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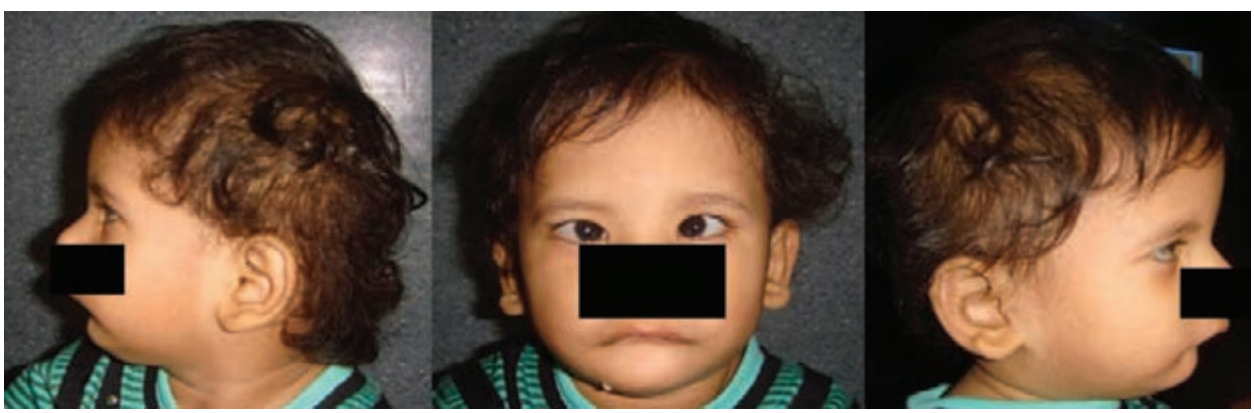

SANKARA NETHRALAYA

Editorial

Dear readers

Corneal collagen cross-linking has become an important treatment modality for several corneal disorders. This is covered in the Perspective article. Approach to abnormal head posture in a patient is elaborated in a simple, practical manner in the article from Elite School of Optometry. A continuing series on Biostatistics talks about inferential statistics. Muscle puzzle follows to keep you guessing. The last page introduces the reader an important controversial area-intraocular implantation in infants, which will be covered in a subsequent issue of Insight.

Dr. S. Meenakshi
Editor
September 2010

Perspective

Corneal collagen cross-linking

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Corneal collagen cross-linking (CXL) through UVA–riboflavin photochemistry is a new approach to increase the mechanical and biochemical stability of the stromal tissue. It has been recognized that ectatic conditions are associated with reduced biomechanical strength of the cornea, possibly related to reduced interfibrillar cross-links. The concept of collagen cross-linking treatment aims to address this pathogenetic mechanism. Although various methods including glutaraldehyde cross-linking are in clinical use in other medical specialties, the method most extensively studied for corneal use is ultraviolet A (UVA)/riboflavin collagen cross-linking. The first studies in photobiology began in 1990s with attempts to identify biological glues that could be activated by heat or light to increase the resistance of stromal collagen. A similar mechanism of natural hardening and thickening of collagen fibers is seen in aging corneas related to glycosylation of age-dependent tropocollagen molecules. Also young diabetics never have progressive keratoconus probably due to natural cross-linking effect of glucose which increases corneal resistance.

So far, the common clinical indication for cross-linking is limited to melting process of the cornea and corneal thinning disorders such as keratoconus, pellucid marginal degeneration and iatrogenic keratectasia after laser *in situ* keratomileusis (LASIK). Though not yet at the standard of care, multiplying uses of cross-linking treatment include bullous keratopathy, corneal melts, infectious corneal ulcers, glaucoma and pathologic myopia. It can be combined with other surgical procedures such as intracorneal rings, conductive keratoplasty, laser thermokeratoplasty and orthokeratoplasty.

TECHNIQUE

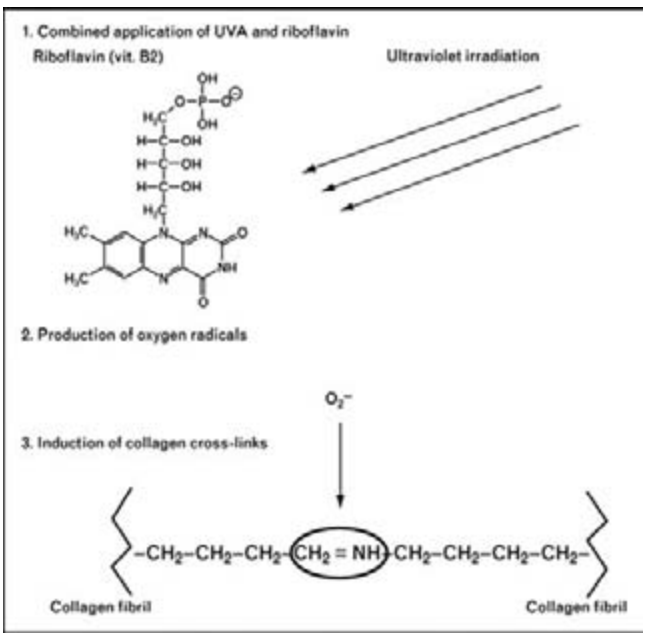
After an abrasion of the cornea, epithelium of 9 mm diameter, drops of a 0.1% riboflavin solution in 20% dextran are instilled onto the cornea every 3 min for 30 min. By means of a slit-lamp inspection using blue light, the surgeon has to assure that riboflavin has appeared in the anterior chamber before the UV irradiation is started. The cornea is exposed to UV light with a wavelength of 370 ± 5 nm and an irradiance of 3 mW/cm^2 for a total time of 30 min; this corresponds to a total dose of 3.4 J or a total radiant exposure of 5.4 J/cm^2 to the cornea. The cropped light beam has a diameter of 9 mm. During the irradiation time, the cornea is rinsed with riboflavin/dextran solution and topical anesthetic every 5 min. This is followed by overnight patching of the eye until the epithelium is healed. The postoperative treatment consists of topical antibiotics and topical corticosteroids.

