Editorial

Lack of research publications with multidisciplinary approach is an area that needs attention from Indian Ophthalmologists. In the previous issue I announced that Current Indian Eye Research plans to publish articles based on researches conducted in the Indian perspective with special thrust on inter disciplinary research. Emerging issues in ophthalmology was promised special place in this journal. In this issue I am happy to publish all the original articles based on multidisciplinary research.

The last decade has seen progress in nanomedicine with development of various nanoparticles for therapy and diagnosis. Authors here report a nano-clustering based synthesis of nanoparticles as a probe for diagnosis of dry eye disease.

Texture information from an image is a useful method of image analysis. Texture quantification of choroidal neovascular membrane on fluorescein angiography image may add useful information in the diagnosis of this disease. In this issue we publish one multi institutional research report on this subject.

Dyslipidemia and hyperhomocysteinemia are considered independent risk factors for retinal vein occlusion, the second most common retinal vascular disorder. We publish one article on relation between these two risk factors in retinal vein occlusion.

Sample size is a very important component of research design. We publish an article on how to calculate sample size in ophthalmic research. I hope this article with useful examples will be of help for serious ophthalmic researchers.

Autologous ex-vivo cultivated limbal epithelial transplantation for the treatment of limbal stem cell deficiency in ocular surface disease following chemical or thermal burns is a relatively new technique of management. We publish a review on this subject in this issue. We also publish a review on osteo-odontokeratoprosthesis, a ray of hope for the hopeless.

I thank all the contributors and our readers for the overwhelming response, the words of encouragement and advice we received after the publication of our first issue.

Reference

Autologous Ex-Vivo Cultivated Limbal Transplantation for the Treatment of Unilateral Limbal Stem Cell Deficiency

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Keywords: Ocular Burns, Limbus, Stem Cells and Therapy:

Ocular surface disease following chemical or thermal burns is a rare but severe form of corneal blindness. Initially corneal surgeons believed that like other corneal diseases, corneal transplantation could restore corneal transparency and vision. In fact, the first successful corneal transplantation, by the Austrian surgeon Dr Eduard Zirm in 1905, was in the left eye of a farmer with bilateral chronic lime burns.¹,² However, with experience corneal transplant surgeons realized that almost all corneal grafts performed for ocular burns failed within a year because of recurrence of epithelial defects and vascularization.³ In the late 1970s and early 1980s Dr Richard Thoft showed in a small series of cases that autologous conjunctival transplantation as opposed to corneal transplantation was effective in stabilizing the corneal surface and moderately improving vision in eyes with ocular burns.⁴ He later proposed that the limbus and not the conjunctiva was the source of corneal epithelium hinting that adult corneal epithelial stem cells could be present at that location.⁵ Soon in 1986, Sun and associates actually demonstrated the presence of stem-cell like cells in the basal layers of the limbus which were slow-cycling, did not express cytological markers for either the conjunctiva or the cornea and were capable of proliferation in-vitro.⁶ This discovery led to a paradigm shift in the understanding of the patho-physiology of ocular burns suggesting that limbal stem cell deficiency (LSCD) was the reason behind corneal epithelial problems in ocular burns.⁷

The obvious implication of this new knowledge was whether limbal stem cell deficiency could be treated by performing limbal transplantation?⁷ Following successful pre-clinical animal trials,⁸ Kenyon and Tseng in 1989 provided the proof-of-principle by describing successful corneal regeneration in patients with unilateral acute and chronic chemical burns following limbal autograft transplantation.⁹ This technique involved removing as much as six clock hours of donor limbal tissue from healthy donor eyes and transplanting it on the recipient eyes after clearing the pathological pannus covering the cornea.⁹ Although this technique was extremely effective, other groups who tried to replicate the results reported rare incidents of iatrogenic LSCD in the donor eyes.¹⁰⁻¹² In 1997 Pellegrini and associates proposed a way around this problem by developing a technique of culturing the limbal cells ex-vivo in a laboratory to form a transplantable sheet of epithelium from less than one clock hour of donor limbal tissue.¹³ Following this, several large clinical trials have established the safety and efficacy of autologous ex-vivo cultivated limbal epithelial transplantation for the treatment of unilateral LSCD.¹⁴⁻¹⁷

Advantages of Ex-vivo Cultivated Limbal Epithelial Transplantation:

Although improving the safety of limbal transplantation was probably the driving force behind its development, this technique offers several other advantages as compared to conventional limbal transplantation.

1. Minimal Donor Tissue: Since first described in 1997, cultivated limbal transplantation has been performed in hundreds of patients with LSCD and till date there are no reports of donor site complications.¹⁴,¹⁵ The authors specifically looked at the donor eyes in 200 cases of unilateral LSCD which underwent autologous cultivated limbal epithelial transplantation and noted that the donor-site epithelialized within two weeks without complications.¹⁷

2. Repeatability: One or two repeat limbal biopsies can be safely obtained from the same donor eye if the primary procedure fails, because more than 90% of the limbus is left untouched by a single biopsy.¹⁰,¹⁸ This is not possible in conventional limbal transplantation as the donor eye is not left with any limbal reserve.

3. Early Corneal Epithelization: Since a ready-made epithelial sheet is transplanted in cultivated limbal epithelial transplantation, corneal epithelization is
almost immediate or is completed by the first week. 
In conventional limbal transplantation, the entire cornea is epithelialized only by six weeks. 

4. **Less Surface Inflammation**: Post-operative ocular surface inflammation subsides faster after cultivated limbal epithelial transplantation as compared to conventional limbal transplantation. 

5. **Less Scarring**: Cultivated limbal transplantation is associated with less scarring on the recipient corneal surface and probably better visual recovery as compared to the conventional technique. 

6. **Amplification in number of transplanted stem cells**: Ex-vivo cultivation results in increase in the number of limbal stem cells obtained by biopsy and this in turn can lead to better long-term survival of the graft. 

**Indications for Autologous Limbal Transplantation:**

Any traumatic or inflammatory damage to the limbus can cause permanent functional damage to the limbal stem cells. This leads to corneal epithelial instability, recurrent or persistent epithelial defects, invasion of the corneal surface by the surrounding opaque conjunctival tissue (conjunctivalization) and consequently severe visual loss. 

The commonest indication of autologous limbal transplantation is unilateral ocular surface burns, due to chemical or thermal injury. Earlier this procedure was performed both during the acute and chronic stages, but it is presently indicated only in the chronic stage, after the ocular surface inflammation has subsided. The other rare indication is iatrogenic LSCD after multiple or extensive ocular surface surgery like excision of ocular surface squamous neoplasia (OSSN). LSCD due to Stevens Johnson syndrome (SJS), ocular cicatricial pemphigoid (OCP), severe allergic eye disease aniridia associated keratopathy have bilateral affliction and need either allogeneic limbal transplantation or keratoprosthesis surgery. However, ex-vivo cultivated autologous limbal epithelial transplantation can be performed in patients who have bilateral LSCD but with asymmetrical involvement having at least one clock-hour of healthy limbus in either eye. 

**Pre-operative Clinical Assessment and Counseling:**

Selecting the proper cases is probably the most important determinant of the outcome of limbal transplantation. The first step is clinically assessing whether the affected eye has visual potential by performing simple macular function tests or electrophysiological testing. Additionally, young children developing unilateral blindness before the age of 8 years, fixing light poorly, and with monocular deviation are likely to have dense amblyopia with poor visual potential. Limbal transplantation may still be performed in amblyopic eyes to provide cosmetic relief, but the poor visual prognosis must be explained to the patient or parents, as appropriate. As there is no objective way of accurately assessing ocular surface inflammation and since these eyes are heavily scarred and vascularized, some surgeons prefer to wait for 3 to 6 months after the acute event before performing limbal transplantation. Lid abnormalities like notches, improper closure, entropion and trichiasis also need to be looked for and addressed. Eyes with severe dry eye disease, with a Schirmer’s test 1 score of less than 10mm at 5 minutes are unsuitable for this procedure and punctual occlusion may be needed prior to surgery. In summary the ocular surface environment must be conducive for the limbal transplantation to succeed. Any cause which may inflict inflammatory damage on the grafted cells post-operatively need to be taken care of prior to planning limbal transplantation. The extent of corneal stromal scarring is also difficult to assess pre-operatively and patients must be counseled that they may need additional surgery in the form of an anterior lamellar or penetrating keratoplasty (PK) for visual improvement despite a successful limbal transplantation. In the authors’ experience it is prudent to perform limbal and corneal transplantation as a two-stage rather than a single-stage procedure. 

