a granulomatous type of anterior uveitis, in HIV-infected individuals and the inflammatory reaction correlates with the level of CD4 cell counts.

Posterior segment disease presents as multifocal choroidal tubercles with discrete yellow lesions mainly at the posterior pole. It may be associated with an exudative retinal detachment with variable vitreous inflammation. Occasionally, however, it may present as a big solitary posterior pole granuloma-like mass lesion. Treatment with long-term systemic anti-tuberculous drugs is effective in most cases. Recently, there has been a report of worsening of ocular tuberculosis in HIV patients after antiretroviral therapy.

Systemic anti-tuberculous therapy (ATT) with drugs such as isoniazid, rifampin, pyrazinamide and ethambutol is important as pulmonary or other foci of disease may coexist. Modified DOTS regimen (Directly Observed Treatment Short course) is the recommended regime in India. Specific ocular treatment should be instituted along with ATT. In individuals with HIV infection, therapy may require longer duration and additional drugs which include newer drugs such as quinolones, newer rifamycins such as rifabutin and macrolides antibiotics.

FUNGAL INFECTIONS

HIV/AIDS patients can develop spontaneous fungal infections in the absence of preceding trauma. *Candida* and cryptococci are the most prevalent ocular fungal pathogens among HIV-positive hosts. *Candida* causes anterior segment keratitis. Fungal keratitides have a more acute and protracted course in the HIV/AIDS population, and are more likely to result in bilateral disease with corneal perforation. Cryptococcus meningitis is the most common cause of AIDS-related neuro-ophthalmologic lesions. Cryptococcal choroiditis may be multifocal, solitary or confluent and may be associated with eyelid nodule, conjunctival mass, granulomatous iritis, iris mass, vitritis, necrotizing retinitis, endophthalmitis and optic neuritis. Culture or

biopsy of lesions is important in HIV-infected patients with ocular surface infections, to differentiate between bacterial and fungal aetiology. Fluconazole maintenance therapy 200 mg/day is currently recommended in all patients.

SPIROCHAETAL INFECTIONS

Ocular syphilis caused by *Treponema pallidum* presents with more aggressive, severe and relapsing manifestations in HIV-positive hosts as compared with immunocompetent hosts. It is frequently the presenting symptom of co-infection with HIV. Disruption of the mucosal epithelial barrier in syphilitic ulcers that contain mononuclear cells (targets of HIV infection) may increase the risk of HIV acquisition.

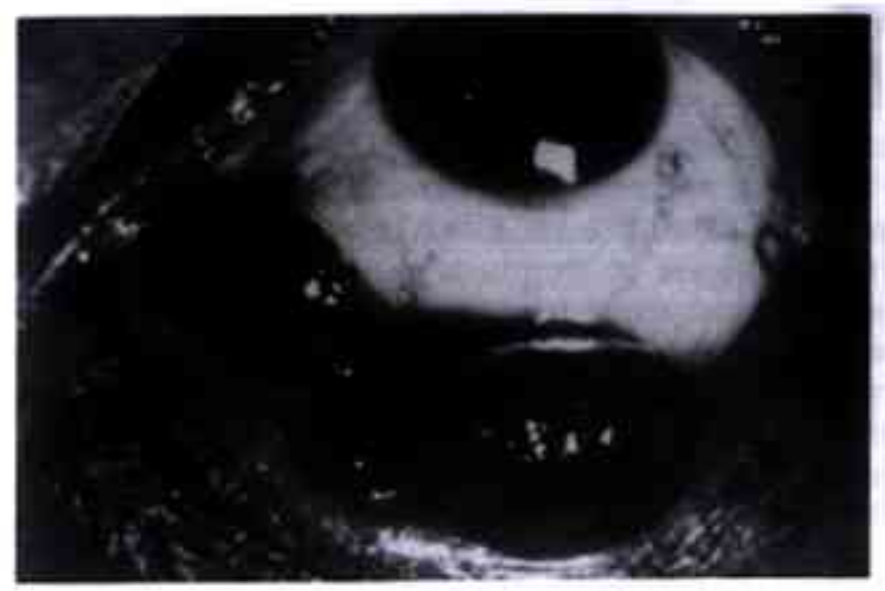
Anterior segment manifestations of syphilis include chancres of the conjunctiva (primary syphilis), conjunctivitis (secondary syphilis) and gummata (late syphilis). Conjunctivitis can be granulomatous and histologically similar to sarcoidosis. It is the most common bacterial cause of uveitis in HIV-positive hosts with an incidence of 0.6%. It tends to be more severe and consists of panuveitis in conjunction with anterior uveitis. Isolated episcleritis and scleritis are uncommon during any stage of the disease, but when present, are usually features of secondary or late syphilis. Retinitis develops during secondary syphilis and manifests with floaters, photophobia, decreased vision and ocular irritation. Clinically two patterns of retinitis are seen—a yellowish-white patch of necrotizing retinitis outside the arcades or a greyish-white geographic area of retinitis in the vicinity of the arcades.

Eighty-five per cent of HIV-positive patients with ophthalmic syphilis have co-existing neurosyphilis. The diagnosis of syphilis involves a good clinical history in addition to serologic screening and confirmatory tests such as the rapid plasma reagin or fluorescent treponemal antibody absorbent tests, respectively. Direct examination using dark-field microscopy or biopsy of suspicious lesions can also be performed.

Treatment of ocular syphilis is that of neurosyphilis. The most effective treatment involves high-dose IV penicillin G 12 to 24 million units/day for 14 days. Doxycyclin 100 mg orally, twice daily, is helpful in penicillin-allergic patients. HIV-positive patients are recommended to have extensive follow-ups subsequently at least for 2 years.

Direct effects of HIV. HIV has been isolated from tears, conjunctiva, cornea, aqueous humour, iris, sclera, vitreous humour and retina, and has been suspected as a cause of intraocular inflammation occasionally, in the absence of other pathogens.

HIV can also infect retinal tissue, especially capillary endothelium and neuroretinal cells. Infection of endothelial cells with immune complex deposition may have a role in formation of cotton-wool spots commonly seen in AIDS patients. "Multifocal punctate retinal infiltrates", an unusual inflammatory condition, which can have associated anterior segment inflammation, may also be a direct effect of HIV infection. Diagnosis requires slit-lamp examination, and therapy is primarily directed at identifying an infectious



Kaposi's Sacroma in AIDS.

aetiology. Topical steroids are often employed but must be used with caution and in combination with appropriate antimicrobial therapy.

NEOPLASMS

Kaposi's sarcoma

Kaposi's sarcoma (KS) is a highly vascular mesenchymal tumour that appears as multiple purple-to-red nodules on the skin and mucous membranes. In approximately 10–20% of individuals with HIV-associated KS, the tumour involves the eyelids and conjunctiva and in rare cases, the orbit. KS can be an initial manifestation of AIDS. The appearance of KS on the eyelids is similar to the lesions elsewhere on the skin. It can mimic a chalazion and can be seen in the upper or lower lids. Conjunctival lesions may be seen in any part of the palpebral or bulbar conjunctiva but are usually more common in the inferior fornix. Kaposi's sarcoma in the conjunctiva can be mistaken for a subconjunctival haemorrhage or pyogenic granuloma. Clinically, it is asymptomatic, but can cause trichiasis, infection and cosmetic disturbance. With orbital involvement, it may lead to ptosis, proptosis and diplopia due to nerve palsies.

Histologically, KS can resemble an angioma, haemangioma, lymphangioma or granulation tissue. It has a complex arrangement of capillary channels and vascular spaces ("slits") without endothelium. Malignant spindle cells are arranged around these incomplete vascular spaces. Clinically and histopathologically, KS is divided into three stages. Stage I and II lesions are flat (less than 3 mm in height), patchy and of less than 4 months duration; Stage III lesions are nodular, greater than 3 mm in height and greater than 4 months in duration. This staging may have prognostic value regarding the course of disease and response to therapy.

Treatment for KS is not necessary if it is asymptomatic and is cosmetically acceptable. The principal goal of therapy in these patients is palliation when disease is disfiguring, painful or interfering with function. Under these circumstances, it may be treated by cryotherapy, surgical excision (if the lesion is small), radiation and/or chemotherapy. Radiation therapy is effective in

treating the eyelid and conjunctival lesions, but can lead to loss of lashes, irritation, conjunctivitis, conjunctival keratinization, cicatricial ectropion and retinopathy.

Systemic agents are warranted for patients with advanced disease such as extensive cutaneous disease, visceral disease or lymphedema. Intralesional vinblastin or interferon alpha have been known to produce good results. Associated systemic KS is best treated with systemic chemotherapy. Anterior segment fluorescein angiography can be helpful in demarcating the 1–2 mm tumour-free zone during surgery. Widespread success of HAART in treating AIDS-related primary KS has reduced the need for other management options. IRIS (Immune Reconstitution Inflammatory Syndrome) complications can be lethal, with KS pulmonary involvement within first 2 months of initiation of HAART. Early treatment with limited systemic chemotherapy can be lifesaving in such cases.