MECHANISM OF ACTION

Application of riboflavin on the cornea along with penetration for approximately 200 μm and irradiation of the riboflavin molecules through UVA leads to loss of the internal chemical balance of the riboflavin molecules, producing oxygen free radicals. The riboflavin molecule becomes unstable and stabilizes only when it is linked to two collagen fibrils. A cross-bridge is created between the collagen fibrils (i.e., cross-linking) to produce a general strengthening of the cornea. Biomechanical measurements have shown an increase in corneal rigidity of 328.9% in human corneas after cross-linking.

POST-OPERATIVE FOLLOW-UP

After corneal cross-linking, the stroma is depopulated of keratocytes approximately 300 μm deep. Repopulation of this area takes up to 6 months. In the first few weeks of the procedure, a thin stromal demarcation line may be visible on the slit lamp at this depth, delineating the anterior cross-linked zone with the posterior unaffected stroma. This results from differences in the refractive index and/or reflection properties of untreated versus cross-linked corneal stroma and represents an effective tool to biomicroscopically easily monitor the depth of effective cross-linking treatment in keratoconus. It has also been shown with the aid of confocal microscopy that the corneal re-innervation is restored after 6 months of treatment.



RISKS AND SIDE EFFECTS

The UV exposure can induce a photokeratitis at the cornea or a cataract in the lens, and thermal or photochemical damage can occur in the retina. Furthermore, the free radicals and oxidizing agents liberated during the exposure of riboflavin-soaked corneal tissues also pose a photochemical risk to anterior-segment structures. The currently used UVA radiant exposure of 5.4 mJ/cm^2 and the corresponding irradiance of 3 mW/cm^2 is below the known damage thresholds of UVA for the corneal endothelium, lens and retina. Regarding the photochemical damage caused by the free radicals, the damage thresholds for keratocytes and endothelial cells are 0.45 and 0.35 mW/cm^2, respectively. In a 400-mm thick cornea saturated with riboflavin, the irradiance at the endothelial level was 0.18 mW/cm^2, which is a factor of 2 smaller than the damage threshold.

Thus, safe clinical application of cross-linking must respect the following criteria: (1) to facilitate diffusion of riboflavin throughout the corneal stroma, the epithelium should be removed; (2) a 0.1% riboflavin solution should be applied for at least 30 min before the UV exposure (during the UV exposure, the riboflavin serves as both a photosensitizer and a UV blocker); (3) the UV irradiance of 3 mW/cm^2 and a wavelength of 370 nm must be homogenous; and (4) the cornea to be cross-linked must have a minimal thickness of 400 mm to protect the endothelium. Damage to the endothelium, the lens or the retina is not expected when these criteria are fulfilled.

CORNEAL CROSS-LINKING FOR DIFFERENT CORNEAL DISEASES

Keratoconus

Early-to-moderately advanced, progressive keratoconus is by far the commonest indication for corneal collagen cross-linking. Patients with an advanced cone/ ectasia, those with substantial stromal scarring, a poor BCVA even with a contact lens and those with a corneal thickness of less than 400 μm at the thinnest point are poor candidates and these are relative contraindications for the procedure.

Clinically, ultraviolet cross-linking treatment appears to be able to halt progression of corneal ectasia in keratoconus patients. The results of some of the studies published in literature are summarized in the below table.

Since this treatment alone does not normalize corneal curvature, attempts have been made to combine it with other surgical modalities. Chan et al. (2007) describe cross-linking treatment immediately after INTACS insertion, with a statistically significant greater mean change in cylinder (2.73 D) and K steep (1.94 D) compared with INTACS alone.

Although these results appear promising, further studies evaluating safety, stability of effect and the effect of combination treatment with intrastromal implants are needed, and in addition, combination of cross-linking technology with other modalities such as the various forms of lamellar keratoplasty remain an intriguing possibility.

PELLUCID MARGINAL DEGENERATION

Corneal CXL described in the management of pellucid marginal degeneration with or without simultaneous customized photorefractive keratectomy improves some parameters in the advanced stages of the disease. The treatment is performed in standard fashion with the exception of slight inferior decentration of the 9-mm diameter irradiated area preserving an untreated surface area 1 mm from the limbus.

POST LASIK KERATECTASIA

Keratectasia is one of the most severe complications after refractive laser surgery. Usually, penetrating keratoplasty

Results of collagen cross-linking in keratoconus

Author (year)	No. of eyes/follow up	Results
Wollensak et al. (2003)[1]	22 eyes/3 years	In all treated eyes, the progression of keratoconus was stopped ('freezing'). In 16 eyes, there was a slight reversal and flattening of the keratoconus by 2 diopters. BCVA improved slightly in 15 eyes.
Sandner et al. (2004)[2]	60 eyes/5 years	No patient had further progression of keratectasia. In 31 eyes, postoperative regression by 2.87 D was observed. BCVA improved slightly by 1.4 lines.
Braun et al. (2005)[3]	27 eyes	Stabilization of keratoconus in all and regression by 2D in 12 eyes occurred. An increased corneal rigidity *in vivo* using a contact ultrasonic device was demonstrated.
Caporossi et al. (2006)[4]	10 eyes/6 months	Refractive results showed a reduction of about 2.5 D in mean SEQ, topographically confirmed by reduction in mean K. Results of surface aberrometric analysis showed improvement in morphologic symmetry with a significant reduction in comatic aberrations.
Chan et al. (2007)[5]	Two groups: Group I Intacs only; Group II Intacs with C3-R	Significantly greater decrease in cylinder, reduction of keratometry and in lower-upper ratio on topography in the Intacs with C3-R group as compared to Intacs-only group.
Hoyer et al. (2009)[6]	153 eyes/7.5 years	Keratectasia significantly decreased in the first year by 2.29 D, in the 2nd year by 3.27 D and in the 3rd year by 4.34 D. Visual acuity improved significantly in at least one line or remained stable (i.e. no line loss) in the 1st year in 48.9 and 23.8%, respectively; in the 2nd year in 50.7 and 29.6%, respectively; and in the 3rd year in 60.6 and 36.4%, respectively.