**Technique of Limbal Biopsy**: A biopsy is taken from a healthy part of the limbus; a 2x2 mm piece of conjunctival epithelium with 1 mm into clear corneal stromal tissue at the limbus is dissected; conjunctiva is excised just behind the pigmented line (palisades of Vogt), and the limbal tissue that contained epithelial cells and a part of the corneal stroma is obtained. 

**Technique of Limbal Culture**: Broadly there are two techniques of limbal cultivation, a) the suspension culture where the a cell suspension of the biopsied limbal tissue is prepared and spread over a suitable substrate; and b) explant culture where the limbal tissue is sectioned into smaller pieces and directly placed on the substrate without separating the epithelial cells from the stroma. Additionally the constituents of the culture medium may or may not contain animal derived products or xenobiotic materials. Xenogenic constituents of a limbal culture system can be in the form of murine feeder-cells, bovine serum, or animal derived growth factors. To avoid the use of animal-derived products four groups, including our own, have independently developed completely xeno-free laboratory protocols of limbal culture. 

In our technique, the tissue is transported to the laboratory in human corneal epithelium (HCE) medium. HCE is composed of modified Eagle’s medium/F12 medium (1:1) solution containing 10% (vol/vol) autologous serum.
CURRENT INDIAN EYE RESEARCH

Table 1. Techniques and outcomes of autologous cultivated limbal transplantation for unilateral limbal stem cell deficiency

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Culture Technique</th>
<th>Substrate</th>
<th>Culture Time</th>
<th>Eyes</th>
<th>Clinical Success (%)</th>
<th>2-line visual gain (%)</th>
<th>Follow-up (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeder-Free and Xeno-Free Cell Cultures</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Sangwan et al(^{17})</td>
<td>2011</td>
<td>Explant</td>
<td>hAM</td>
<td>10 to 14</td>
<td>200</td>
<td>71</td>
<td>60.5</td>
<td>3</td>
</tr>
<tr>
<td>Kolli et al(^{26})</td>
<td>2010</td>
<td>Explant</td>
<td>hAM</td>
<td>12 to 14</td>
<td>8</td>
<td>100</td>
<td>63</td>
<td>1.6</td>
</tr>
<tr>
<td>Di Girolamo et al(^{27})</td>
<td>2009</td>
<td>Explant</td>
<td>CL</td>
<td>10</td>
<td>2</td>
<td>100</td>
<td>50</td>
<td>0.9</td>
</tr>
</tbody>
</table>

| Feeder-free but not Xeno-free Cell Cultures |
| Barandan-Rafii et al\(^{29}\) | 2010 | Explant           | hAM       | 14           | 8   | 88                    | 63                     | 2.8               | 0.5 to 4         |
| Pauklin et al\(^{30}\)    | 2010 | Explant           | hAM       | 14           | 30  | 77                    | 73                     | 2.4               | 0.8 to 6         |
| Shortt et al\(^{31}\)    | 2008 | Suspension        | hAM       | 14 to 21     | 3   | 78                    | 22                     | 0.8               | 0.5 to 1.1       |
| Shimakazi et al\(^{32}\) | 2007 | Explant           | hAM       | 14.6         | 16  | 50                    | 37.5                   | 2.5               | 0.5 to 7.1       |
| Nakamura et al\(^{33}\)  | 2006 | Explant           | hAM       | 15 to 16     | 2   | 100                   | 67                     | 1.2               | 0.5 to 1.6       |
| Sangwan et al\(^{34}\)   | 2006 | Explant           | hAM       | 11 to 15     | 88  | 73                    | 37                     | 1.5               | 0.3 to 3.3       |
| Sangwan et al\(^{35}\)   | 2003 | Explant           | hAM       | 10 to 14     | 2   | 100                   | 50                     | 1                 | 1                |
| Gruterich et al\(^{36}\) | 2003 | Explant           | hAM       | 21           | 1   | 100                   | 100                    | 3.1               | 3.1              |
| Tsai et al\(^{37}\)      | 2000 | Explant           | hAM       | 14 to 21     | 3   | 100                   | 50                     | 2                 | 0.3 to 10        |

| Neither Feeder-Free nor Xeno-Free Cell Cultures |
| Di Iorio et al\(^{38}\)  | 2010 | Suspension        | Fibrin    | NA           | 166 | 80                    | NA                     | NA                | NA               |
| Rama et al\(^{39}\)      | 2010 | Suspension        | Fibrin    | 14 to 16     | 107 | 68                    | 54                     | 2.9               | 1 to 10          |
| Gisoldi et al\(^{40}\)   | 2010 | Suspension        | Fibrin    | 14 to 16     | 6   | 83                    | 83                     | 2                 | 0.9 to 2.8       |
| Kawashima et al\(^{41}\) | 2007 | Explant           | hAM       | NA           | 2   | 100                   | 67                     | 2.7               | 1.7 to 3.7       |
| Nakamura et al\(^{42}\)  | 2004 | Explant           | hAM       | 23           | 1   | 100                   | 100                    | 1.6               | 1.6              |
| Rama et al\(^{43}\)      | 2001 | Suspension        | Fibrin    | 14 to 16     | 18  | 74                    | 33                     | 1.5               | 1 to 2.2         |
| Schawb et al\(^{44}\)    | 2000 | Suspension        | hAM       | 21 to 28     | 10  | 60                    | 36                     | 1.1               | 0.5 to 1.6       |
| Schawb et al\(^{45}\)    | 1999 | Suspension        | hAM       | 28 to 35     | 17  | 76                    | 16                     | 0.9               | 0.2 to 2         |
| Pellegrini et al\(^{33}\)| 1997 | Suspension        | 3T3s      | 16 to 19     | 2   | 100                   | 50                     | NA                | NA               |

hAM= human amniotic membrane; CL=contact lens; NA=data not available

(AS), 2mM l-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 2.5 µg/ml amphotericin B, 10 ng/ml human recombinant epidermal growth factor, and 5 µg/ml human recombinant insulin. Under strict aseptic conditions, the donor limbal tissue is shredded into small pieces. Human amniotic membrane (hAM), prepared and preserved by our eye bank is used as a carrier. A 3x4cm hAM sheet is de-epithelialized using 0.25% recombinant trypsin and EDTA solution for 15 minutes. The shredded bits of limbal tissue are explanted over the center of de-epithelialized hAM with the basement membrane side-up. A similar parallel culture is also prepared as a backup. A submerged explant culture system without a feeder-cell layer is used. We used the HCE medium to nurture the culture. The culture is incubated at 37°C with 5% CO₂ and 95% air. The growth is monitored daily under an inverted phase contrast microscope and the medium is changed every other day. The culture is completed when a monolayer of the cells growing from the explants became confluent, typically in 10 to 14 days.

Technique of Limbal Transplantation: Any symblepharon which prevented adequate separation of the lids is released to permit the insertion of a wire speculum (no additional...
surgery to treat the symblepharon is performed). A peritomy is performed and the corneal fibrovascular pannus is excised. If an impending or frank corneal perforation is noted at this stage a PK is performed prior to placing the limbal graft.23 The hAM and monolayer of cultivated limbal epithelial cells is spread over the cornea, epithelial side up.17,18 The graft is then secured to the peripheral cornea by interrupted, circumferential 10-0 nylon sutures and to the surrounding conjunctival edge by interrupted 8-0 polyglactin sutures. Alternately, using a sutureless technique, the graft is secured to underlying ocular surface with fibrin glue (TISSEEL™ Kit from Baxter AG, Austria) and the margins of the graft are tucked under the surrounding conjunctival edge. Bandage contact lenses are not applied at the end of surgery.