Squamous cell carcinoma

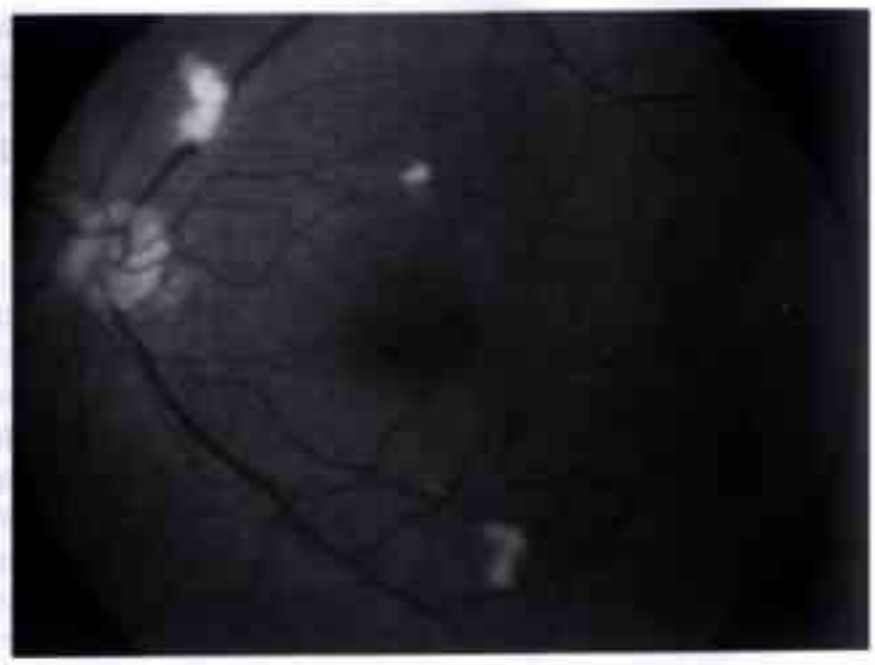
SCC is the third most common neoplasm associated with HIV after KS and lymphoma. When the tumour is found at the limbus, gonioscopy has to be performed to rule out intraocular extension. Most commonly noted clinical characteristics of conjunctival SCC are corneal overriding (90%), fast growth rates (with a rate of 1 mm in 83%), tumours larger than 1 cm (17%), changes in conjunctival colour (66%) and nasal locations (66%).

Twenty per cent of benign pterygia may harbour SCC/Carcinoma *in situ* and hence pathology review should be done for all conjunctival lesions.

Treatment is wide excision of the lesion with frozen section evaluation for monitoring of the margins. HAART has been known to cause complete regression of invasive conjunctival SCC in an HIV-positive patient, which raises the possibility of antiviral therapy as an alternative to radical surgery.

Lymphomas

Non-Hodgkin's lymphoma (NHL) accounts for 3.5–5% of AIDS-defining illnesses and tends to be of a higher grade of malignancy in HIV patients and can affect eyelids and conjunctiva. NHL should be suspected in all HIV patients of any age who have prominent vitreous cells, with or without subretinal exudation. Intraocular lymphoma usually is seen with CD4 cell counts of less than 50 cells/ml. Intraocular lymphoma can also cause anterior chamber reactions and manifest as a pseudo-hypopyon uveitis. Primary NHL has also been found in eyelids of patients with AIDS, presenting as rapidly enlarging erythematous lesions. Lid swelling, ptosis, proptosis, gaze palsies and ophthalmoplegia have been reported. NHL can mimic KCL. Hence any AIDS patient with dry eye, especially if unresponsive to therapy, should be viewed with suspicion, more so, if it is unilateral and/or the patient has associated systemic symptoms such as fever, malaise or weight loss. Reported posterior segment manifestations of NHL include necrotizing retinitis, multifocal choroiditis, retinal vasculitis, vitritis and a subretinal mass lesion.



Cotton Wool spots in Retinal Microvasculopathy.

Treatment options include radiotherapy and/or chemotherapy such as vinblastin. Interferon alpha has also been used as a treatment option.

HIV MICROVASCULOPATHY

Retinal vasculopathy

Microvasculopathy is the most common ocular manifestation of AIDS, seen in about 40–60% of HIV-positive patients. Clinically, it manifests as cotton-wool spots, which are seen as white spots with feathered edges on the surface of retina located in the posterior pole and may be confused with small patches of cytomegalovirus (CMV) retinitis. They have rounded borders, are distributed along the vascular arcades and represent focal areas of ischaemia in the nerve fibre layer, leading to accumulation of cytoid bodies in the axons of nerve fibre layer. Fluorescein angiography reveals microaneurysms, telangiectasias, focal non-perfusion areas and areas of capillary loss, probably reflecting a more generalized microvasculopathy. Most patients with retinal microvasculopathy are asymptomatic. Treatment is not indicated in most cases. The prevalence of microvasculopathy is inversely proportional to $CD4^+$ count.

Conjunctival microvasculopathy

Seventy to eighty per cent patients have some form of asymptomatic conjunctival microvascular changes. These include segmental vascular dilatation and narrowing, microaneurysm formation, comma-shaped vascular fragment and visible granularity to the flowing blood column (sludging). These are seen more commonly near the inferior limbus and have a good correlation with the occurrence of retinal microvasculopathy. Exact cause is not known. It is postulated to be due to increased plasma viscosity, endothelitis or immune complex deposition. No treatment is required.

OTHER MANIFESTATIONS

Keratoconjunctivitis sicca

Dry eye occurs in 20–38.8% of HIV-positive hosts in the later stages of AIDS. It is thought to be due to lymphocytic infiltration of the lacrimal gland. Afflicted individuals are more susceptible to bacterial keratitis, and abnormalities in the composition of the tear film are present. Aetiology is multifactorial and is due to the combined effects of HIV-mediated inflammatory destruction of primary and accessory lacrimal glands and to the direct conjunctival damage due to the HIV virus itself. The virus is also postulated to play a significant role in spontaneous corneal thinning and perforation, which is observed in some infected patients. HAART does not help in significantly reducing the prevalence of KCS.

Management options include artificial tears, long-acting lubricants and punctal occlusion in severe cases.

Angle closure glaucoma

Acute angle closure glaucoma has been described in association with uveal effusion syndrome in patients infected with HIV. Cause of angle closure glaucoma is not known. Intraocular inflammation is minimal but can be severe in case of primary choroidal inflammation with secondary exudative retinal detachment. B-scan ultrasonography and ultrasound biomicroscopy help in diagnosis. Treatment includes cycloplegics, corticosteroids, aqueous suppressants, hyperosmolar agents and surgical drainage of suprachoroidal fluid.

IATROGENIC COMPLICATIONS

Immune recovery uveitis

Immune recovery uveitis (IRU) or IRIS is a non-infectious intraocular inflammation which develops in patients with inactive CMV retinitis who have had a substantial elevation in $CD4^+$ count with HAART. IRU is the leading cause of new visual loss in persons with AIDS seen in about 16–63% of HAART responders. The severity of the inflammation depends on the degree of immune reconstitution, extent of CMV retinitis, amount of intraocular CMV antigen and previous treatment. Clinical findings include anterior chamber or vitreous reaction, panuveitis with hypopon, optic disc oedema, cystoid macular oedema, epiretinal membrane formation, cataract, vitreomacular traction syndrome and proliferative vitreoretinopathy. Treatment with corticosteroids (subtenon or systemic or intravitreal) is effective in controlling inflammation and improving vision in some cases. However, surgery may be required in patients with vitreomacular traction syndrome, epiretinal membrane formation, cataract and proliferative vitreoretinopathy.

Cidofovir

Intravenous or intravitreal cidofovir is used for CMV retinitis in HIV-positive patients. However, 23–71% cases show side effects such as anterior non-granulomatous uveitis and ocular hypotony. Cidofovir may also give rise to ciliary body necrosis. Symptoms are pain, photophobia and red eye. Oral probenecid before intravitreal injection prevents uveitis in 50% of cases. Topical steroids and mydriatics are helpful in treating severe ocular hypotony.

Rifabutin

Rifabutin is used for prophylaxis of *M. avium* infections and can cause sight-threatening anterior uveitis, commonly associated with hypopyon. Fine, white, stellate, corneal endothelial deposits, in the periphery are also seen. Treatment includes topical corticosteroids, mydriatics and dose reduction of rifabutin.

Proteosome inhibitors can result in cataract formation and IRIS symptoms.

MEDICAL MANAGEMENT OF HIV INFECTION

HAART has changed the face of AIDS patients by leading to dramatic decreases in HIV-related morbidity and mortality in the developed as well as developing world. It is defined as "an antiretroviral regimen that can reasonably be expected to reduce the viral load by <50 copies/ml in treatment-naive patients".

The standard HAART regimen since the late-1990s has consisted of combination therapy of three antiretroviral drugs from the three major drug categories, namely nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). The goal of HAART is to achieve sustained viral suppression, minimize drug resistance and simplify dosage pattern. The immune recovery resulting from HAART is due to an absolute increase in CD4 cell count first through an increase in memory T-cells followed by the renewed production of naive CD4 T-cells.

Since the introduction of HAART, the incidence of ocular opportunistic infections causing retinitis, such as CMV, varicella zoster virus (VZV), tuberculosis and toxoplasmosis, has dramatically decreased. Before the introduction of HAART, CMV retinitis affected 30–40% of HIV-infected individuals, with visual loss primarily due to CMV involvement of the posterior retina and retinal detachment; it was also suggested that upon careful examination, 30% of patients with CD4 cell counts below 50 cells/μl would be harbouring CMV retinitis. However, IRU secondary to HAART has become a major visually threatening condition. High rates of ocular syphilis have been documented as well among patients receiving HAART.

CONCLUSIONS

Ocular disease is a late sequela of HIV infection. With newer and more effective treatment and widespread use of HAART, the lifespan of HIV-infected individuals is likely to increase and with it will multiply the number

of cases of ophthalmic disease. With implementation of HAART, the frequency of some ophthalmic manifestations such as KS is decreasing, but other complications such as IRU are on the rise. The long-term effect of treatment is still not fully understood, and therefore it is important for the clinicians to continuously monitor the patients look for iatrogenic complications in the eye. Finally, HIV has a very varied ophthalmic spectrum and it is important to maintain a high level of suspicion for timely detection and treatment of ocular morbidity, to give the patient a better quality of life.