is the treatment of choice to achieve an optical rehabilitation in such cases. Riboflavin-UVA corneal cross-linking increases the biomechanical stability of the cornea and thus is a therapeutic option to arrest and partially reverse the progression of LASIK-induced iatrogenic keratectasia.

BULLOUS KERATOPATHY

Bullous keratopathy is a clinical condition involving endothelial pump dysfunction and corneal decompensation. Without sufficient endothelial pump function, the cornea swells and fluid accumulates in the extracellular spaces between collagen fibers and lamellae. In corneal edema, the interfiber collagen space increases due to fluid accumulation. *In vitro* experiments in porcine eyes suggested anti-edematous effect of collagen cross-linking using riboflavin and UVA (374 nm). This effect was shown to be the strongest in the anterior, intensely cross-linked zone of about 250 μm stromal depth and is probably due to the additional intra- and interfibrillar chemical bonds preventing further fluid influx and stromal swelling.

In a clinical interventional case series of three patients with bullous keratopathy due to pseudophakia, corneal transplant rejection and Fuchs' endothelial dystrophy, Wollensak et al. tested this anti-hydration effect of corneal cross-linking. Optical coherence tomography pachymetry measurements of central cornea was reduced by 90.33 ± 17.04 μm on average 3 days after cross-linking and by 93.67 ± 14.22 μm after 8 months. The bullous changes of the epithelium were markedly improved resulting in the loss of pain and discomfort. Ehlers et al. also reported a reduction in corneal thickness in 10 evaluated eyes ($n=11$) following cross-linking in the treatment of corneal edema caused by endothelial decompensation. The majority in their study also experienced improvement in vision. Thus, in future, cross-linking in bullous keratopathy may be recommended as a symptomatic treatment in these eyes with a restricted visual potential, reducing pain, formation of epithelial bullae and corneal clouding by edema.

In a further laboratory ($n=10$ eye bank corneas) and clinical case, Krueger et al. (2008) described the intrastromal administration of riboflavin into two Intra Lase laser created stromal pockets followed by exposure to high-energy UVA irradiation (15 Mw/cm^2), during two sequentially staged sessions, thus overcoming the limited depth of penetration of riboflavin UVA cross-linking, making it possible to achieve stromal compaction throughout the edematous cornea.

CORNEAL ULCERATION

Uncontrollable corneal sterile or infectious ulceration may lead to corneal perforation necessitating tectonic corneal transplantation. Recently, CXL has been used for the treatment of corneal melting and ulcers. In theory, riboflavin-UVA treatment, in addition to increasing tissue firmness by collagen cross-linkage, reduces the load of proteolytic enzymes induced by inflammation of various etiologies. Ehlers et al. (2009) in their small series of 14 eyes suggested that in some eyes ($n=6$), the riboflavin-UVA treatment can halt the ulcerative process and promote healing. In sterile keratitis, it is speculated that free oxygen radicals may directly inactivate proteolytic enzymes or damage leukocytes to an extent, which reduce production of such enzymes. In microbial keratitis, the effect may be exerted by direct killing of bacteria or amoebae. In both cases, enhanced resistance to enzymatic melting of the cornea induced by CXL treatment may be an important mechanism.

SCLERAL CXL FOR GLAUCOMA AND PATHOLOGIC MYOPIA

In severe myopia, the pathologic change is in the biomechanically weakened sclera with progressive thinning, probably due to a disturbed feedback mechanism of emmetropization after visual deprivation. At the wavelength of 465 nm, 35% of light is absorbed by the sclera, 57% is reflected and transmission of the non-reflected light is approximately 8%, and therefore is sufficient for scleral CXL. Several animal scleral CXL studies using riboflavin and a wavelength of 365–465 nm at 4.2 mW/cm^2 has shown an increase in sclera stiffness at the stress–strain curve.

The myopic optic nerve head is also more susceptible to the several likely forms of intraocular pressure (IOP)-related insult at all levels of IOP. The myopic scleral canal may be unusually large, abnormally shaped and/or tilted, leading to elevated levels of IOP-related stress for a given level of IOP. The myopic peripapillary sclera and lamina may be thin, leading to higher IOP-related laminar and scleral wall stress and deformation. Additionally, extracellular matrix of myopic eye may be abnormally weak, causing larger laminar deformations for a given level of IOP. The size of the axially myopic eye should increase IOP-related scleral stress for any given level. We know that impaired CXL is an important factor in the weakening process of the myopic sclera. Wollensak et al. demonstrated an impressive stiffening effect of CXL *in vivo* in rabbit sclera after glyceraldehyde treatment for 14 days. Biomechanical tests showed that the proposed method of scleral reinforcement induced a highly significant improvement in the biomechanical parameters of the sclera.