Postoperative management: All patients receive 1% prednisolone acetate eye drops eight times a day tapered to once a day in 35-42 days and 0.3% ciprofloxacin hydrochloride eye drops four times a day for 1 week, in both the biopsied and transplanted eye. The latter are continued till the epithelial defect completely resolves. No systemic antibiotics or steroids are needed. Patients are examined on postoperative days 1, 7, 42 and at an interval of 90-180 days thereafter, as customized by the clinical appearance of the transplant. Each examination includes a complete history, visual acuity assessment with Snellen’s charts, intraocular pressure measurement and detailed ocular examination with slit-lamp bio-microscopy.

Clinical Outcomes of Cultivated Limbal Transplantation: The techniques and outcomes of autologous cultivated limbal transplantation described by various groups are summarized in Table 1.13,17,26-44 Success was defined clinically in most studies; a few studies additionally used impression cytology or symptom scoring. With our technique the hAM usually disappeared (it either disintegrates or is incorporated as a part of the corneal stroma) by 4 weeks and the recipient ocular surface stabilized by 6 weeks. The donor site completely epithelialized without scarring within two weeks of limbal biopsy. Overall, the success rate of autologous cultivated limbal transplantation varied from 50 to 100% and a two-line improvement in visual acuity after cultivated limbal transplantation alone was seen in 22 to 100% cases (Table 1). More than 90% of failures occurred by the end of one year and more than half of these by six months after transplantation.15,16

Although on cursory review it appears that there is no clinical advantage that one culture technique holds over the other, comparing success rates among different culture techniques may be misleading as the indications for surgery, sample size, and follow-up duration are variable among different studies. Shimakazi et al32 and Nakamura et al33 compared the explant and suspension culture techniques, finding no significant difference in the outcomes. It is noteworthy in this context that with similar indications for surgery, clinical criteria for success, and follow-up Sangwan et al (explant culture, 71%, 200 eyes),17 Rama et al (suspension culture, 68%, 107 eyes)16 and DiIorio et al (suspension culture, 80%, 166 eyes)38 reported similar and impressive success rates of cultivated limbal

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Eyes</th>
<th>Clinical Success (%)</th>
<th>2-line vision gain (%)</th>
<th>Complications</th>
<th>Follow-up (Months)</th>
<th>Mean</th>
<th>Range</th>
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<tr>
<td>Miri et al45</td>
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<td>25</td>
<td>NA</td>
<td>NA</td>
<td>Filamentary keratitis (4)</td>
<td>41</td>
<td>3</td>
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<tr>
<td>Miri et al46</td>
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<td>12</td>
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<td>81.3</td>
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<td>46</td>
<td>12</td>
<td>120</td>
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<tr>
<td>Santos et al47</td>
<td>2005</td>
<td>10</td>
<td>80</td>
<td>61</td>
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<tr>
<td>Ozdemir et al48</td>
<td>2004</td>
<td>15</td>
<td>87</td>
<td>80</td>
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<tr>
<td>Dua et al49</td>
<td>2000</td>
<td>6</td>
<td>100</td>
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<td>Rao et al50</td>
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<tr>
<td>Basti et al10</td>
<td>1999</td>
<td>3</td>
<td>100</td>
<td>100</td>
<td>LSCD in donor eye (1)</td>
<td>NA</td>
<td>9</td>
<td>15</td>
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<tr>
<td>Frucht-Pery et al51</td>
<td>1998</td>
<td>9</td>
<td>100</td>
<td>100</td>
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<td>NA</td>
<td>15</td>
<td>60</td>
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<tr>
<td>Tan et al11</td>
<td>1996</td>
<td>9</td>
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<td>77</td>
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<td>Morgan et al52</td>
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<td>6</td>
<td>83</td>
<td>83</td>
<td>Donor site micro-perforation (1)</td>
<td>3</td>
<td>2</td>
<td>24</td>
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<tr>
<td>Kenyon et al9</td>
<td>1989</td>
<td>26</td>
<td>77</td>
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<td>18</td>
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</table>

NA= data not available; LSCD: Limbal Stem Cell Deficiency
epithelial transplantation with mean follow-up ranging from 1.5 to almost 3 years and maximum follow-up of up to 8 years. It is noteworthy that of the various groups only Sangwan et al.,17 Kolli et al.,26 Di Girolamo et al.,27 and Zakaria et al.28 described completely xeno-free techniques of culturing limbal epithelium while others used at least one or more animal derived products for culture. None of the studies reported any donor-site complications. Around 18 to 38% of all eyes treated with autologous cultivated limbal epithelial transplantation also needed a PK for visual improvement.14,15 The authors have found that adopting a staged approach of performing limbal transplantation first, followed at least six weeks later by PK resulted in better clinical outcomes as compared to a single-staged approach of combined limbal transplantation and PK.23 Therefore PK and limbal transplantation should not be combined unless PK is absolutely unavoidable as in the case of an impending or frank corneal perforation discovered after removal of the vascular pannus. The authors also found that a repeat limbal biopsy from the healthy eye followed by ex-vivo cultivation and transplantation of the cultured cells on the affected eye can successfully restore the ocular surface and improve vision in at least two thirds of cases with failure of therapy with primary autologous cultivated limbal epithelial transplantation.18 Therefore combining the efficacy of primary (71%) and repeat autologous cultivated limbal epithelial transplantation (67%) almost 90% of cases of unilateral LSCD can be treated successfully without any adverse impact on the healthy donor eye.17,18

Conclusions:

In terms of clinical efficacy there is hardly any difference between conventional and cultivated autologous limbal transplantation as treatment options for unilateral LSCD (Table 2).5-11,45-52 Proponents of ex-vivo cultivation cite the safety of the donor eye as the main advantage of their technique. However, ex-vivo cultivation requires specialized expertise and a licensed (by the Human Tissue Authority in the United Kingdom) laboratory. It can take up to 2 weeks to generate a sheet of desired dimensions, and it is expensive, costing approximately 10 300 Pounds Sterling or 12 000 Euros at current exchange rates for cultivation alone.46 Furthermore, many groups practicing this technique continue to use xeno-biotic materials for cell culture (Table 1). Not surprisingly, conventional limbal transplantations are still widely performed worldwide and cultivated limbal transplantation is restricted to few select centers around the globe.14,15 There are however certain clinical scenarios where cultivated limbal epithelial transplantation can be a superior alternative to conventional limbal transplantation. For example, in patients with bilateral but asymmetrical LSCD, even one clock hour of healthy limbal area in either eye can be utilized to restore the ocular surface and improve vision in both eyes.22

The only study that compared conventional and cultivated limbal transplantation reported slower epithelialization rate, prolonged ocular surface inflammation and significantly more scarring with the conventional technique.53 However, for cultivated limbal epithelial transplantation to emerge as the more popular technique of treating unilateral LSCD the economic and logistic barriers of cell culture have to be overcome first. Moreover the actual mechanism by which limbal transplantation works is still debated. It is unclear whether this therapy replenishes the stem cell reserve16 or revives the surviving stem cells by improving the micro-environment.14 It is also widely accepted that the cause of failure of limbal transplantation is multi-factorial and poorly understood.16,17 In the large clinical trials Rama et al and Sangwan et al investigated the cause for failure and found that eyes with more severe injuries and post-operative complications were more likely to fail.16,17 Rama et al also found that the proportion of holoclone forming cells (actual stem cells) in culture needed to be more than 3% of the total cell population for the transplant to have higher chances of success.16

In summary, the last two decades have witnessed tremendous progress in the understanding of ocular surface disease due to corneal burns and this has in turn led to therapeutic innovations, at the cutting edge of which stands ex-vivo cultivated limbal epithelial transplantation. Stem-cell based therapy for LSCD has already benefitted hundreds of patients worldwide and continuous research and medical development in this field holds promise for an even more exciting future.