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Compounding prisms – experience with strabismus

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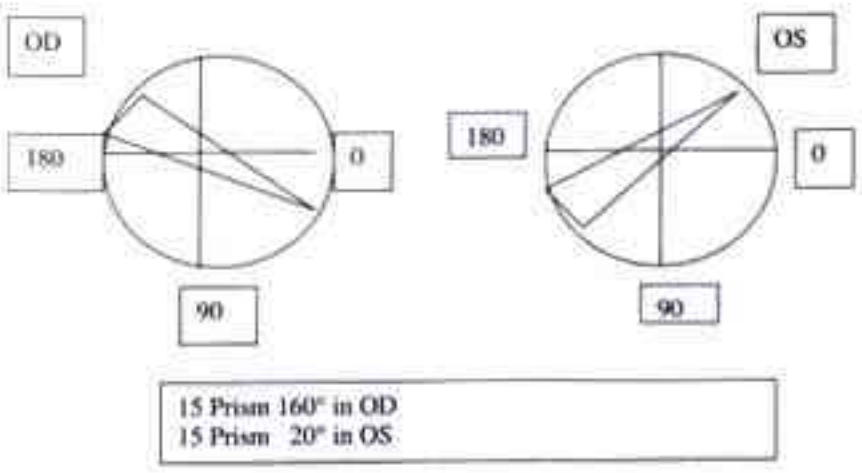
INTRODUCTION

Prescribing prism to phoria and tropia patients is common in optometry and ophthalmology practices. Prism prescription to heterophoria patients is usually based on Sheridan or Percival criteria, however prism prescription to tropia patients is based on their diagnostic profile. In other words, prescribing prism to tropias can be to stabilize or establish tropias, to eliminate diplopia and/or to improve cosmesis in some unresolvable cases.[1] A case report on prescribing prism for constant tropia is discussed below.

CASE REPORT

A 35-year-old man was referred to orthoptic department of a tertiary eye care hospital with complaints of diplopia. The patient had a history of penetrating keratoplasty after which developed binocular diplopia. He also had a history of increased intraocular pressure before penetrating keratoplasty. He denied diplopia history anytime in his life before. He was on antiglaucoma medications. Binocular diplopia manifested when patient came for post-operative check-up after a month.

Visual acuity in the right eye was 6/5, N6 without any correction. The vision in left eye was 6/18 with $+2.00/-3.50 \times 40$ and near addition of +3.00 DS to achieve N10. Extraocular motility check did not show any abnormality. Hirschberg test showed left esotropia. On doing Maddox rod test, the following finding was noted: 27 Prism BO and 13 Prism BU (right eye), for both short and long sights. On utilizing the compound prism table,[2] the amount of prism was found to be 30 prism at 25°. The resultant prism can also be arrived by using the following formula: $P^2 = H^2 + V^2$ and $\tan a = V/H$, where H is the horizontal deviation in prism (diopter), V the vertical deviation in prism (diopter) and P the prism (diopter). The patient was given equal-amount prisms for both eyes.



With this equal distribution between the right and left eyes, patient still felt diplopia though reduced. So, 30 prism at 20° was fully tried in front of the left eye. The patient felt that he was able to see fused images. The 6/60 letter was shown and the patient was able to see the single image. Near and distant scanning of the visual space was easy and devoid of doubling of images. However, the patient felt peripheral images with the Fresnel prism. The patient also expressed relatively decreased vision over the Fresnel prism. On checking the visual acuity, the right vision was 6/5 and the left eye vision was 6/24. The patient was given demo Fresnel prism correction over a dummy glass and asked to come after a day. On examination the next day, the patient was subjectively happy that he was able to see all the objects as a single image except for slight peripheral distortion in the left field of view. Finally, the patient was prescribed full correction in the left eye with Fresnel prism and advised to report after three months of usage.

DISCUSSION

Prescribing prism for strabismus in order to remove diplopia is commonly practiced. The ideal method of quantifying the amount of deviation especially to prescribe prisms is Maddox rod method for both long and short eyesight. This case report highlights the importance of compounding prisms before prescribing. This case report also highlights the procedure to use the compounding prism table to arrive at the amount of prism and at which axis it should be prescribed. This case report also highlights the equal distribution of the prism between the eyes as the first mode and if it does not work, to try to prescribe prism in one of the eyes, preferably the deviating eye. The effects of large amounts of prescribed prism in one lens can cause asthenopia, diplopia, cosmesis and nausea.[3] Due to the

limitation of glass prisms, wherein we could not prescribe high prisms, Fresnel prism will be the ideal choice for deviation of this magnitude. It is also important to note that the Fresnel prism would also impair the visual acuity and contrast sensitivity function of the user.

Ideal approach to patients reporting with sudden onset of diplopia will be to first rule out potentially life-threatening aetiologies. In adults, especially those with hypertension or diabetes, vasculopathic ischaemic infarction is a common cause of fourth-nerve paresis. Midbrain or cerebellum tumours, aneurysm and ischaemia can also cause paresis, resulting in diplopia. In this case, all these potential causes were ruled out. The next history will be to know the characteristic representation of the diplopia – horizontally, vertically or diagonally displaced. This information will help us to decide the type of prism to be used to negate the diplopia. For example, in diagonally displaced images, vertical prism is not warranted for secondary vertical deviations. A simple method to identify primary vertical deviation is by following a general guideline: secondary verticals tend to be very small and primary verticals are larger. If an intermittent exotrope has a 25Δ horizontal displacement and only 2Δ or 3Δ vertically when the eyes are strabismic, this is most likely a secondary vertical, and correction with vertical prism is not necessary. If the patient instead reports 13Δ to 20Δ vertical separation in addition to the horizontal deviation, he or she is unlikely to be able to align the eyes vertically even if the patient could pull his or her eyes straight horizontally. The latter is more likely to be a primary vertical, and horizontal and vertical prism will likely be required to relieve the patient's symptoms.

The next step is to determine whether diplopia is worse at long or short, upgaze or downgaze, or in any particular field of gaze. A deviation that is the same in all fields of gaze with either eye fixating is called a comitant deviation. A deviation that varies in size from gaze to gaze or varies when the right eye fixates vs. when the left eye fixates is considered incomitant or non-comitant. A non-comitant deviation often indicates a muscle underaction or paresis.

Once you determine comitancy, it required to know if the patient has the potential for normal correspondence. One method requires merely a Maddox rod, which should already be available as an accessory lens in your trial lens set or commonly on the other end of a cover paddle.

For a patient with normal correspondence and diplopia, one simple way to determine an acceptable amount of prism that eliminates the diplopia is by Maddox rod test. The clinical finding that a patient may need higher amounts of prism as smaller targets are used may be explained by Panum's area. Panum's area allows for some imprecision in ocular alignment without the perception of diplopia. The size of Panum's fusional area is smallest at the fovea and increases in size as you move farther into the retinal periphery. Small targets restricted to the central area of retina will be perceived as diplopic, easier than larger objects that can take advantage of the larger extent of Panum's area in the retinal periphery.[4]

After arriving at the total prism, the patient is allowed to wear the trial prism for a while and experience the visual space for at least a day before prescribing for constant use. Glass prisms up to 6 prism is easily worn. Beyond 6 prism, it is usually acceptable to go in for Fresnel prism. Ideally, it is better to give equal amount of prisms in both eyes. However, as in this case if the patient prefers total prism in one of the eyes, it is better to prescribe in one eye alone.

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Principles of real-time polymerase chain reaction and its application in clinical microbiology

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INTRODUCTION

Polymerase chain reaction

With the advent of polymerase chain reaction (PCR) in the mid-1980s, scientific progress of molecular biology has been revolutionized several fold. PCR has become a standard laboratory procedure that is being applied in all biological sciences. The most basic application of PCR is that it can amplify a small amount of template DNA (or RNA) into large quantities in a few hours. This is performed by mixing the DNA with primers (forward and reverse) on either side of the specifically identified DNA, *Taq* polymerase (of the species *Thermus aquaticus*, a thermopile whose polymerase is able to withstand extremely high temperatures), free nucleotides and buffer.

Principle of real-time PCR

Also called quantitative real-time polymerase chain reaction (Q-PCR/qPCR) or kinetic polymerase chain reaction, real-time PCR is used to amplify and simultaneously quantify a portion of DNA of interest. DNA is quantified as it accumulates in the reaction in *real time* after each amplification cycle. Like in other PCR amplification reactions, primers are designed to amplify the target. Unlike in PCR, the amplification is detected by any one of the following:

1. A fluorescent dye such as SYBR green that intercalates with double-stranded DNA is used. As the DNA amplifies, the dye gets incorporated and an increase in product leads to increased fluorescence intensity (Figure 1).
2. In addition to the primers, a short-sequenced DNA complementary to the DNA target called probe labelled to a fluorescent dye is used. In addition to the fluorescent dye, the probe also has at its close proximity a quencher. As long as they are in close proximity, the fluorescence emitted by the dye is not detected. As the reaction proceeds, during the annealing stage of the PCR, both probe and primers anneal to the DNA target.
   A. The *Taq* polymerase used in the reaction has 5′ to 3′ exonuclease activity. The probe bound to the target DNA will be cleaved during the strand elongation by the *Taq* polymerase. An increase in the product targeted by the reporter probe at each PCR cycle therefore causes a proportional increase in fluorescence due to the breakdown of the probe and release of the reporter.
   B. Fluorescence emitted is detected and measured in the real-time PCR thermocycler and its geometric increase corresponding to exponential increase of the product is used to determine the threshold cycle (CT) in each reaction.

More than one gene target can be identified and quantified in real-time PCR by multiplexing the primers in the same reaction and using specific probes with different coloured labels, provided that all genes are amplified with similar efficiency.

Advantages of real-time PCR

A significant advance is the development of instruments that allowed real-time monitoring of a PCR reaction using fluorescence within the reaction vessels. The technology is very flexible with the development of many alternative instruments and fluorescent probe systems. The technical importance of real-time PCR assays is that it can be completed rapidly with no procedures required for post-amplification identification of the amplification products, as they are available as

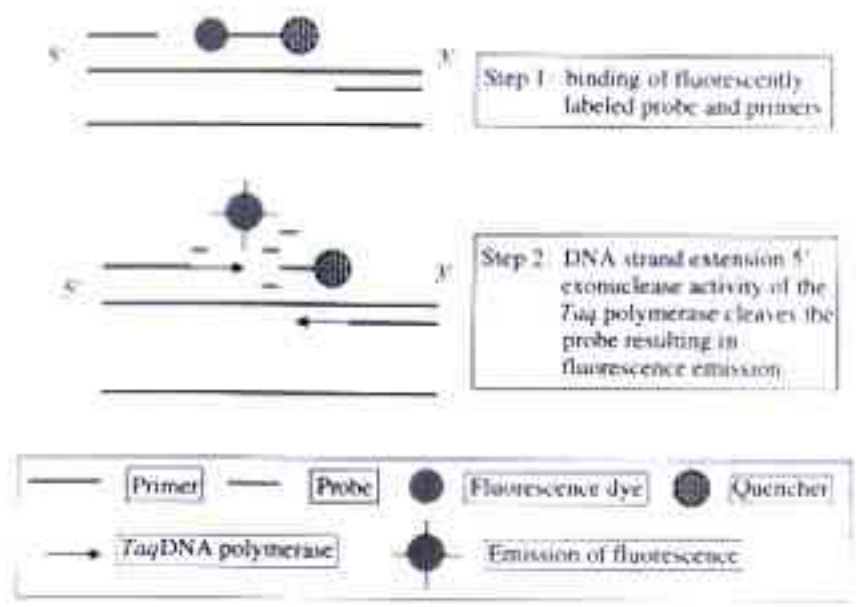


Figure 1

the reaction proceeds. The results are highly accurate compared with size analysis on gels after standard PCR. The most important result is the ability of the scientists to analyse the progress of the reaction with accurate quantification of the target sequence over a wide dynamic range, provided suitable standards are available. Further investigation of the real-time PCR products within the original reaction mixture using combination of probes and melting analysis sequence variants including single-base mutations can be detected. The development of fluorescent methods for a closed-tube polymerase chain reaction has greatly simplified the process of nucleic acid quantification.