An entirely new scleral-based approach for the prevention and treatment of progressive myopia may become possible. Using sub-Tenon's injections with a large injection volume, it may be possible to achieve a treatment effect on the entire sclera because of the easy spread of the injected fluid in the sub-Tenon's space around the globe. However, the UV-A technique allows CXL in a clearly defined scleral target area, which may be an advantage in disorders such as myopic scleral staphyloma. Also, with riboflavin/UV-A irradiation, the extent of the treatment area is easily controllable because of the visible UV-A–light-induced fluorescence on the scleral surface. In the future, it may be possible to treat patients with progressive myopia using scleral CXL with riboflavin and blue light at 465 nm.

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Abnormal head posture

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Abnormal head postures are frequent in pediatric ophthalmology. The medical term is torticollis, from the Latin "tortus" and "collum" neck. This term, used on its own, is applied to muscular or neurologic disorders that cause unnatural positions of the head. The eye-related conditions that lead to abnormal head postures are termed as ocular torticollis.

OCULAR CAUSES OF HEAD POSTURES

Children adopt head postures as a result of several ocular conditions. When the patient derives a demonstrable advantage by adopting the head position, it is more correctly termed a compensatory head posture.

Compensatory head postures have four advantages. They serve to

1. optimize visual acuity
2. maintain binocularity
3. center the field of binocular vision and
4. miscellaneous benefits.

FACE TURN: OCULAR CAUSES OF A RIGHT FACE TURN

1. Nystagmus
   a. Congenital
      i. Conjugate form
      ii. Disconjugate form
   b. Acquired
      i. Periodic alternating nystagmus
      ii. Spasmus nutans
2. Incomitant strabismus
   a. Horizontal muscle abnormalities
      i. Right eye/left eye
         a. Innervational causes
         b. Mechanical causes
   b. Vertical muscle abnormalities
      i. Left superior oblique dysfunction
      ii. Left inferior oblique dysfunction
   c. Paradoxical face turn
3. Uncorrected refractive errors
4. Eccentric fixation
5. Right homonymous hemianopia
6. Miscellaneous etiologies
   a. Oculomotor apraxia
   b. Monocular blindness
   c. Very high myopia with esotropia

CONGENITAL NYSTAGMUS

1. It forms with conjugate null zones. The eyes are normally aligned but the nystagmus null zone lies in the same side for both eyes. This is the typical pattern of congenital nystagmus. The child adopts a face turn to the opposite side to fixate in the null zone and optimizes binocular visual acuity.
2. It forms with disconjugate null zones. This is usually seen with infantile esotropia. The patient presents with a large-angle esotropia with an adduction fixation preferences with either eye. They develop nystagmus on attempting to abduct either eye.

PARADOXICAL FACE TURN

In rare cases, a patient with diplopia due to an acquired incomitant strabismus will adopt a head posture that brings the eyes into their maximum deviation.

UNCORRECTED REFRACTIVE ERRORS

The most common uncorrected refractive errors that cause face turns are myopia and against the rule astigmatism. Correction of refractive error usually eliminates the face turn. However, if a glass prescription is too weak, the child may adopt a face turn in order to fixate through the peripheral parts of the lenses.

CHIN UP: OCULAR CAUSES OF A CHIN-UP POSTURE

1. Nystagmus
   a. Congenital
   b. Acquired
2. Strabismus
   a. Elevation deficits
      i. Right eye/left eye
         a. Innervational causes
         b. Mechanical causes
      ii. Both eyes

   b. Pattern strabismus
      i. 'A' pattern esotropia
      ii. 'V' pattern exotropia
3. Ptosis
4. Uncorrected refractive errors
5. Supranuclear gaze disorders
6. Superior visual field defects

'A' AND 'V' PATTERNS

The two entities that cause chin-up postures are an 'A' pattern esotropia and a 'V' pattern exotropia. In both situations, the horizontal heterotropia is minimal in the downgaze field, whereas it increases progressively as the eye move up to primary position and then into upgaze field.

PTOSIS

Ptosis, congenital or acquired, of one or both eyelids can lead to chin-up posture if the lid margins are at or below the level of the pupil.

VISUAL FIELD DEFECTS

Patients who have lost a significant portion of their superior visual fields may adopt a chin-up posture to centralize the range of residual field relative to the body. The most common causes are retina and optic nerve diseases that lead to altitudinal field defects.

CHIN-DOWN: OCULAR CAUSES OF A CHIN-DOWN POSTURE

1. Nystagmus
   a. Congenital
   b. Acquired
2. Strabismus
   a. Depression defects
      i. Right eye/left eye
         a. Innervational causes
         b. Mechanical causes
   b. Pattern strabismus
      i. 'A' pattern exotropia
      ii. 'V' pattern esotropia
3. Uncorrected refractive errors
4. Supranuclear gaze disorders
5. Inferior visual field defects

HEAD TILTS: DIFFERENTIAL DIAGNOSIS

1. Nystagmus
   a. Congenital
   b. Acquired
2. Strabismus
   a. Vertical muscle problems
      i. Right eye/left eye
         a. Innervational causes
         b. Mechanical causes
   b. Cyclotropia
   c. Horizontal muscle problems
   d. Paradoxical head tilt
   e. Ocular tilt reaction
3. Refractive errors

MANAGEMENT

Management consists of identification of the cause and correction of refractive errors. The strabismus conditions usually need surgical correction to correct the anomalous head posture. Anomalous head posture can be corrected through optical methods in some patients. For example, a patient with nystagmus who adopts right face turns for null point. So, here the eyes are to left. The prisms are placed with the apices to the left (apex to the side of the eye; in this particular case, the prism has to be oriented in base in fashion in the left eye and base out in the right eye). Fresnel prism may be used instead of a ground in prism.