References:

6. Schermer A, Galvin S, Sun TT. Differentiation-related expression of a major 64K corneal keratin in vivo and


Osteo-odonto keratoprosthesis (OOKP) surgery is a technique developed half a century ago by the Italian ophthalmologist Strampelli and uses the patient’s own tooth root and alveolar bone to support an optical cylinder.1 This multi-staged procedure is indicated in cases of severe bilateral corneal blindness, when conventional corneal transplantation or even the Boston type 1 keratoprosthesis is doomed to failure. Although initially conceptualized by Pellier de Quengsy, a French ophthalmologist, the first keratoprosthesis implantation in a human was performed by Nussbaum in 1855. This was a quartz crystal implant that remained in the eye for six months. Subsequently owing to the inevitable extrusion of the early implants and also due to the increasing popularity of penetrating keratoplasty, the initial enthusiasm surrounding keratoprosthesis died down. However, as experience with penetrating keratoplasty grew, surgeons the world over realized that there were still some forms of corneal blindness that were not amenable to treatment by replacing the diseased cornea with a healthy donor cornea. Thus interest in keratoprosthesis was renewed and numerous designs and techniques were subsequently described.2

An ideal keratoprosthesis should be a suitable replacement of the cornea being optically clear, bio-integrable, resistant to infection and most importantly it should be long-lasting.3,4 Keratoprosthesis can be classified based on the material of the optical cylinder (optic) and the support for the optical cylinder (haptic). Most models have a non-biological haptic (Boston KPro, Pintucci, Leon-Barraquer, Legeais and AlphaCor), while few use biological haptics (Strampelli OOKP, Casey and Temprano). Among these the Boston type 1 keratoprosthesis is currently the most popular while the OOKP has the longest follow-up and best retention rate. The OOKP can also be used in bone dry eyes with keratinized ocular surface and it is the only potentially vision restoring surgery that is possible in such eyes. The original technique of OOKP described by Strampelli has been modified by Falcinelli to improve visual results and retention of the device.5,6 Therefore this technique is now also referred to as the modified OOKP or MOOKP.

**Indications:**
Patients with bilateral corneal blindness resulting from severe end-stage Stevens-Johnson syndrome (SJS), ocular cicatricial pemphigoid (OCP), chemical burns, trachoma, dry eyes or multiple corneal graft failure may be considered for OOKP surgery. However, this technique is best reserved for bilateral corneal blindness with dry and keratinized ocular surfaces. In wet eyes the Boston type 1 keratoprosthesis is preferred over the OOKP.

**Contraindications:**
This procedure should not be recommended for patients who are otherwise well adjusted to their visual handicap, children under the age of 17, or in cases of doubtful or no visual potential. Another important consideration is the patient’s expectations from surgery. Since the final look of
the eye after all stages of OOKP is unnatural, the procedure should not be considered in patients who aspire to have both vision and cosmesis (Figure 1). There have been anecdotical reports of patients who after regaining vision and looking at themselves in the mirror have asked for the OOKP to be removed or even committed suicide.

Pre operative assessment:

The most important preoperative examination involves determining the visual potential. This can be done by checking accurate light projection in all quadrants, B-scan ultrasonography, and electrophysiological tests like flash ERG and VEP. However certain macular pathologies like a macular hole or scar can still be missed. The intra-ocular pressure is determined digitally and past records are checked to find out if the patient has been treated for glaucoma. A-scan is performed to measure the axial length which is used to calculate the power of the optical cylinder. After ruling out any contraindications to the procedure as described above, the patient is explained about the risks and complications, the need for frequent and lengthy follow-up. The patient must be encouraged to take an informed decision after consultation with family and friends.

Pre-operative oral assessment:
The buccal mucosa is inspected to look for areas of keratinisation or scarring. Patients who smoke or chew betel nut should be advised to discontinue such habits before surgery. For patients with poor oral hygiene, a prophylactic antiseptic mouthwash may be advised prior to surgery. Similarly the condition and health of the teeth are also inspected. The ideal donor tooth is the canine because it is mono-radicular. It is not mandatory to perform radiographs, but they can help in identifying the proximity of the floor of the maxillary antrum to the root of the tooth which can help avoid inadvertent oro-antral fistula creation during surgery.

Surgical technique:

Stage 1A: Before the implantation of the keratoprosthesis a complete iridectomy, lens extraction and anterior vitrectomy is performed. During this procedure the surgeon gets an opportunity to examine the posterior segment by performing intra-operative indirect ophthalmoscopy. After suturing the limbal wound, the cornea is covered by the anterior part of the PMMA optical cylinder protruding through the mucous membrane covered bony lamina. Note that the cosmetic appearance is unnatural and there is significant pseudoproptosis induced by the OOKP.

Stage 1B: The ocular surface is de-epithelized and a buccal mucous membrane graft is placed on it. The graft is sutured to the four recti and underlying epiclara as well as the surrounding Tenon’s capsule and conjunctiva (Figure 2 E and F).

Figure 1: Slit-lamp biomicroscopy photograph of the left eye of a 44 year old woman before and after osteo-odonto keratoprosthesis (OOKP) surgery. (A) Pre-operative photograph shows a dry and keratinized ocular surface with loss of corneal transparency. This patient developed blindness after an episode of Stevens Johnson Syndrome when she was 17 years of age. (B) Two years after OOKP all stages of OOKP surgery were completed, her vision in this eye is 20/20 unaided for distance and N6 for near with +3.0D Sphere. The photograph shows the anterior part of the PMMA optical cylinder protruding through the mucous membrane covered bony lamina. Note that the cosmetic appearance is unnatural and there is significant pseudoproptosis induced by the OOKP.
Stage 1C: A monoradicular tooth (preferably canine) is harvested to prepare an osteo-odontolamina. The root and surrounding jaw bone is removed using a cutting mechanised saw. The bone is thinned on one side to expose the dentine and a small hole is drilled through it. The PMMA optical cylinder is cemented in place and the assembled osteo-dental lamina is placed in a sub-muscular pocket just below the lower eye lid of the fellow eye for a period of 8 to 10 weeks.

Stage 2: The osteo-odontolamina along with its fibrovascular capsule is removed from the submuscular pocket and cleaned, the soft tissue excised from the posterior surface and trimmed from the anterior. The buccal mucosa is incised and a flap hinged inferiorly is raised to expose the cornea. A Flieringa ring large enough to accommodate the lamina is secured to the episclera. The center of the cornea is marked and a central opening just large enough to fit the posterior part of the optical cylinder is made with a trephine and scissors. After adequate anterior vitrectomy the lamina is fitted into place and sutured to the episclera. The buccal mucosal flap is repositioned and an opening is made in it to expose the optical cylinder (Figure 2, M to P).
Stage 1B and 1C are frequently combined together as the first step and Stage 1A and Stage 2 as the second step. This separates the extra-ocular and intra-ocular parts of the procedure and makes the procedure a two-stage affair. Others combine stage 1B and 1C as a single procedure reducing the number of procedures to three. We prefer a four-staged procedure as it gives time for the patient to recover from each operative insult and reduces the chances of inflammatory complications which can be quite unpredictable in patients with SJS or OCP.

Post operative care and follow up:
Systemic antibiotics, corticosteroids and ocular hypotensive agents are administered till the patient can be discharged. Topical antibiotic ointments are prescribed for the operated eye. Patients are usually seen after one week of discharge from the hospital and again at one month, three months and six monthly thereafter. At the follow-up visits the best spectacle corrected vision is assessed. Additionally, the digital assessment of the intra-ocular pressure, health of the buccal mucous membrane and stability of the optical cylinder is also assessed. Fundoscopy is carried out to check the optic disc and macula, B-scan to detect early peripheral detachments and visual field assessments are made 6 monthly for diagnosis and monitoring glaucoma.