Real-time PCR has found applications in many branches of biological science. Applications include gene-expression analysis, the diagnosis of infectious diseases, quantification of DNA/RNA of infectious agents and human genetic testing. Real-time applications for mutation detection include detecting alterations associated with inherited disease, acquired alterations in oncology, and microbial or viral mutations associated with drug resistance in infectious diseases.

Real-time PCR applications

Real-time PCR can be applied to traditional PCR applications as well as new applications that would have been less effective with traditional PCR.

With the ability to collect data in the exponential growth phase, the power of PCR has been expanded into applications such as:

- viral quantitation,
- quantitation of gene expression,
- array verification,
- drug therapy efficacy,
- DNA damage measurement,
- quality control and assay validation,
- pathogen detection and
- genotyping.

REAL-TIME PCR IN CLINICAL MICROBIOLOGY

The introduction of real-time PCR assays in the clinical microbiology laboratory has led to significant improvements in the diagnosis of infectious disease. With the explosion of this technique since its introduction, several hundred reports have been published describing applications in clinical bacteriology, parasitology and virology. There are few areas of clinical microbiology which remain unaffected by this new method. It has been particularly useful to detect slow-growing or difficult-to-grow infectious agents. However, its greatest impact is probably its use in the quantitation of target organisms in samples. The ability to monitor the PCR reaction in real time allows accurate quantitation of target sequence over at least six orders of magnitude. The closed-tube format which removes the need for post-amplification manipulation of the PCR products also reduces the likelihood of amplicon carryover to subsequent reactions reducing the risk of false-positives. As more laboratories begin to utilize these methods, standardization of assay protocols for use in diagnostic clinical microbiology is needed; also participation in external quality control schemes is required to ensure quality of testing.

The quantification assay basically has five standards, and the copies of DNA present are known. Along with the standard, a negative control which does not contain any DNA but the reagent and the DNA extract from the specimen, where the copy number needs to be determined is run; as and when the reaction proceeds, the assay can be visualized. The system plots a graph against the cycle and the fluorescence emitted. The copy number in the specimen is estimated from the graph.

POSITIVE AND NEGATIVE CONTROLS

The positive control should be at a concentration near the lower limit of detection of the assay to challenge the detection system, yet at a high enough level to provide consistently positive results. However, an optimal negative control is a sample containing non-target nucleic acid to demonstrate that non-specific PCR amplification and detection of amplified product is not happening.

In addition to the gene of interest, a housekeeping gene target is used to rule out the presence of PCR inhibitors. It is important to monitor the kinetics of the amplification of housekeeping gene to know the presence of PCR inhibitors which will be shown by the absence of amplification.

REAL-TIME PCR IN THE STANDARD PROCEDURES FOR CLINICAL AND RESEARCH PURPOSES ARE STUDIED ON THE FOLLOWING INFECTIOUS AGENTS AT THE MICROBIOLOGY LABORATORY, SANKARA NETHRALAYA

Human immunodeficiency virus

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that can lead to acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. Infection with HIV occurs by the transfer of blood, semen, vaginal fluid or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The four major routes of transmission are: unprotected sexual intercourse, contaminated needles, breast milk and transmission from an infected mother to her baby at birth (vertical transmission). The persistence of proviral human immunodeficiency virus type 1 (HIV-1) DNA reservoir represents one of the major drawbacks to the total eradication of HIV-1. Although serological methods are often helpful in the detection of HIV antibodies, HIV-infected individual in "window period" are not detected as there is no antibody response. The RNA detection of the virus by real-time PCR technique is the only sensitive method recommended for this "window period". In addition, the quantitative determination of

proviral HIV-1 DNA load offers significant therapeutic information, especially when the HIV-1 RNA levels drop below the detectable limits during the highly active antiretroviral therapy (HAART) treatment. Moreover, the detection of HIV-1 proviral DNA is an important diagnostic marker in the evaluation of the progress of HIV-1 infection. Several gene targets are used for designing the primers, which include terminal repeat (*LTR*) region, *gag, pol, envelope*.[2] The virus can be detected in any of the human body fluids, whole blood, serum and plasma. The rate of detection is reported to be infrequent in the tears when compared with other specimens.

Figure 2 shows the real-time PCR performed with five standards and clinical specimens for detection of HIV.

Chikungunya real-time PCR

Chikungunya is a RNA virus belonging to the family Togaviridae, genus *Alphavirus*. The disease Chikungunya also known as Chikungunya virus disease or Chikungunya fever is characterized by severe, sometimes persistent, joint pain (arthritis), as well as fever and rash. The disease is spread by the bite of infected mosquitoes and the clinical symptom resembles that of dengue fever. Four to 12 weeks later the febrile period, individuals have been reported to develop iridocyclitis and retinitis with a typically benign clinical course. Less-frequent ocular lesions include episcleritis.

The virus-isolation technique is cumbersome, and serological diagnosis is so often not reliable. The best method of detection of the virus is real-time PCR. Real-time PCR is the best method to quantify the virus copy numbers. Real-time PCR has been standardized targeting Capsid (C), Envelope E1 and E2, and part of non-structural protein (*NSP*)[1] gene of the virus.[3,4]

In our laboratory, intraocular specimens and serum/plasma specimens collected from affected individuals are subjected for the detection of Chikungunya virus.

Figure 3 shows the real-time PCR performed with five standards and clinical specimens for detection of Chikungunya virus.

Human cytomegalovirus real-time PCR

Cytomegalovirus is a DNA beta-herpes virus and is ubiquitous in nature. Infection caused by the virus is usually silent in immunocompetent individuals, although acute CMV infection may also cause a brief mononucleosis-like malaise in immunocompetent adults. It is proven to establish latency in macrophages. Following infection, the virus resides in endothelial cells, macrophages or granulocyte stem cells and may cause re-infection if the host is rendered immunosuppressed, as by HIV or by immunosuppressive agents used during transplantation and chemotherapy. From infected pregnant women, the virus gets transmitted to foetuses causing congenital defects, as they have poorly developed immune systems. As normal population is known to harbour baseline copy number of virus, estimation of the quantity of virus is very important to distinguish normal subjects from the diseased, especially in blood samples. Theocular infections caused by the viruses include retinitis, which is more commonly seen among immunocompromised individuals with AIDS, renal or bone