Introduction to Biostatistics-6

Inferential Statistics, Part I

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INTRODUCTION

Inferential statistics are used for drawing conclusions (testing of hypothesis) and make predictions (estimation) about the population from a sample, based on the descriptions of data. Thus, statistical inference mainly consists of two aspects:

1. Testing of hypothesis – done by using various statistical tools like Chi-square test, independent *t*-test, paired *t*-test, etc.
2. Estimation of population value – interval estimate (confidence interval).

Before knowing various statistical tools, it is better to understand the basic concepts or terminologies that are commonly used in inferential statistics. In this issue (Part I), we will be dealing with this.

BASIC TERMINOLOGIES

Population and sample

A population can be defined as including people with all characteristics one wishes to understand. The population is the complete collection to be studied. But it is very large, making a complete enumeration of all the values in the population impractical or impossible. When understanding a scientific study, the aim is usually to be able to generalize the results to the population as a whole. Therefore, we need a sample which is a representative of the population.

A critical difference between a population and a sample is that with a population, our interest is to identify its characteristics, whereas with a sample, our interest is to make an inference about the characteristics of the population from which the sample was drawn (see Figure 1).

HYPOTHESIS

A hypothesis is a tentative proposition relating to certain phenomenon, which the researcher wants to verify when required. Hypothesis can be derived from many sources such as observation, theory, past experiences and case studies.

Hypothesis testing allows us to determine whether enough statistical evidence exists to conclude that a belief (i.e. hypothesis) about a parameter is supported by the data. It decides the truthfulness of a prespecified statement concerning a certain population parameter. The statement being tested is often referred to as a hypothesis. There are two types of statistical hypothesis: null and alternative hypotheses.

Null hypothesis

"Null" means nothing, and the null hypothesis always states that there is no significant difference between the population parameter and the sample statistic that is being compared. The process change or treatment makes no difference, or the process is operating properly. It is denoted by H_0 (pronounced as H "nought").

Alternative hypothesis

Alternative hypothesis which is the opposite of the null is a statement expressing a relationship between two variables or indicating difference between the groups. The process change or treatment has an effect, or something is wrong with the process. It is denoted by "H_1".

Usually, the null hypothesis and the alternative hypothesis represent two contradictory statements. They are said to be mutually exclusive. For example, in a clinical trial to find out whether the drugs A and B have equal effect or not, the null hypothesis for the trial will be there is no significant effect between the drugs and the alternative hypothesis will be there is a significant effect between them.

PARAMETER AND STATISTIC

A parameter is a numerical quantity, usually unknown, that describes certain population characteristic. Statistic is a quantity, calculated from a sample of data, used to estimate a parameter. The statistic x, s and p are used to estimate the population characteristics μ, σ and P (see Table 1).

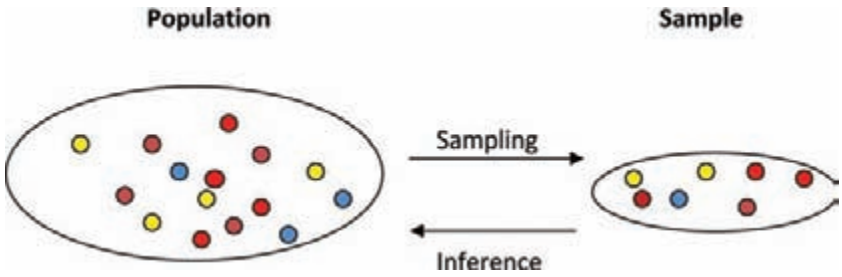


Figure 1. Population and sample.

Table 1. Statistic and parameter.

Quantity	Sample (statistic)	Population (parameter)
Size	n	N
Mean	$\bar{x}$	μ
Standard deviation	s	σ
Proportion	p	P
Variance	s^2	σ^2

ERRORS

In a research study, one seeks to demonstrate that a null hypothesis is either true or false. No decision-making process is completely error-free. This leads to two classic types of errors.

Type I error (false-positive): It is the false rejection of null hypothesis, i.e. it occurs when one rejects the null hypothesis when it is true. The probability of a type I error is the level of significance of the test of hypothesis, and it is denoted by α. Statistically, in a study, type I error is set at a value of 0.05 (5%) or 0.01 (1%).

Type II error (false-negative): It is the false acceptance of null hypothesis, i.e. it occurs when one accepts the null hypothesis when it is false. It is denoted by β. The power of a test is one minus the probability of type II error $(1-\beta)$. The maximum acceptable probability of a type II should be 0.2 (20%) (see Table 2).

ONE-TAILED AND TWO-TAILED TESTS

In the one-tailed test (unidirectional), the alternate hypothesis H_1 is one-sided, i.e. the outcome of the study will be only in one direction, above or below the mean. The test statistic falls in the critical region on only one side of the direction (see Figure 2a and b).

In the two-tailed test (non-directional), the alternate hypothesis H_1 is formulated to test for difference in both the directions. Hence the test statistic is tested for occurrence with the two critical regions on the two extremes of the distribution (see Figure 2c). A two-tailed test requires us to consider both sides of the normal.

For example, in a clinical trial for treating glaucoma by two different eye drops A and B, if the alternative hypothesis states that either A is less effective than B or A is more effective than B, then the one-tailed test is more appropriate.

Symbolically,

H_0: $\mu_A = \mu_B$
H_1: $\mu_A < \mu_B$ or $\mu_A > \mu_B$ (unidirectional)

Suppose the alternative hypothesis tries to determine whether there is any significant effect between the two eye drops, then the two-tailed tests should be preferred.

Table 2. Type I and II error.