Clinical outcomes
Anatomical retention rates and visual outcomes in eyes with blindness due to sequelae of inflammatory disease have been better with the OOKP as compared to purely synthetic prostheses. The overall results with OOKP are good compared with those reported in literature for other available methods in patients with end stage ocular surface disease due to severe inflammatory syndromes like SJS and OCP. Tan et al reviewed the largest eight case series of OOKP published in the scientific literature with sample sizes ranging from 4-181 eyes. The most common indications for surgery were severe cases of SJS and ocular burns. Anatomical survival rate in all the studies was 87.8% (range 67-100%) at 5 years, and three studies showed survival rates of 81% (range 65-98%) at 20 years. Visual acuity was more than 6/18 in 52% (range 46-72%) of the eyes with OOKP surgery. The most common intraoperative complication was vitreous hemorrhage (0-52%) and the most common long-term blinding complication was glaucoma (7-47%). Endophthalmitis ranged from 2-8%. The most common repeat surgical procedure was mucosal trimming due to mucosal overgrowth at the optical cylinder and mucosal grafting for extrusion of the OOKP or mucosal ulceration.

In our series we performed OOKP in 31 eyes of 30 patients blinded by SJS and one patient blinded by severe ocular burns. The anatomical success rate was 100% at a mean follow-up of 14.2±7.2 months. The visual acuity improved from hand motions or light perception to 20/40 or better in 18 eyes, 20/50 to 20/200 in 9 eyes, 20/400 in two eyes and remained hand motions or light perception in 2 eyes. Retinal detachment occurred in 2 eyes, mucosal ulceration in 9 eyes and mucosal overgrowth in one eye.

Conclusion:
Although the surgical procedure of OOKP is very tedious, the overall results are very satisfying. Most patients regain good vision and maintain this for long periods of time. There is no doubt that the retention rate of the OOKP is still unparalleled among all keratoprostheses designs. However, setting up of an OOKP practice requires a dedicated team of surgeons and a multi-disciplinary approach. Complications are common but mostly manageable, provided they are addressed early and appropriately. The most important caveat in OOKP surgery is choosing the right patients, who do not have unrealistic expectations, are committed to come for long follow-ups and are ready to face the prospect of additional surgery for management of complications. It is entirely heartening for an OOKP surgeon to see patients completely blinded for decades to resume the near normal life of a sighted individual free of dependence after OOKP.

References


Synthesis Of Nanoparticles As A Probe For Diagnosis Of Dry Eye Disease

Mohammad Azharuddin1, Anjan Kr Dasgupta1, Himadri Datta2

Abstract

Purpose: To study whether dry eye is protein conformational disease by synthesis of metallic nanoparticles as a probe. Methods: 20 dry eye patients and 18 control individuals were recruited for this study. Schirmer’s test was performed for diagnosis of dry eye patients. Collection and extraction of tear proteins was done with the help of Schirmer’s strip. Synthesis of nanoparticles was done using tear proteins as template. Results: Nanoparticles synthesized from dry eye tear proteins have been compared with those from control subjects and are seen to have a significantly higher degree of clustering. The observation supports our recent observation that the dry eye tear proteins are more prone to aggregation, and the nanoparticle clustering can be explained by the report that unfolded proteins facilitate formation of clustered nanoparticles. Conclusion: The reducing property of tear proteins is exploited for synthesis of metallic nanoparticles. The degree of clustering of nanoparticles depends on the pathological origin of the tear proteins and consequently this dependence can be useful for prognosis of dry eye disease.

Keywords: Dry eyes, Tear proteins, Nanoparticles, Protein aggregation.

Nanoparticles have been used as drug delivery agents and diagnostic probes1-3. Unconventional use of nanoparticles has also been reported. For example plasmonic properties of nanoparticles have been used to detect glycation of proteins4, to trace conformational and folding properties of proteins5, to enhance efficacy of drug6, as contrast agents (in MRI or microscopy for example)7. In the field of sensing and detection there have been several reports, notable ones being detection of bacteria8, detection of cancer cells9, sensing various metabolites and altering thermodynamic properties of energy rich fuels10. In the field of therapy and diagnostics various smart nanoparticles (e.g. designed core shell nanoforms) have been reported which performs intelligent therapy, e.g. releasing a drug in a targeted fashion11. There have been fewer reports on nanoparticle dictated photothermal therapy. Selected use of nanoparticles has also been reported in proteomics genomics and flow cytometry12,13.

Dry eye is a multi-factorial disease which leads to burning and irritation on the ocular surface. The most common symptom of the disease is that the quantity of tear production decreases. Diagnosis of dry eye by Schirmer’s test is routinely followed by many ophthalmologists worldwide. Although extensive researches have been conducted on the disease, starting from SDS-PAGE14 protein analysis to proteomics15 and lipidomics16 still the mechanism for the disease progression is obscure. Treatment of dry eyes with sodium hyaluronate17, autologous serum18, trehalose19 and artificial tear eye drops20 have been experimented in animals. These treatment methods are still not frequently used by ophthalmologists because of the inconsistency in results. Our study would provide a new insight into the disease diagnosis and treatment.

Despite the multidimensional progress in nanomedicine in the last decade in particular, the nano-clustering has been ignored as a useful tool in diagnosis. In this paper we have reported a simple cluster formation based diagnostic tool in dry eye disease. The report though in its preliminary form has the unique perspective, that the whole property has been holistically mapped such that there is a disease signature embedded in the nanocluster morphology.

Our work also provides a nanotechnology route to trace protein folding disease in general and dry eye disease in particular as we have shown the extent of nano-clustering may be an implicit function of the disease status.

Methods

The study adhered to the tenets of the declaration of Helsinki. Informed consent was obtained from both the

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groups (Dry eyes and Control) prior to the start of the research.

**Subjects**

Twenty dry eye patients (20 female: 10 male; mean age 29 ± 1.7 years) were recruited from the outpatient department. Eighteen control individuals (11 female: 7 male; mean age 28 ± 2.1 years) were enlisted from among the hospital staff. The inclusion and exclusion criteria were same as mentioned in22.

**Collection and extraction of tear fluid from Schirmer’s strip**

Tear fluids were collected using Schirmer’s strip. Following the collection process the wet portion of the strip was transferred into 0.5ml eppendorf with a punctured bottom. The punctured eppendorf was then placed on top of 1.5ml eppendorf with addition of 100µl extraction buffer (50mM NH₄HCO₃) on top of the strip and then centrifuged at 13,000 rpm for 15 minutes at 4°C 23. Total time needed from collection to extraction process was 20 minutes. All the experiments were conducted immediately after protein extraction.

**Total protein estimation by Bradford method**

Biorad protein kit assay was used for total tear protein estimation for dry eye patients and control. Absorption was measured (Evolution 300 UV-VIS, Thermo Scientific) at 595nm. Total tear protein concentration was normalized to 0.1mg/ml for both groups for carrying all the experiments.

**Synthesis and characterization of gold nanoparticles (GNPs) using tear proteins**

To 1ml of tear proteins 1mM chloro auric acid (HAuCl₄ purchased from Arora Matthey, Kolkata, India) was added and kept under stirring condition at room temperature until the solution color changes to red. Size and correlation coefficient of the synthesized nanoparticles was measured by Zeta Sizer Nanoseries- NANO-ZS. Transmission electron microscopy (TEM) was done on a copper grid (Siemens Elmiskon 101 TEM).