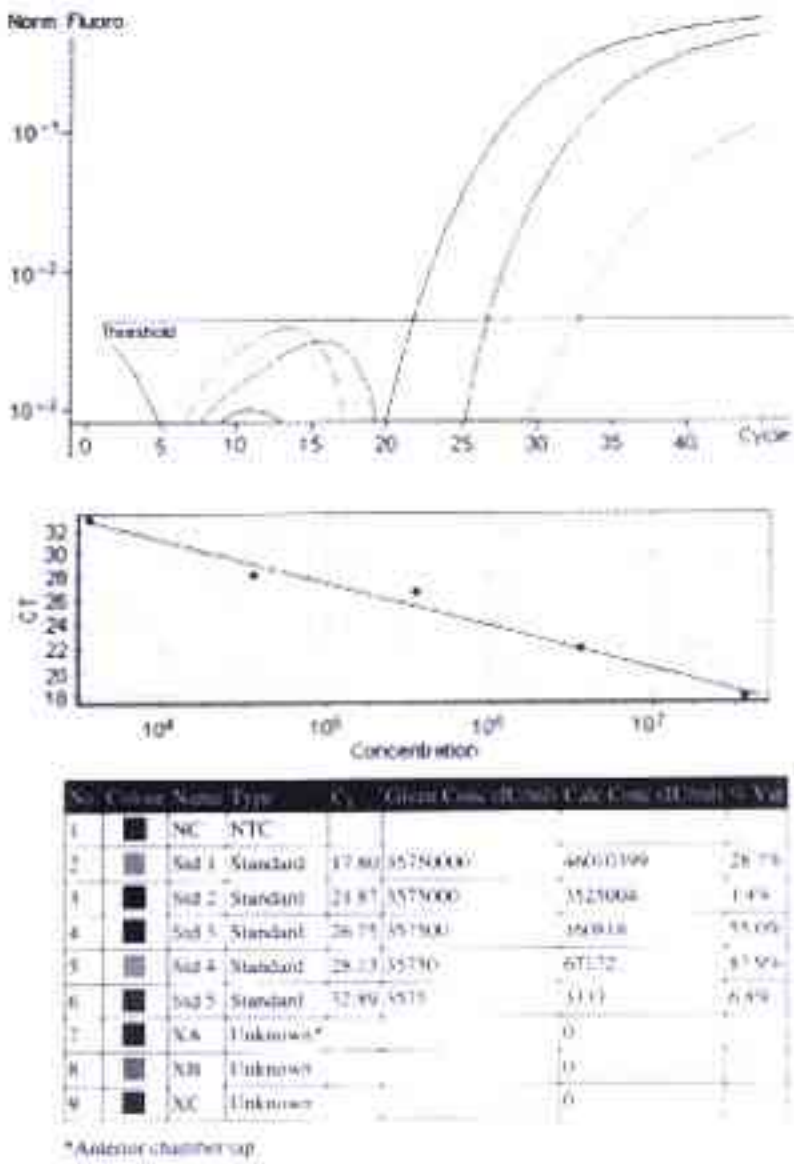


Figure 2

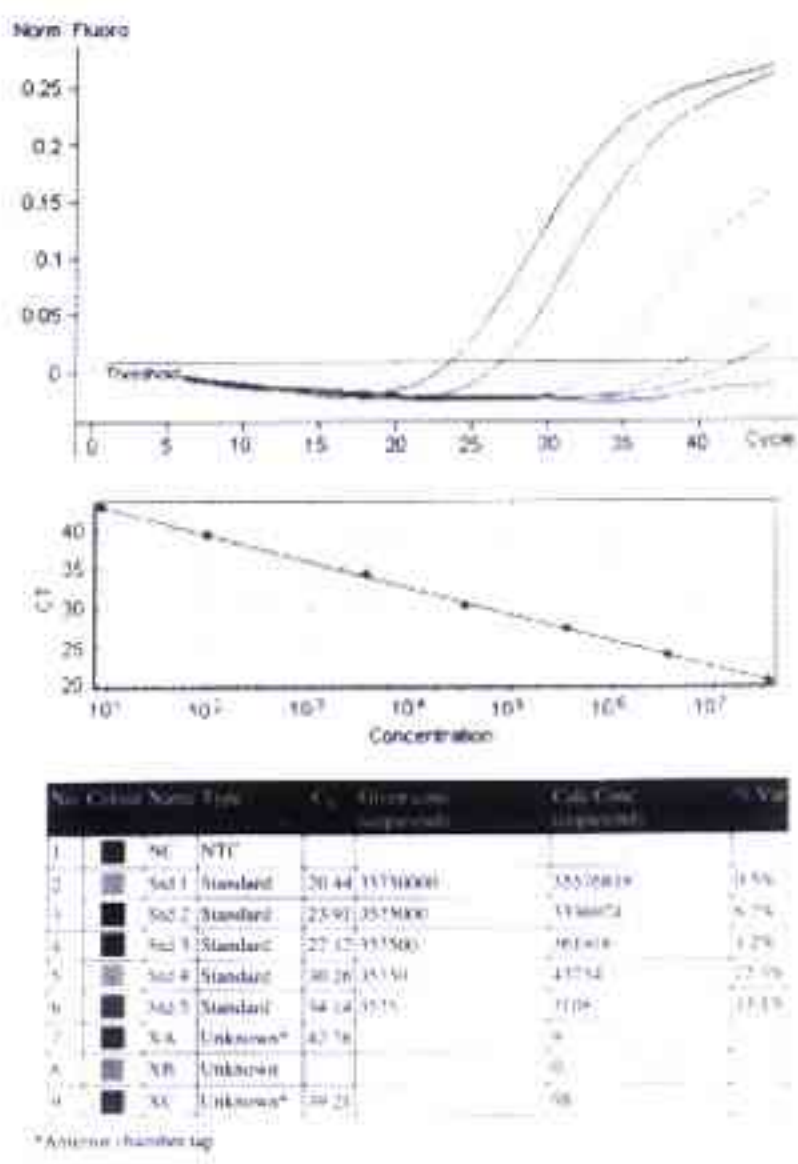
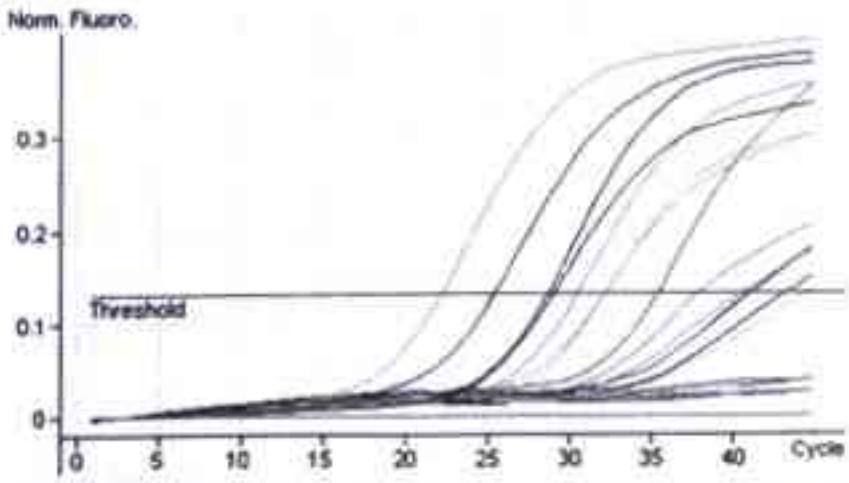


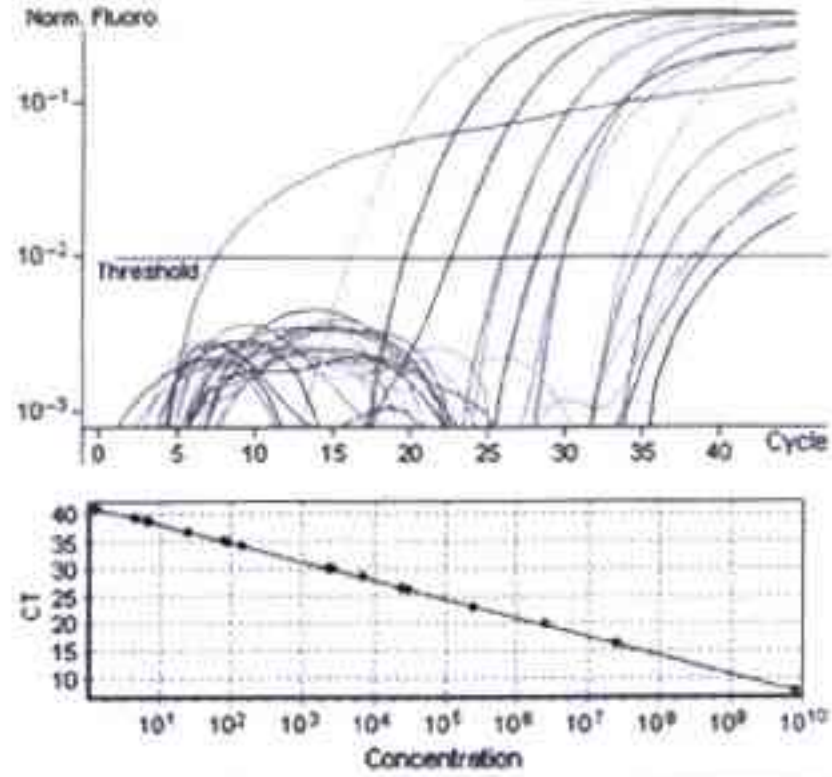
Figure 3



No.	Colour	Name	Type	Ct	Concentration (copies/ml)	Calculated concentration (copies/ml)	% Var
1		NC	NTC				
2		Std 1	Standard	22.37	25000000	39821500	59.3%
3		Std 2	Standard	28.84	2500000	270305	89.2%
4		Std 3	Standard	25.63	250000	3223094	1189.2%
5		Std 4	Standard	32.31	25000	18485	26.1%
6		Std 5	Standard	35.54	2500	1523	39.1%
7		3680/08	Unknown	41.23		19	
8		6038/08	Unknown	40.62		30	
9		6110/087	Unknown	41.10		21	
10		6614/08	Unknown			0	
11		6815/08	Unknown	43.37		4	
12		6816/08	Unknown			0	
13		6532/08	Unknown	44.70		1	
14		XA	Unknown			0	
15		XB	Unknown	32.43		16786	
16		XC	Unknown			0	
17		XD	Unknown	38.08		214	
18		XE	Unknown*	30.69		64496	
19		XF	Unknown			0	
20		XG	Unknown*	29.21		202184	
21		XH	Unknown			0	

*Anterior chamber tap.

Figure 4



No.	Colour	Name	Type	Ct	Concentration (copies/ml)	Calculated concentration (copies/ml)	% Var
1		NC	NTC	39.02		5	
2		Std 1	Standard	16.20	25000000	24841155	0.6%
3		Std 2	Standard	19.75	2500000	2228401	10.9%
4		Std 3	Standard	22.76	250000	290901	16.4%
5		Std 4	Standard	26.28	25000	26745	7.0%
6		Std 5	Standard	29.91	2500	2267	9.3%
7		XA	Unknown				
8		XB	Unknown				
9		XC	Unknown*	7.63		829847442n	
10		XD	Unknown	36.55		25	
11		XE	Unknown	40.91		1	
12		XF	Unknown				
13		XG	Unknown	34.74		86	
14		XH	Unknown	38.54		7	
15		XI	Unknown				
16		XJ	Unknown	38.42		7	
17		XK	Unknown	34.89		78	
18		XL	Unknown	29.92		2265	
19		XM	Unknown	33.98		144	
20		XN	Unknown	28.30		6756	
21		XO	Unknown	26.10		30133	

*Vitreous aspirate

Figure 5

marrow transplant recipients. Many gene targets are used in real-time PCR technique, namely morphologically transforming regions (mtrII), immediate early antigen (IE) and glycoprotein regions of the virus.[5,6] PCR techniques for the detection and quantification of HCMV is applied to peripheral blood, intraocular specimens and urine.[5]

Figure 4 shows the real-time PCR performed with five standards and clinical specimens for detection of human cytomegalovirus.

Herpes simplex virus (HSV) real-time PCR[7]

Herpes simplex virus is a widespread human pathogen and causes morbidity and mortality in immunosuppressed individuals. Quantification of viral load is recommended to know the decrease in viral load following institution of acyclovir therapy by real-time PCR. The gene targets used are many, which include glycoprotein, DNA polymerase gene, etc. Clinical specimens used include peripheral blood, body fluids, vesicles, etc.

Figure 5 shows the real-time PCR performed with five standards and clinical specimens for detection of human herpes simplex virus.