		Real result	
		No change	Significant change
Observed result	No change	Measure: effect	Type I error (α) False-positive
	Significant change	Type II error (β) False-negative	Measure: Power $(1-\beta)$

Symbolically,

H_0: $\mu_A = \mu_B$
H_1: $\mu_A \neq \mu_B$ (bidirectional)

In the majority of the studies, it is important to always use a two-tailed test. If a one-tailed test is used, the direction should be specified in the study design prior to data collection. As shown in Figure 2c, a two-tailed test halves the level of significance (i.e. 0.025 or 2.5%) in each tail of the direction.

For a two-tailed test, 2.5% of the rejection region is placed in the positive and negative tails of the distribution, thereby Type I error is reduced by 2.5%. Whereas in one-tailed test, 5% the rejection region is placed only in one tail of the distribution, thus the Type I error is more (5%). Hence for this reason, one-tailed tests are rarely used in health research.

SIGNIFICANCE LEVEL

The significant level for a given hypothesis is a value for which a *P*-value less than or equal to a significant level is considered statistically significant. The levels of significance in a study are 5% (0.05), 1% (0.01) and 0.1% (0.001). If a test of significance gives a P-value lower than the level of significance, then the null hypothesis is rejected. Choosing a level of significance is an arbitrary task, but for many applications a level of 5% is chosen.

STANDARD ERROR

The standard error of a statistic is the standard deviation of the sampling distribution of that statistic. It gives a measure of how well a sample represents the population. In general, the larger the sample size, the smaller the standard error (see Figure 3). In other words, when the sample is representing the population, then the standard error will be small. The standard error is usually designated as SE. It is derived by dividing the sample standard deviation (s) by the square root of the sample size (n).

$$\mathrm{SE} = \frac{s}{\sqrt{n}}$$

SAMPLING ERROR

It is the error caused by observing a sample instead of the whole population. The sampling error can be found by subtracting the value of a parameter (population characteristic) from the value of a statistic (sampling characteristic).

Following are reasons to cause for a sampling error:

Biased sampling procedure
Improper sample size
Non-randomization
Non-probability sampling method

The sampling error in a study can be reduced by selecting an unbiased, optimal sample size with proper probability sampling and randomization.

EFFECT SIZE (ES)

In research, statistical significance testing has received many valid criticisms in recent years, primarily because the outcome of statistical significance testing relies too heavily on sample

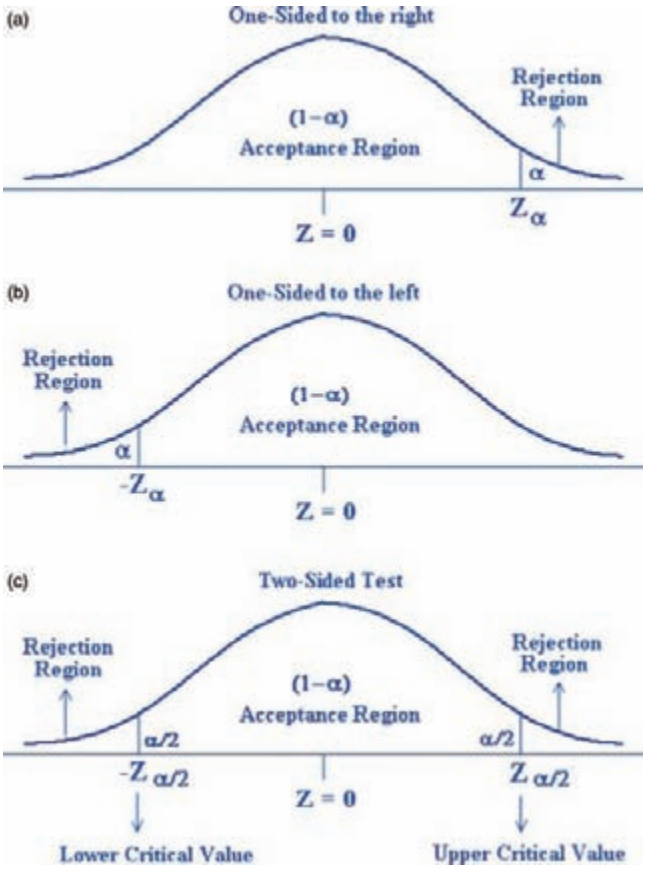


Figure 2. (a, b) One-tail test showing rejection region on any one side of the mean. (Z=standard normal variable; α=level of significance); (c) Two-tailed test showing rejection region on both sides of the mean. (Z=standard normal variable; α=level of significance).

size, and the issue of practical significance is often ignored. Effect size has been proposed as a supplement or an alternative to statistical significance testing. In inferential statistics, an effect size helps to determine whether a statistically significant difference is a difference of practical concern. It measures the size of any observed effects.

Generally, effect size is calculated by the following formula:

$$\text{ES} = \frac{\text{Mean of experimental group} - \text{Mean of control group}}{\text{Standard deviation} \ldots \text{pooled}}$$

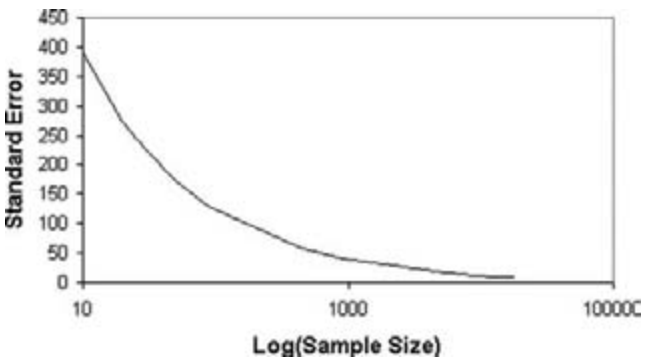


Figure 3. The relationship between the sample size and the standard error.

Effect size is interpreted using Cohen's method.