**Results**

Figure 1 shows the synthesized GNPs formed by reduction method using human tear proteins as template. The formation of GNPs is visible by the appearance of ruby red solution. The size distribution of the GNPs formed was measured by dynamic light scattering represented in Figure 2. This figure show that (GNPsC) formed by control individual tear proteins are much smaller in size as compared to the (GNPsD) synthesized by dry eyes patient tear fluids. The range for control tear GNPs is 5-20nm whereas for dry eyes it lies in the range 10-90nm. The formation of large sized GNPs in case of dry eye tear proteins in comparison to control individuals is indicative of the fact that dry eye patient tear proteins are prone to protein misfolding or aggregation because in only case of denatured and aggregated proteins there is a tendency to form clustered nanoparticles24.
Our hypothesis that dry eye tear proteins leads to larger sized or clustered nanoparticles formation is further verified by TEM images shown in Figure 3, 4A and B. Figure 3 shows TEM image of GNPsC, it can be seen from the image that control tear proteins leads to smaller sized GNPs without any cluster formation indicative of the fact that there is no protein aggregation or misfolding. Whereas in case of GNPsD as represented in Figure 4A and B, there is a strong indication that dry eye patients tear proteins are more prone to protein misfolding leading to clustered nanoparticles formation with a much higher size distribution compared to GNPsC.

Discussion

Protein mediated synthesis of metallic nanoparticles have been carried out earlier. It has been reported that there is an impressive relation between the folding status and the nanosurface topology. The unfolded proteins in which more hydrophobic surface is exposed are likely to offer a different template for synthesis of nanoparticles as compared to proteins in their native state in which there is a known hydrophobic collapse (hydrophobic groups in their native state been mostly concealed from the aqueous environment), as higher hydrophobic surface would lead to formation of nanoparticles with extended clustering propensity. The whole process may be considered as a snap shot of the folding status of the proteins.

While in this paper we show that the method may be a reliable indicator of dry eye disease, what remains to be explored is a general validity of this folding based templating approach in exploring protein conformational disease in general.

Furthermore the different classification of dry eye disease as for example evaporative dry eye can also be compared using this method. The method can be particularly useful for determining the efficacy of a drug which would cure dry eye disease. This drug discovery tool has particular importance as presently there is no known drug for treatment of dry eyes and the mechanism is yet to be discovered. The fact that there is abundance of unfolded
proteins leading to such extended nanocluster formation also indicates that dry eye may be a class of protein conformational disease.

Our study was conducted on patients with Schirmer’s score <5mm, from the Schirmer’s strip tear proteins was extracted and total tear protein estimated and normalized for further experimental procedure. The idea that tear proteins could form a template for nanoparticles synthesis came from earlier work. After the synthesis of GNPs by dry eyes and control individuals tear proteins, the size measurement and particle formation analysis was done by DLS and TEM. The DLS data shown in Figure 2 represents size distribution histogram for GNPsC and GNPsD respectively. The figure suggests that control tear proteins forms smaller GNPs with respect to dry eye tear proteins. This could be due to the fact that diseased state tear proteins are in a misfolded or aggregated structure which serves as a less preferred template for monodisperse particle formation as opposed to healthy tear proteins where there is no protein aggregation. The presence of extended structures in dry eye tear proteins increases the hydrophobicity which also results in decrease reduction capacity for nanoparticles formation.

To further validate our hypothesis TEM study was performed, from the TEM images it is apparent that GNPsC and GNPsD lead to monodisperse and clustered nanoparticles formation. Figure 3 demonstrate that control tear proteins forms smaller and monodisperse gold nanoparticles owing to the absence of any extended or misfolded protein aggregates. This observation is contradicted in case of dry eye patient tear protein (shown in Figure 4A & B), here the particle size as well as monodispersity is different. Dry eye tear proteins lead to bigger size GNPs formation along with it there is formation of cluster, which may be due to extended protein aggregates in dry eye tear proteome.

Conclusion

To conclude we can say that dry eyes falls into protein conformational related disease. This study would help in the near future for the correct and accurate treatment of the disease.

References


Purpose: Both hyperhomocysteinemia and dyslipidaemia are considered as an independent risk factor in retinal vein occlusion. This study was done to find out the correlation of plasma homocysteine with serum lipids in patients with retinal vein occlusion.

Material & Methods: A total of 84 retinal vein occlusion cases and 65 age and sex matched controls were assayed to explore the relationship of plasma homocysteine with serum lipid profiles in this observational, cross sectional, open, comparative, eight month study. Results: Plasma homocysteine, total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol levels were elevated significantly (P <0.001) and HDL cholesterol was decreased significantly (P <0.001) in the patients with RVO as opposed to the control subjects. Significant negative correlation was found between homocysteine and HDL cholesterol in RVO patients (r = -0.273, P < 0.029).

Conclusions: Patients with low HDL cholesterol should be screened for HHcys as association of low HDL cholesterol and HHcys might have a synergistic effect on the retinal circulation. Future study is needed to see whether treatment of HHcys will increase the HDL cholesterol level and that can be an important preventive measure against development as well as treatment of retinal vein occlusion.

Keywords: Homocysteine (Hcys), Hyperhomocysteinemia (HHcys), High density lipoprotein (HDL), Low density lipoprotein (LDL), Retinal vein occlusion (RVO).
males and 30 females) age and sex matched controls were included in the study. Presence of any of the following conditions like pregnancy, lactation, malignancy, sepsis, liver and renal failure, recent vascular accidents (< 6 months), previous thromboembolic events, inflammatory disorders, thyroid disorder, diabetes mellitus, vitamin intake (B6, B12, and folate), alcohol, drugs (methotrexate, fibrates) and smoking were excluded from the study population by detailed history, clinical and biochemical examination. The study was approved by the institutional ethics committee and informed consent was obtained from all the study populations, in accordance with the Declaration of Helsinki.

Fasting blood samples were collected from the patients and the controls. The blood samples were collected by vein puncture in EDTA vial and plain vial using disposable syringe. The blood collected in EDTA vial was immediately centrifuged at 1000g at 25°C for 3 minutes and plasma was separated and analyzed for Hcys. Samples were stored tightly capped at 2-8 °C for up to 48 hours if testing was delayed. Plasma Hcys was estimated by enzymatic method in autoanalyser (Toshiba TBA40FR Biochemistry analyser) with a Reagent kit, supplied by Lilac Clinical chemistry division16. (Linearity extends to 50 ìmol/L)

The blood collected in plain vial was kept in tilted position for 30 minutes at room temperature and then centrifuged to separate serum for the estimation of lipid profile. Serum total cholesterol was measured after enzymatic hydrolysis and oxidation17. The High density lipoprotein (HDL) cholesterol level was determined after precipitating the chylomicrons, Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) fragments, using phosphotungstic acid and magnesium chloride18. Serum triglyceride level was determined after enzymatic hydrolysis with lipases19. The serum LDL and VLDL cholesterol levels were determined using the formula of Friedwald T (1972)20. Serum lipid profiles were estimated in autoanalyser (Toshiba TBA40FR Biochemistry analyser) with a Reagent kit, supplied by coral.

Statistical analysis was performed using the Student’s t-test and Pearson’s correlation coefficient by SPSS software (Versions 16.0).

Results
The mean age of RVO patients and control participants were 44.1 ± 15.2 years and 50.2 ± 10.6 years respectively. Of the 84 RVO cases 59 patients were BRVO, 22 were CRVO and 3 were HCRVO.

Hcys levels were increased significantly in the patients with RVO (mean total Hcys, 17.86 ± 5.13 ìmol/L) as opposed to the control subjects (mean total Hcys, 12.05 ± 2.11 ìmol/L; P < 0.001). (Figure- 1)

Total cholesterol (209.2 ± 53 mg/dl) levels were elevated significantly as opposed to the control (159.2 ± 30 mg/dl) (P <0.001). Triglyceride (175.3 ± 45.6 mg/dl) levels were
elevated significantly as opposed to the control (96.4 ± 22.1 mg/dl) (P <0.001). LDL cholesterol (127.7 ± 33.7 mg/dl) levels were elevated significantly as opposed to the control (93.3 ± 17.2 mg/dl) (P <0.001). VLDL cholesterol (34.6 ± 9 mg/dl) levels were elevated significantly as opposed to the control (19.3 ± 4.6 mg/dl) (P <0.001) as well as HDL cholesterol (30.8 ± 6.6 mg/dl) (P <0.001).