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Introduction to biostatistics 2

Descriptive statistics

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INTRODUCTION

In the previous article, we discussed in detail about the types of variables and their implications in statistics. In this article, we will discuss the various methods by which an investigator could measure and describe data in a study.

Descriptive statistics are used to describe the basic features of the data in a study. They are numbers that are used to summarize and describe data.[1] Together with simple tabular and graphical analyses, they form the basis of virtually every quantitative as well as qualitative analyses of data. In a research study, we may have lots of measures or we may include a large number of people on any measure. Descriptive statistics help us to simplify large amounts of data in a sensible way.

Following are the methods used in descriptive statistics:

1. tabular representation,
2. graphical representation,
3. central tendency and
4. dispersion.

TABULAR REPRESENTATION

As poets give beautiful and meaningful presentation to the words by arranging them properly, the main objective of a statistical table is to summarize the data in a simple and systematic way so as to be easily analysed and interpreted for specific requirements. According to D.W. Paden and E.E. Lindquist, "tabulation is a medium of communication of great economy and effectiveness for which ordinary prose is inadequate ...".

The main objectives of tabulation are

- to simplify complex data,
- to facilitate comparison,
- to help reference,
- to economize space,
- to depict trend and pattern of data and
- to facilitate statistical processing.

Essential parts of a table

Following are the essential parts of a good statistical table:

- table number,
- title,
- head notes,
- captions and stubs,
- body of the table,
- footnote and
- source note.

GRAPHICAL REPRESENTATION

Facts and figures do not catch our attention unless they are presented in an interesting way. Graphical representation of data is one of the most commonly used modes of presentation, which makes the reading more interesting, less time-consuming and easily understandable.

There are different types of graphs, including bar graphs, pie charts, frequency polygon, histogram and scatter diagram.

Bar diagram

It is a method of presenting data in which frequencies are displayed along one axis and categories of the variable along the other, the frequencies being represented by the bar lengths. There are different types of bar diagrams.

a) **Simple bar diagram**: A simple bar diagram is used to represent only one variable. For example, the number of surgeries performed during Jun'08 to Dec'08 is listed below:

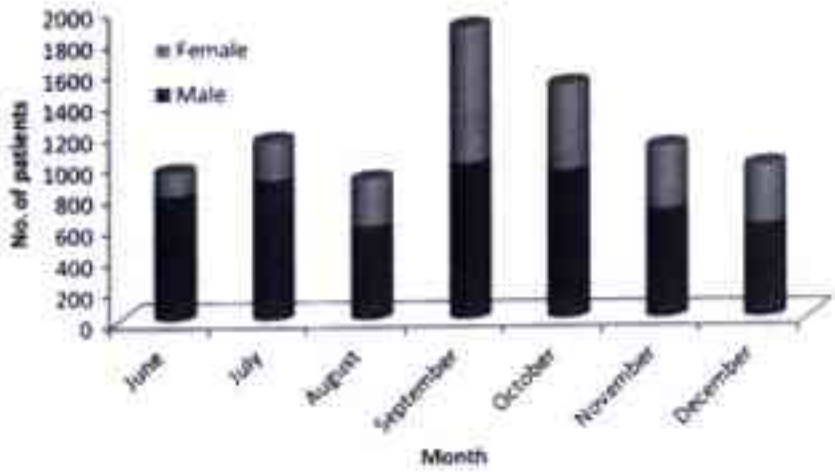
Month	Number of surgeries
June	950
July	1150
August	900
September	1875
October	1500
November	1100
December	975

The same data is represented as a simple bar diagram as shown below:

b) **Sub-divided bar diagram**: In a sub-divided bar diagram, the bar is sub-divided into various parts in proportion to the values given in the data, and the whole bar represents the total. Such diagrams are also called component bar diagrams. For example, the number of males and females who underwent surgery in June–December 2008 is shown below:

	Number of surgeries	
Month	Male	Female
June	800	150
July	900	250
August	600	300
September	1000	875
October	950	550
November	700	400
December	600	375

The above data is being represented as a sub-divided bar diagram as shown below:

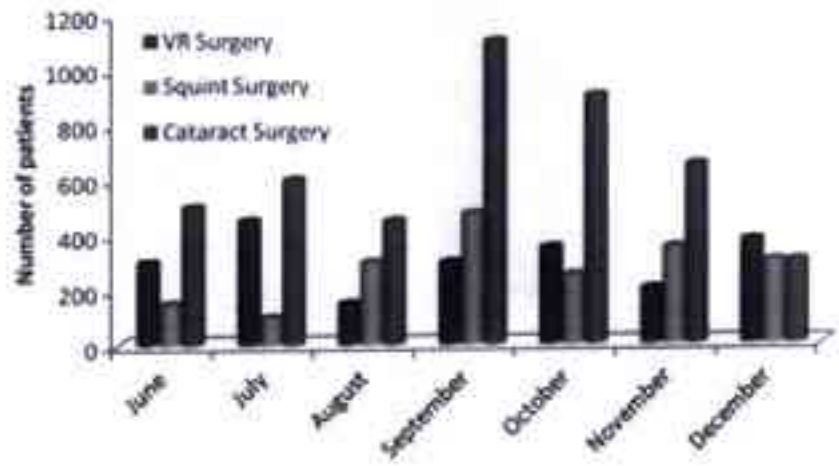


Multiple bar diagram

It is used for comparing two or more sets of statistical variables. Bars are constructed side by side to represent the set of values for comparison. For example, number of different types of ophthalmic surgeries performed in the above months are shown below:

	Number of surgeries		
Month	VR surgery	Squint surgery	Cataract surgery
June	300	150	500
July	450	100	600
August	150	300	450
September	300	475	1100
October	350	250	900
November	200	350	650
December	375	300	300

The data is represented as a multiple bar diagram as shown below:



Pie charts

A pie chart (or a circle graph) is a circular chart divided into sectors, illustrating relative magnitudes, frequencies or percentages. In a pie chart, the arc length of each sector is proportional to the quantity it represents. The result of a clinical study of 200 patients to grade hypertension (HTN) in smokers is depicted as follows:

Grade of HTN	No. of patients	Percentage
Mild	32	16
Moderate	70	35
Severe	98	49

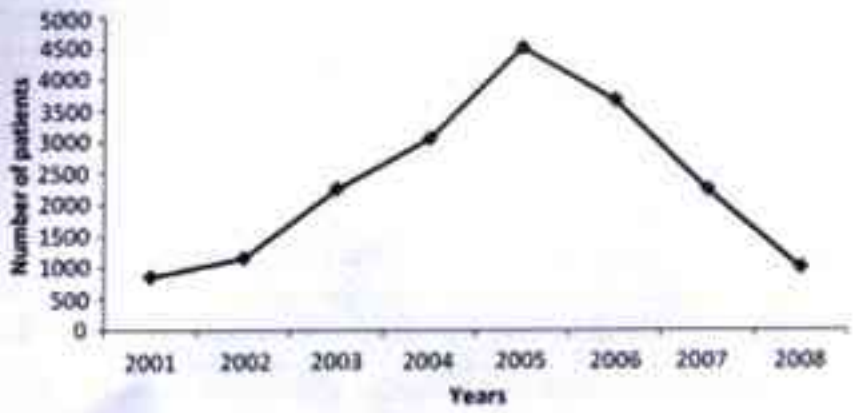
The above result is expressed as a pie chart as shown below:

Frequency polygon

Frequency polygons are a graphical device for understanding the shapes of distributions. It is used to represent the time-series data and also for predicting the trend and comparing distributions.[2] In frequency polygons, *x*-axis should be time-series data, whereas *y*-axis should indicate the frequency of each time interval. For example, the number of patients screened for glaucoma in 2001–2008 is shown below:

Years	No. of patients screened	Years	No. of patients screened
2001	850	2005	4500
2002	1150	2006	3680
2003	2250	2007	2250
2004	3050	2008	1000

The above data is shown as frequency polygon as given below:



Histogram

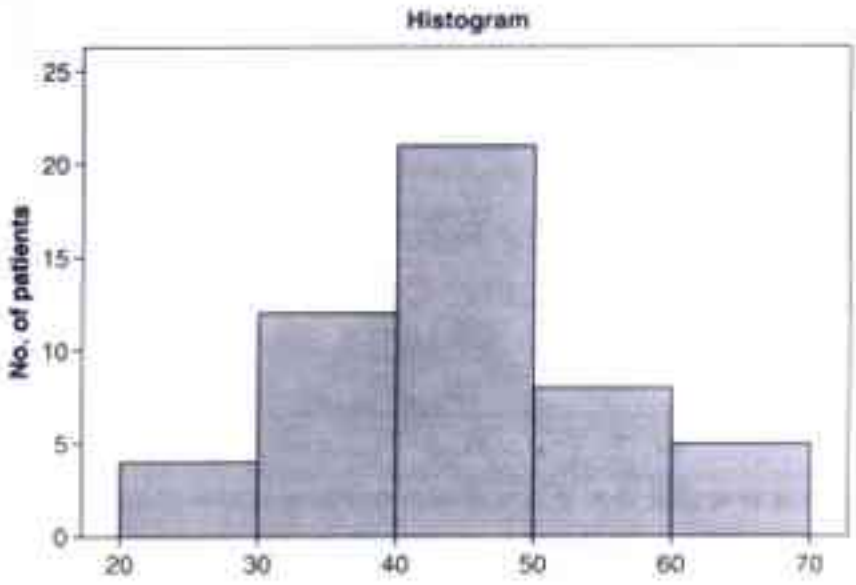
Histogram is a graphical display of tabulated frequencies (frequency distribution), shown as bars. It shows what proportion of cases fall into each of several categories. It conveys the following information:

(a) The general shape of the frequency distributions (normal, chi-square, etc.),
(b) Symmetry of the distribution and whether it is skewed and
(c) Modality — unimodal, bimodal or multimodal.