<0.1=trivial effect
0.1–0.3=small effect
0.3–0.5=moderate effect
>0.5=large difference effect

To know if an observed difference is not only statistically significant, but also important or meaningful, effect size has to be determined. The statistical significance does not tell about the most important thing: the size of the effect. To overcome this, a researcher has to report the effect size, together with an estimate of its likely "margin of error" or "confidence interval".

POWER

It is the probability of rejecting the null hypothesis when it is false. In other words, when a relationship exists between the independent and dependent variables in the population, statistical power measures the probability of detecting that relationship from the sample data. If a study has high power, then there is a high chance that the study will find a statistically significant result. If the power is less, say <50%, then the study is really not helpful. Many investigators choose sample size to obtain an 80% power in the study.

The power of a test is defined as $1-\beta$=1−probability of a type II error = P(rejecting H_0/H_1 true)

The power in a study depends on the following:

Critical alpha or level of significance (α)
Sample size (n)
Effect size (ES).

P-VALUE

There is a wide range of statistical tests available, depending on the nature of the investigation. However, the end result of any statistical test is a P-value. The 'P' stands for probability and measures how likely it is that any observed difference between groups is due to chance. In other words, the P-value measures the strength of evidence against the null hypothesis. It measures consistency by calculating the probability of observing the results from sample of data, assuming that the null hypothesis is true. The general rule is that a small P-value means that evidence against the null hypothesis is present, whereas a large P-value means that little or no evidence against the null hypothesis exists. To permit a decision between the null hypothesis and the alternative hypothesis, significance limits are often specified in advance, at a level of significance α. The level of significance of 0.05 (or 5%) is often chosen. P can take any value between 0 and 1.

Interpretation of P-value:

P-value	Interpretation
$P < 0.01$	Very strong evidence against H_0 or Result is highly significant
$0.01 \leq P \leq 0.05$	Strong evidence against H_0 or Result is significant
$P > 0.05$	No evidence against H_0 or Result is not significant

CONCLUSION

In any research study, to begin with, always formulate the null hypothesis. Initially, null hypothesis is accepted and considered true for all analytical comparisons. Statistical testing using probability (*P*) value evaluates only null hypothesis and does not evaluate the accuracy of the proposed alternative hypothesis. The level of confidence for rejecting the null hypothesis using a *P*-value is arbitrary. Thus, in a study, if one obtains a *P*-value >0.05, then there is weak evidence against the null hypothesis, a very likely a chance finding. On the other hand, if a *P*-value is <0.05 or <0.01, then there is strong evidence against the null hypothesis, very unlikely to be a chance finding.

Apart from showing a statistically significant difference between the groups in a study, a researcher should also determine the power and effect size in their study to make it more meaningful.

In the next issue, we will be discussing various statistical tools used for testing hypothesis.

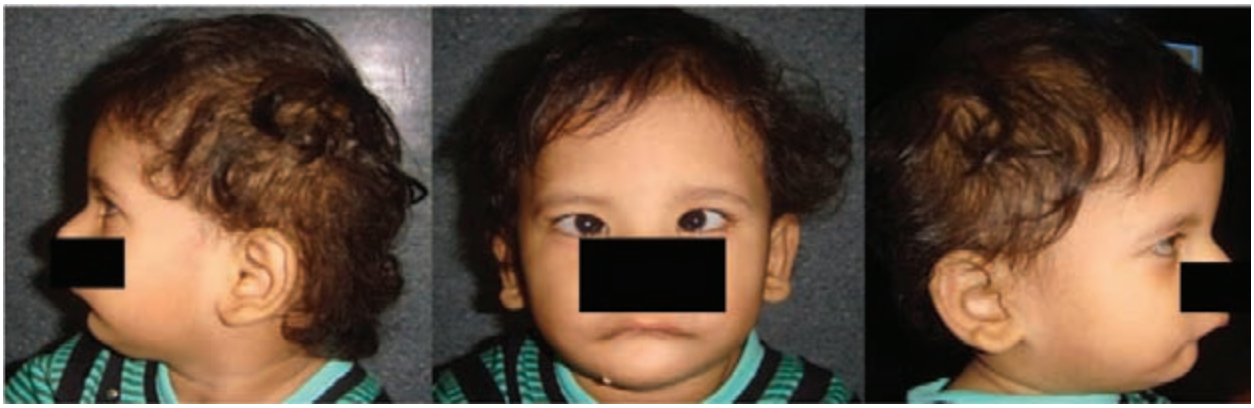
Muscle Puzzle

B. Mahalakshmi, Benazir Ansari, and Sumita Agarkar

Department of Pediatric Ophthalmology

An 18-month-old boy presented to pediatric ophthalmology clinic, with inward deviation of the eyes noted since 5 months of age. He was one of twins, and sibling was apparently normal. External examination revealed following features: prominent epicanthal folds, hypognathia, tongue tie, dental abnormalities, mask-like face, low set ears, mental retardation and dysmorphic features. There was no lagophthalmos. Cover test revealed a right convergent squint which was alternating. Abduction was limited in both eyes even on doll's eye movement. Modified Krimsky test showed 35–40 prism diopters baseout. Rest of ocular examination and fundus evaluation was within normal limits. MRI of the brain and orbit was done in view of bilateral lateral rectus paresis. In MRI, cisternal segments of the 6th and 7th nerves were not visualized. Periventricular leukomalacia were noted.

WHAT IS YOUR DIAGNOSIS?



MOBIUS SYNDROME

Mobius syndrome is a congenital, nonprogressive disorder clinically characterized by a loss of facial expression, impaired stomatognathic system functions, incapacity to close the eyelids and facial dysmorphic features.