There was also significant negative correlation found between homocysteine and HDL cholesterol in RVO patients (r = -0.273, P < 0.029). (Table-1)

**Discussion**

A retinal arteriol and its corresponding vein share a common adventitial sheath. Thickening of the arteriol appears to compress the vein. This causes secondary changes, including venous endothelial cell loss, thrombus formation, and potential occlusion. Similarly, the central retinal vein and artery share a common adventitial sheath at the arteriovenous crossings posterior to the lamina cribrosa so that atherosclerotic changes of the artery may compress the vein and precipitate the CRVO. It therefore appears that both arterial and venous disease contribute to RVO. Venous occlusion causes elevation of venous and capillary pressure with stagnation of the blood flow. This results in hypoxia of the retina drained by the obstructed vein, which in turn results in damage to the capillary endothelial cells and extravasation of blood constituents. Tissue pressure is increased, causing further stagnation of the circulation and hypoxia, so that a vicious cycle is established.3

HHcys was reported as an independent risk factor for CRVO11-12, 21. An Odds Ratio (OR) of 3.0 for fasting HHcys in patients with CRVO was reported by Lattanzio et al14 and an OR of 1.3 was reported by another study in a Chinese population22. Janssen et al23 have observed an overall OR of 8.9 (95% CI, 5.7–13.7) for Hcys. The meta-analysis by Cahill et al24 have shown that raised plasma Hcys levels and low serum folate levels were associated with retinal vascular occlusion. Dayal S et al26 have observed that deficiency of either methionine synthase or folate produces oxidative stress leading to the endothelial dysfunction in the cerebral microcirculation of mice. The direct cytotoxic effect on retinal vascular endothelial cells by Hcys and Hcys thiolactone has also been reported in a case report by Poloshek et al26.

The results of the present study indicated that hcy levels were increased significantly in the patients with RVO (mean tHcys, 17.86 ± 5.13 ìmol/L) as opposed to the control subjects (mean tHcys, 12.05 ± 2.11 ìmol/L ; P < 0.001). (Figure-1)

There are various mechanisms reported regarding endothelial dysfunction by Hcys. These include diminished bioavailability of nitric oxide27, abnormal expression of various thrombotic factors28. Hcys can form stable disulfide bonds with protein cysteine residues and, in the process, alters or impairs the function of many proteins like albumin, fibronectin, transthyretin, annexin II, and factor V29. Hcys may be metabolised into hcy-thiolactone which is a highly reactive compound that contributes to Hcys toxicity in humans30 (Hcys-thiolactone hypothesis) leading to endothelial dysfunction. HHcys can lead to upregulation of the inflammatory response in the vascular smooth muscle cells that characterizes early atherogenesis31. This study also observed that total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol levels were elevated significantly (P <0.001) as well as HDL cholesterol was decreased significantly (P <0.001) in the patients with RVO as opposed to the control subjects. There are various mechanisms reported regarding endothelial dysfunction...
by LDL Cholesterol. It is initially converted into oxidized LDL cholesterol by free radicals. The oxidized LDL is taken up by scavenger receptor on monocyte macrophages leading to foam cell. It decreases the expression of endothelial nitric oxide synthase and in turn inhibiting nitric oxide mediated vasorelaxation. It also stimulates Interleukin-1, tumor necrosis factor-alpha and interferon-gamma which cause leukocyte recruitment and adhesion to the endothelial cell.

Although Jadav et al did not find any correlation between Hcy with lipid profile in ischemic heart disease patients but our study observed a significantly negative correlation with HDL Cholesterol in RVO patients (Table-1). This can be explained by possible action of Hcys in reducing the expression of peroxisome proliferator-activated receptor (PPARα) and decreased the ApolipoproteinA-I promoter activity and its protein levels. In addition to its influence on ApolipoproteinA-I, hyperhomocysteinemia inhibits reverse cholesterol transport by reducing circulating HDL via inhibiting ApolipoproteinA-I protein synthesis and enhancing HDL cholesterol clearance in mice.

It was well established that both HHcy and Dyslipidemia can induce atherosclerotic changes. Our study showed that low HDL cholesterol was aggravated by HHcys in RVO cases as evidenced by strong negative correlation between them. So the Patients with low HDL cholesterol should be screened for HHcys as association of low HDL cholesterol and HHcys might have a synergistic effect on the retinal circulation. Future study is needed to see whether treatment of HHcys will increase the HDL cholesterol level and that can be an important preventive measure against development as well as treatment of retinal vein occlusion.

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Abstract

Purpose: To quantify the Choroidal Neovascularization (CNV) image textures using Differential Box-Counting method. Method: DFA images of 10 individuals including pathological subjects were used for textural quantification. Pre-processing was carried out for the generation of vessel-free fundus images for better discrimination with more detailed texture information. Subsequently, to quantify textural information fractal based method is used. Result: The fractal features; fractal dimension, standard deviation and Lacunarity measures found to be promising in pathological images over normal fundus images. Conclusion: The empirical evaluation of pre-processed fundus images with DBC features has shown the minute difference between normal and CNV affected images.

Keywords: Texture, Choroidal Neovascularization (CNV), Fractal Dimension, Lacunarity, Differential Box Counting (DBC)

Digital fundus angiography (DFA) images have been widely used by the ophthalmologists for treatment planning and diagnosing retinal vascular and non-vascular pathology. Visual inspection of the retinal non-vasculature may reveal severity of diseases related with Age related Macular Degeneration (AMD); specifically choroidal neovascularization (CNV), which is a major cause of adult blindness due to proliferation of blood vessels and requires extensive analysis from specialist. Endeavoring to reduce the effect of CNV includes obtaining and analyzing DFA images of the optic fundus at regular intervals. Early detection of changes to the blood vessel patterns can prevent major vision loss.

An automatic assessment of optic fundus abnormalities initially requires the segmentation and removal of the vessels from the background. Next, obtained vessel-free images are used for texture quantification.

Texture information is one of the most important image features that can be used in many fields including medical image analysis extensively. A fundamental characteristic of texture is that it cannot be analyzed without a frame of reference of tonal primitive being stated or implied.

The texture features are obtained from several methods: statistical, geometrical, model-based, transform-based methods and Local Binary Operator (LBP).

Fractal analysis has been useful in image processing for characterizing shape and gray-scale complexity. The statistical self-similarity and scale-invariance are two fundamental principles of fractal geometry. Fractal geometry and fractal models are applied to most of natural objects which are not ideal but semi-fractal. Fractal dimension gives detail characteristics on the statistical self-similarity and the spatial distribution irregularity of biomedical images.

Sarkar and Chaudhuri proposed a differential box-counting (DBC) method advantageous with its wide independent scale range and low computational complexity. The DBC method was testified to be an effective method and used for texture image classification successfully.

Materials and Methods:

We have tested our method on publically available database (DRIVE) consisting of digital fundus angiogram images. It consists of 40 images legitimate data. The images are acquired in digital form using 3 CCD cameras.
with 45° FOV. The image resolution is 768 X 584 pixels, 8 bits per pixel. The training set of 20 images including three pathological images and remaining non-CNV images9 including images of diabetic retinopathy) were considered for texture quantification. The labeled images were obtained through manual segmentation and also tested our method on 10 individual subject’s DFA images provided by the ophthalmologist.

The green channel shows better contrast over the other channels of RGB image. Hence green channel was extracted from RGB image. Next, the gray scale image obtained through green channel image was taken as input for vessel removal. The resulting image was then used for texture quantification through DBC method.