For example, the weight of 50 patients is given as follows:

Weight (in kg.)	No. of patients
20–30	4
30–40	13
40–50	20
50–60	8
>60	5

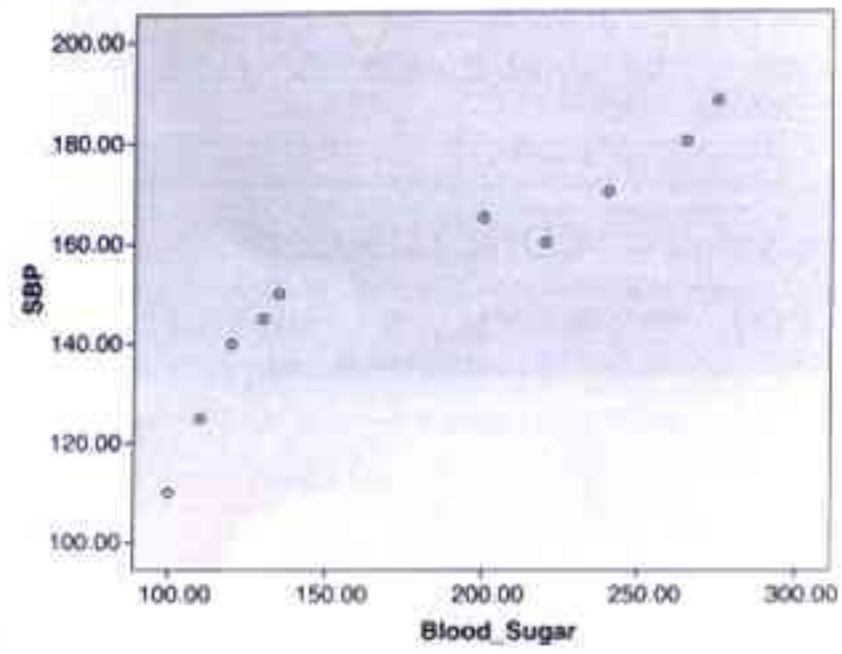
The above result is expressed as a histogram as follows:



Scatter diagram

Scatter diagram is a collection of points, each having the value of one variable determining the position on the horizontal axis and the value of the other variable determining the position on the vertical axis. It can suggest various kinds of correlations between variables with a certain confidence level. The correlation may be positive (rising), negative (falling) or null (uncorrelated). If the pattern of dots slopes from lower left to upper right, it suggests a positive correlation between the variables being studied. If the pattern of dots slopes from upper left to lower right, it suggests a negative correlation.

For example, the relationship between blood pressure (systolic BP, mmHg) and fasting blood sugar level (mg/dl) in 10 diabetic patients is shown in the scatter diagram as given below:



The above scatter diagram shows a strong positive linear correlation between the blood sugar and blood pressure levels.

CENTRAL TENDENCY

A measure of central tendency is a single number used to represent the "centre" of a group of data. Different variables may possess different numerical characteristics. So different measures of central tendency may better summarize the variable. The basic measures of central tendency are mean, median and mode.

Mean: Mean is the arithmetic average in which all the data are added and the total sum is divided by the total number of observations. It is the commonest measure of central tendency. Note that the symbol for the sample mean is a modified Roman letter X.

Median: Median is the middle-most observation and it divides the distribution into half. It is mainly used for the calculation of average of abnormal data. It is also called as the 50th percentile.

Mode: Mode is the maximum number of occurrences in a set of observations. It is used for determining the average of qualitative data.

MEASURES OF DISPERSION

The purpose of measures of dispersion is to find out how the data values are spread out or how far each element is from some measures of central tendency (average).[5] There are several ways to measure the variability of the data. They are as follows:

(i) range,
(ii) standard deviation and
(iii) quartile deviation.

Range: Range is the difference between the highest and lowest data element. It is the simplest measure of spread, but it gives only a rough idea about the presence of dispersion.

Standard deviation: It is a measure of the dispersion of data about a mean value. A low standard deviation indicates that the data is clustered around the mean, whereas a high standard deviation indicates that the data is widely spread with significantly higher or lower figures than the mean.

Quartile deviation: It is half the difference between the upper and lower quartiles in a distribution. It is a measure of the spread through the middle half of a distribution.

CONCLUSION

A good understanding of descriptive statistical concepts can assist researchers in presenting and interpreting the results of their studies. Such an understanding also helps clinicians to apply research results in their practice and to communicate with patients. In the next issue, we will discuss about testing of normality.

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Technology Update

Multifocal visual-evoked potential

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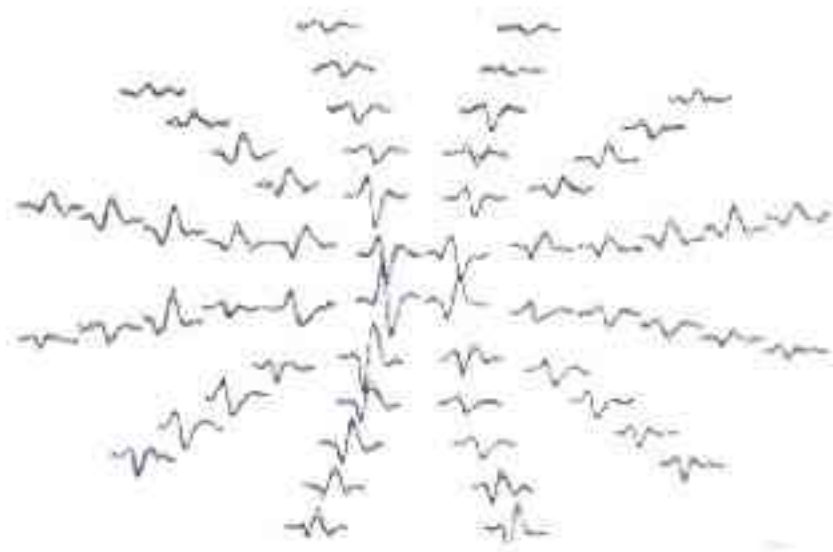
Visual-evoked potential (VEP) is extensively used to objectively assess the functional integrity of the visual pathway. It is generated at the visual cortex in response to either flash or pattern visual stimulation. The conventional VEP is a global response and does not reveal information about the local defect in the visual pathway. It primarily reflects the activity of the central visual field due to the magnified projection of macula at the visual cortex.

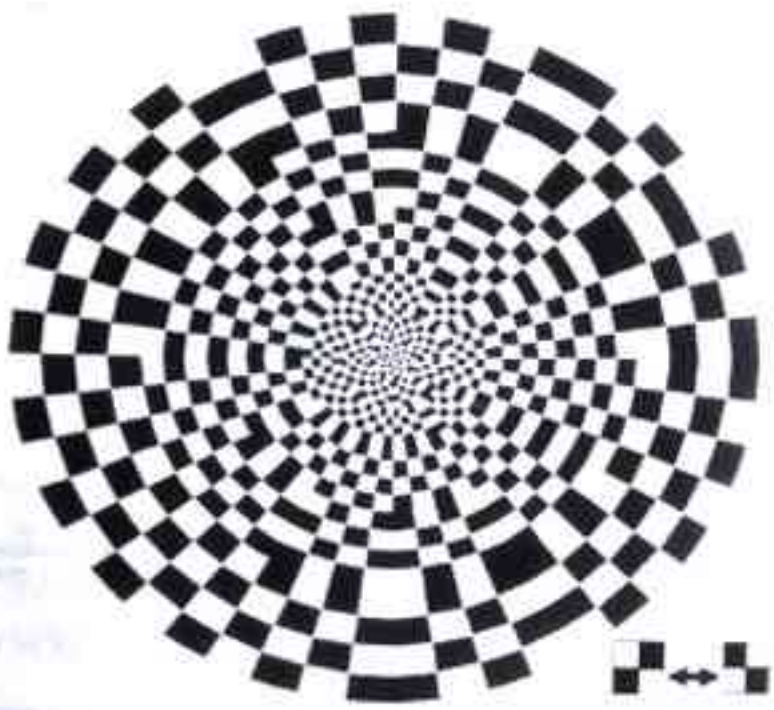
The multifocal VEP (mfVEP) reveals subtle local defects and allows for the topographical assessment of the visual field. Its origin is from the V1 areas of the visual cortex (Zhang and Hood, 2004). Baseler *et al.* (1994) developed the mfVEP that works in response to pattern-reversal stimulation with multifocal technology.



mfVEP responses from 60 locations

MULTIFOCAL VEP STIMULUS

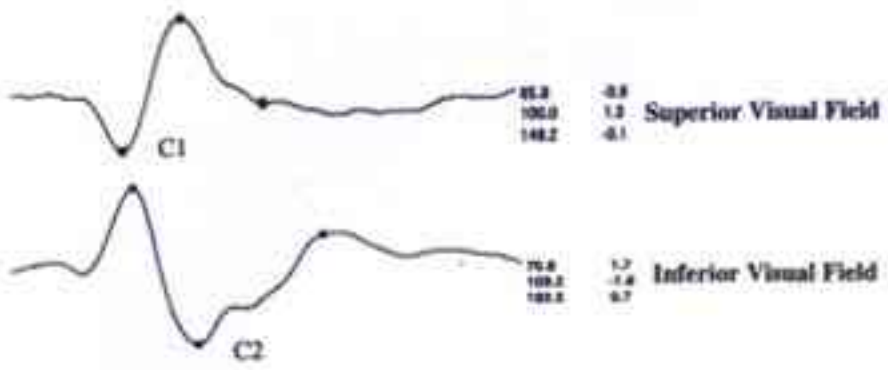
The stimulus of mfVEP is termed as Dartboard pattern that contains 60 sectors. It subtends 44.5 of visual diameter when viewed at 32 cm from the monitor (Hood and Greenstein, 2003). Each sector contains 16 checks of which eight are black and eight are white. The stimulus checks are scaled, based on cortical magnification factor. The black-and-white checks in each sector reverses independently according to a pseudorandom sequence known as a binary m-sequence. The responses are mathematically extracted by cross-correlating the continuous VEP signal with the stimulus sequence during real-time recording.