Mobius syndrome is often evident in neonatal period as children have difficulty in sucking and lack of facial expression while crying due to facial nerve palsy. If facial paresis is incomplete, as in half of the cases, the syndrome may not be recognized until later in childhood. Some patients are unable to close their eyes during sleep or awake resulting in conjunctivitis or corneal ulceration. There is probably more than one mechanism in the pathogenesis of facial paralysis, where agenesis of the motor neurons being the commonest. Patients usually have a convergent squint with preservation of vertical gaze and convergence. Most patients cannot abduct their eyes beyond midline due to bilateral sixth nerve palsies. Physiological studies have shown aplasia of the ocular muscles. Ptosis, nystagmus or strabismus might accompany these findings. Epicanthal folds are frequently seen.

The nasal bridge is often high and broad, particularly during infancy and early childhood. The broadness of the nasal bridge extends downward in a parallel fashion to include the nasal tip, thus resulting in a midfacial prominence. The mouth aperture is often small. The angles of the mouth droop and may allow saliva to escape. Attempts at opening the mouth further results in a little change. As the child grows older, a definite but slow improvement can be observed in the ability both to open the mouth and to feed adequately. Poor feeding during the first year of life frequently results in poor growth. Unilateral tongue hypoplasia is frequent, but bilateral hypoplasia can occur. Muscular fasciculations of the tongue may be present. Poor palatal motility, inefficient sucking and swallowing, coarse voice and speech impairment are often present as a result of ninth and twelfth cranial nerve paresis. Frequently, the mandible is mild to moderately hypoplastic and when combined with the small mouth aperture imparts the appearance of low midfacial hypoplasia. The pinnae may be deficient in cartilage and protrude laterally. Limb defects occur approximately in 50%. Talipes deformities constitute 30%. The remaining 20% include primary hypoplasia of digits, syndactyly or more severe reduction deformities of the limbs. Ten to 15% are mentally retarded. The degree of mental retardation is usually mild.

Intraocular lens implantation in infants—no small matter this!

Harsha G. Mangalgi

Department of Pediatric Ophthalmology

Congenital cataract management in a visually immature child poses many challenges to the pediatric ophthalmologist.

The critical period for surgery for visually significant unilateral congenital cataract ranges from birth to 6 weeks of age, whereas sensory deprivation amblyopia can occur in bilateral cataracts, if surgery is delayed beyond 3–4 months for bilateral dense cataract. Hence, the need for early surgery is well established.

Introduction of new microsurgical techniques, instrumentation and high-molecular weight viscoelastics have enabled surgeons to remove cataracts safely at an early age.

In the management of surgical aphakia, which can be as amblyogenic as the cataract itself, the various options available include spectacles, contact lenses and, recently, primary implantation of intraocular lenses (IOLs).

IOL implantation as a modality of aphakic correction in an infantile eye is debatable.

Major problems of primary IOL implantation in infants include difficulty in selecting the appropriate diopteric power of the IOL. Technical difficulties are many, considering the small dimension of the infant eye with a small capsular bag, decreased scleral rigidity and increased tissue reactivity, leading to excessive postoperative inflammation.

More on this topic in the next issue's Perspective.

REGISTRATION FORM
Please complete this form using block letters and return it to the below mentioned address

1. PARTICIPANT DATA
Family Name_____
First Name_____
Institution_____
Home address
_____
_____
Zip code _____ City _____
Country_____
Tel._____
Mobile _____
Fax _____
E-mail _____
(Mandatory)

2. REGISTRATION FEES

	Please tick	Fees(INR/$)
International delegate		
National delegate		
OPAI member		
International Trainee*		
Indian Trainee*		

3. SUMMARY
I wish to attend (Please tick)
Day 1☐ Day2 ☐ BOTH ☐

***Trainee certificate attached: YES/NO**

4. PAYMENT BY
❑ Demand Draft ❑Cheque

The demand draft/cheque has to be issued in favor of **"MEDICAL RESEARCH FOUNDATION" payable at CHENNAI, INDIA**

Conference Venue:

Main Auditorium,
7th Floor, Sankara Nethralaya (Main Campus),
No. 18, College Road, Nungambakkam,
Chennai - 600 006
Tamil Nadu, India

Ph No: 91-044-2845-1477 (12 lines)
Mobile: 91 93828 32910
Fax No: 91-044-28254180
E mail: chrysalis2011@snmail.org

Functional day – 16/1/2011

Members	Early bird registration (15/11/2010)	Advance registration (until 31/12/2010)	On the spot registration (16/1/2011- 17/1/2011)
National delegate	1000 INR	1200 INR	2000 INR
International delegate	150$	200$	250$
Trainee national (Proof required)	500 INR	750 INR	1000 INR
Trainee International (Proof required)	75 $	100$	200$
OPAI member	750 INR	1000 INR	1500 INR

Aesthetics day – 17/1/2011

Members	Early bird registration (15/11/2010)	Advance registration (until 31/12/2010)	On the spot registration (16/1/2011- 17/1/2011)
National delegate	1200 INR	1500 INR	2500 INR
International delegate	200$	250$	300$
Trainee national (Proof required)	750 INR	1000 INR	1500 INR
Trainee International (Proof required)	100$	150$	250$
OPAI member	1000 INR	1200 INR	2000 INR

Functional and aesthetic day- 16/1/2010 & 17 /1/2010

Members	Early bird registration (15/11/2010)	Advance registration (until 31/12/2010)	On the spot registration (16/1/2011- 17/1/2011)
National delegate	1500 INR	2500 INR	3000 INR
International delegate	175 $	225 $	275 $
Trainee national (Proof required)	1000 INR	1500 INR	2000 INR
Trainee International (Proof required)	125$	175 $	300 $
OPAI member	1200 INR	1500 INR	2500 INR