Dartboard stimulus

NORMAL VARIATION OF MFVEP RESPONSES

The local folding of the primary visual cortex and location of the electrode in relation to the calcarine fissure contribute to the intersubject variability of the response (Hood and Zhang, 2000; Hood and Greenstein, 2003). An interocular variability of about 5 ms exists between the nasal and temporal fields along the horizontal meridian, where the left eye leads the right eye in left visual field and vice versa. This is due to the difference in the conduction time of action potentials from unmyelinated temporal ganglion nerve fibres that travel farther to reach the optic disc than nasal fibres (Sutter and Bearse, 1999). The responses are also reversed in polarity along the horizontal meridian since the cells generating the responses in the visual cortex are oriented in opposite direction (Baseler et al., 1994; Hood and Greenstein, 2003).



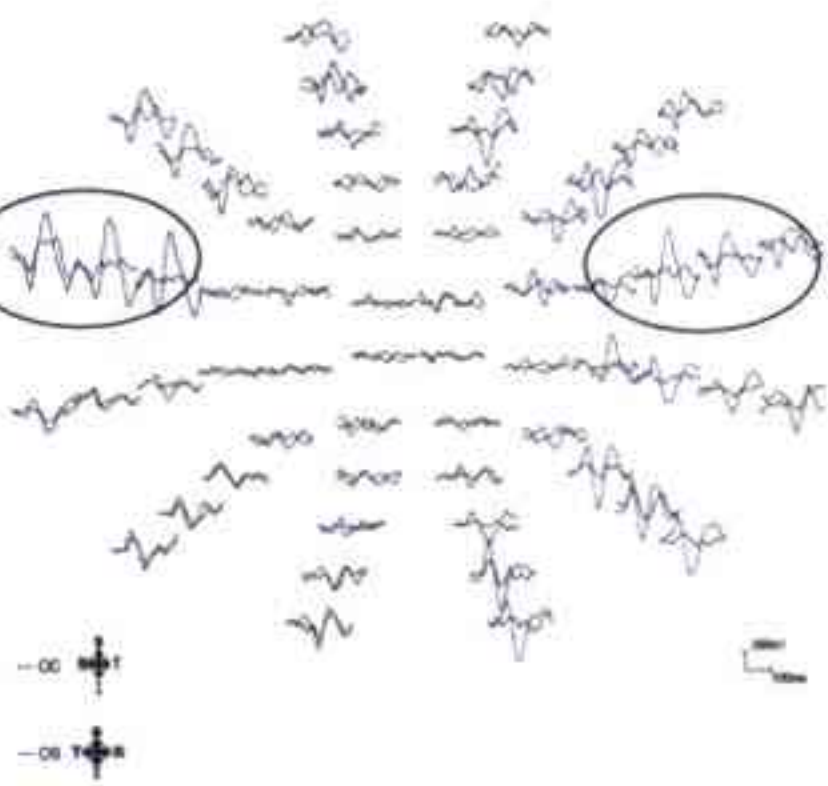
Components of mfVEP response

NOMENCLATURE OF PEAKS

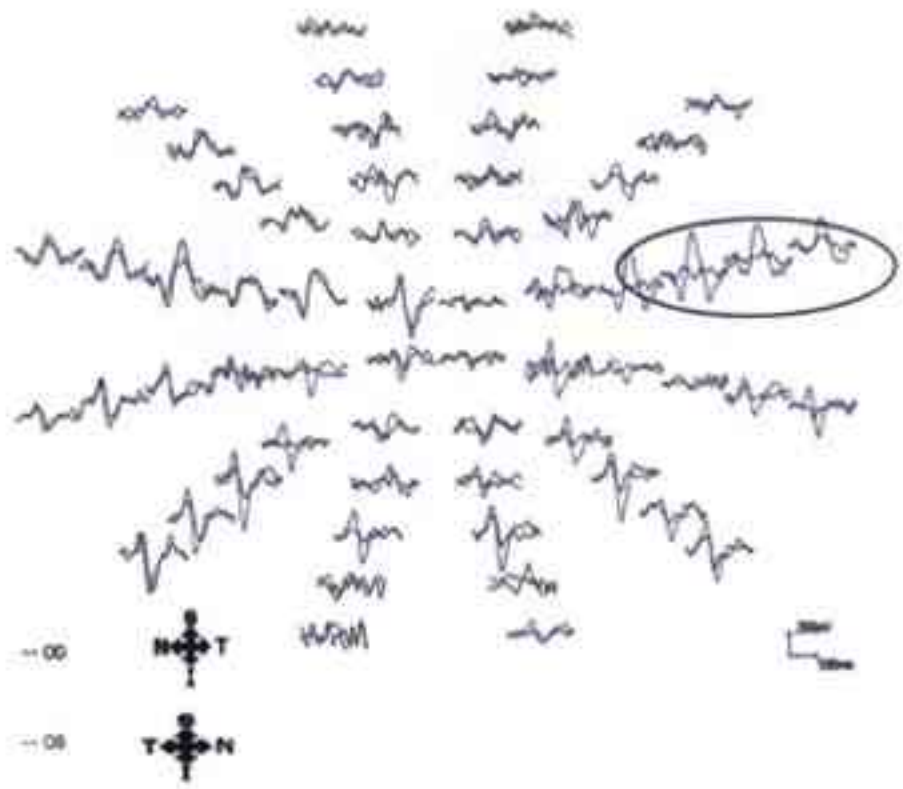
The typical mfVEP waveform is a biphasic wave which is extracted mathematically from the first slice of the second-order kernel. It consists of three peaks, namely C1, C2 and C3 where the C1 component arises at around 65 ms and C2 component arises at around 95 ms (Hood *et al.*, 2003).

BASIC TECHNOLOGY AND CLINICAL PROTOCOL

The stimulus is delivered by a cathode ray tube monitor at a frame rate of 75 Hz. The luminance of 100–200 Cd/m^2 is used for white checks and <1 Cd/m^2 is used for black checks. The band-pass filter is set between 3 and 100 Hz. The room light should be on par with illumination ideal to the stimulus luminance. Till date, there is no protocol recommended by the International Society for Clinical Electrophysiology of Vision Standard (ISCEV) for performing mfVEP. The procedure should be performed in the undilated pupil and any abnormality in pupil size should be noted. Gold disc surface electrodes are used to record mfVEP. Active electrodes are placed 4 cm above the inion and 4 cm lateral to and 1 cm above the inion on either side which are referred to as midline and lateral channel, respectively. The reference electrode is placed on the inion. The ground electrode is placed on the forehead (Hood and Greenstein, 2003).



Pituitary adenoma (bitemporal hemianopia)



Optic neuritis in left eye (non-recordable waveforms)

PREPARATION OF PATIENT

The patient should sit comfortably in front of the instrument. The position of the patient is monitored throughout the test with a camera unit in instrument setting. The refractive error is corrected with a refractor unit built within the instrument.

CLINICAL APPLICATIONS OF MFVEP

It

(a) helps to detect the local defect in the visual field,
(b) helps to understand the topography of the disease,
(c) is better than cVEP for retrochiasmatic lesions,
(d) helps to assess the patients whose visual field report is unreliable,
(e) is possible in some children who do not respond to HVF 30-2 testing,
(f) assists in differentiating between and monitors the progression of diseases in subjects with optic neuritis/compressive optic neuropathy,
(g) is performed along with multifocal electro-retinogram to differentiate retinal diseases from the optic nerve diseases, and
(h) is used in assessing the subjects with unexplained visual loss.

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9th Annual Meeting
of the
Uveitis Society Of India

9th USI Meet

December 4th, 5th & 6th 2009
at Sankara Nethralaya,
Chennai, India

Confirmed International Faculty

Dr. Narsing Rao, USA

Dr. Dean Elliot, USA

Dr. John Kempen, USA

Dr. Russel Read, USA

Dr. Manfred Ziehrut, Germany

Dr. S P Chee, Singapore

USI & Host Faculty

Important Dates

Conference Dates: 4th, 5th and 6th December 2009

Basic outline of the Programme

4th December 2009 :

- Morning Session : Indian Uveitis Patient Interest association meet
- Afternoon Session : Basics of uveitis: Instruction courses & Symposia

5th and 6th December 2009 :

- Didactic lectures by experts in the field
- Free papers and challenging case presentations with interactive sessions followed by discussion by panel members

5th December, 2009 : Uveitis Society of India General Body Meeting Followed by Banquet

Registration Form

Name : Dr/Mr/Ms: .....

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E-mail ID: .....

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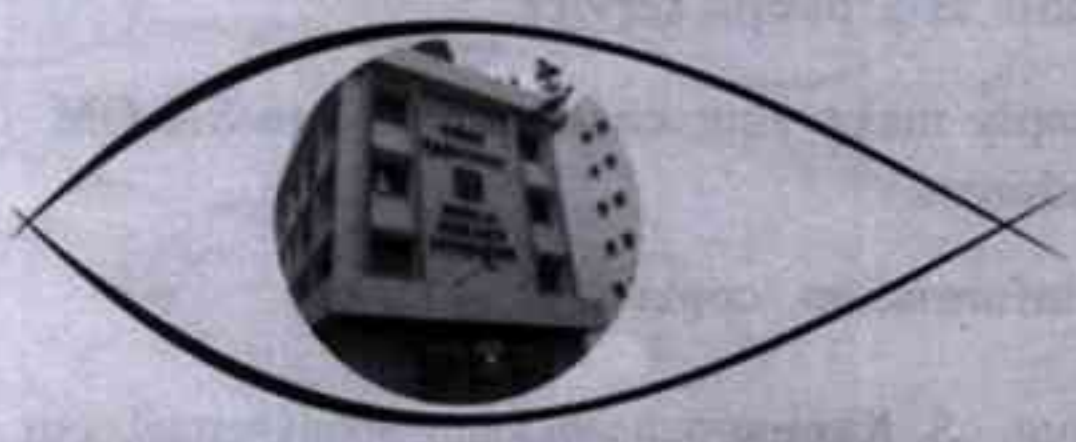
Please find enclosed my DD No .....

Dated: ..... for Rs. ..... towards registration.

DD should be drawn in favour of : Medical Research Foundation, Chennai, and mailed to the organising secretary.



Registration fee

Important Dates

I.Early Bird Registration Begins **Feb 1, 2009**

II.Early Bird Registration Ends **Sep 30, 2009**

Early Bird payment: Rs. 1500

Late/Spot registration: Rs. 2000

Organising Secretary

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