Differential Box-counting method requires a meshing of the fractal object and formulation of a probability in each generated box. Finally the log of the number of boxes counted is plotted alongside the log of the scaling factor for each stage of partitioning, yielding a set of points on a line. The measures at different scales are obtained through counting the minimum number of boxes of different size, which entirely cover the whole surface instead of directly measuring an image surface. The DBC\(^6\) can also be used in FD evaluation of multi-fractal spectrum computing methods. By covering a fractal object with boxes of length \(r\), the FD is estimated as:

\[
FD = \lim_{r \to 0} \frac{\log(N(r))}{\log(r)}
\]

Where \(N(r)\) is total number of boxes needed to completely cover fractal object and \(r\) is the scaling factor. The slope of the best-fitting straight line is obtained from least-square regression by plotting \(\log(N(r))\) versus \(\log(r)\).

Assuming the image of size \(M \times M\) pixels scaled down to a size \(s \times s\) where \(M/2^e\) \(s > 1\) and \(s\) is an integer. Therefore we have \(r=s/M\). Consider the image as a 3-D space with \((x, y)\) denoting 2-D position and the third coordinate \((z)\) denoting gray level. The \((x, y)\) space is partitioned into grids of size \(s \times s\). Each grid is consisting of the column of boxes of size \(s \times s \times s'\). If the total number of gray levels is \(G\) then,

\[
\left\lfloor \frac{G}{s'} \right\rfloor = \left\lfloor \frac{M}{s} \right\rfloor
\]

Let the minimum and maximum gray level of the image in the \((l, j)\)\(^{th}\) grid fall in box number \(k\) and \(l\), respectively. The equation below is the contribution of \(N_r\) in \((l, j)\)\(^{th}\) grid.

\[
n_r(i, j) = l - k + 1
\]

Taking contributions from all grids, \(N_r = \sum_{i,j} n_r(i, j)\)

Because of the differential nature of computing \(n_r\), it is called DBC approach. It gives a better approximation to the boxes intersecting the image intensity surface, which is quantized in space and gray value, in particular when there is sharp gray level variation in neighboring pixels in the image.

Lacunarity is one of the most reliable fractal features defined as the measure of lumpiness in an image; heterogeneity. It aids in discrimination of the visual perception sham in case of two almost identical fundus images regarding fractal dimension.

**Results:**

The fractal features; fractal dimension, standard deviation and Lacunarity measures found to be promising in pathological images over non-CNV fundus images. The results were obtained by taking non-CNV and pathology (CNV affected) images. Prior to crop sections, images were pre-processed, to improve the texture feature discrimination, for textural feature quantification through DBC method to extract prominent fractal feature. Both, non-CNV and pathology image cross-sections of macular region were taken (Figure 1 (a),(b)) for further analysis and the values of fractal features were obtained. The most promising outcomes are drawn [Table 1].

<table>
<thead>
<tr>
<th>Table 1: Comparison of fundus images for Fractal features quantification</th>
<th>FD Avg</th>
<th>FD SD</th>
<th>FD Lacunarity</th>
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</table>

FD Avg = Average fractal dimension, FD SD = Standard deviation in FD, FD Lacunarity = Lacunarity in FD, Image_N = Normal image, Image_P = Pathology image
Discussion

Values of non-CNV images revealed almost same dimensions, standard deviation and lacunarity. Whereas CNV affected images demonstrated drastic change in all the fractal feature values. Lacunarity estimated in Table 1 shows a clear discrimination between non-CNV and pathology DFA image textures. The empirical evaluation of pre-processed fundus images with DBC features thus can reveal the difference between non-CNV and CNV images.

Acknowledgement:

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References

Unusual Manifestation Of Ocular Tuberculosis Presenting As Corneal Fistula

Somnath Mukhopadhyay, Debjani Mishra

Abstract

Ocular involvement of tubercular bacilli is diverse and extensive. Predominant anterior segment involvement includes interstitial keratitis, phlyctenular nodules and scleritis. We describe a rare manifestation of ocular tuberculosis presenting as corneal fistula.

Keywords: Tuberculosis; Ocular tuberculosis; Corneal fistula.

Tuberculosis is fast emerging as the most common cause of mortality and morbidity globally. It is estimated that about one-third of world’s population is currently infected with Mycobacterium tuberculosis. During the last two decades with the emergence of HIV infection especially in African and in Asian countries, the incidence of extra-pulmonary and multi-drug resistant tuberculosis is on the rise. Among other organs of the body having high oxygen tension, eye is vulnerable to tuberculous infection. Ophthalmological involvement of tuberculosis is diverse and inclusive of both anterior segment (lid granuloma, scleritis, phlyctenular keratoconjunctivitis and interstitial keratitis) and posterior segment (choroidal tubercles, sub-retinal abscess, retinal vasculitis, neuroretinitis and optic neuritis) involvement. We present an interesting case of tubercular corneal fistula.

Case Report

A 22 year male patient presented to Institutional cornea clinic about a month ago with a history of corneal ulcer in right eye persisting for more than six weeks. He was initially treated elsewhere with a combination of topical natamycin and moxifloxacin for about a month without improvement. No history of trauma or ocular foreign-body was obtained. His presenting vision in the involved eye was 6/60 with accurate projection in all directions. Slit-lamp evaluation revealed presence of a corneal fistula near 7 o’clock area with positive Seidel’s test. A 1mm zone of cellular infiltration was seen around the fistula. Iris was incarcerated around the fistulous tract (causing a pupillary drag) with a central hole through which aqueous seepage was documented. Anterior chamber was maintained except the area of iris-adherence. Iris pigment dispersion was noted on the anterior lens capsule. No abnormal anterior chamber reaction and endothelial deposit was noted in right eye. Fundus evaluation by indirect ophthalmoscope was normal in both the eyes. Vision and anterior segment evaluation of the fellow eye was normal.

A decision was made to perform a full thickness patch graft in the right eye. Medical records of the patient revealed that he was getting anti-tubercular treatment (ATT) for pulmonary tuberculosis (PTB) in the Department of Chest Medicine of our hospital. Systemic examination revealed a high ESR but normal HIV, HBV and HCV serology. Full...
thickness patch graft was performed (SM) under peribulbar anaesthesia with sedation taking a donor disc that was 0.25mm larger than the zone of infiltration in the recipient corneal bed. The removed recipient button was sent for microbiological examination. He was put on topical moxifloxacin (0.5%) four times a day for two weeks and topical prednisolone acetate (1%) with a slow taper. The recipient button showed presence of Mycobacterium Tuberculosis bacilli in Z-N stain. Till the last follow-up six months after operation, he had a clear graft with no recurrence.

Discussion

Haematological dissemination of tuberculous bacilli is responsible for choroidal involvement. Granuloma formation (tuberculoma) causes lid involvement, choroidal mass lesion and sub-retinal abscess formation. Some manifestations (phlyctenulitis and interstitial keratitis) are supposed to be due to immunogenic response to mycobacterium. Due to its avascularity, cornea is immune-privileged. We may assume that absence of other known causes of corneal fistula formation (trauma, chemical insult, stem cell deficiency disorders, collagen vascular disease and hepatitis infection) in a patient on ATT for PTB along with microbiological report of recipient disc point towards tuberculous aetiology of corneal fistula formation.

References:
Sample Size Calculation For Research Studies In Ophthalmology

Arun Sharma

ABSTRACT

An ideal research is one where no sample is required, meaning thereby that all eligible persons are included in the study. It is next to impossible due to time, money and other resource constraints. In ophthalmology, we have to be careful about few things in sample size estimation. First that each person has two eyes and that one person at a time may present one or both of his/her eyes for the purpose of research. So here, each eye is a sample and the sample size calculated is the number of eyes and not number of individuals. However, if right eye of person is different from the left eye and this has a bearing on the research, then we have to treat right eye as a separate entity from left eye and this will have a bearing on the process of sampling. Sample size depends on type of research design, whether it is descriptive, analytical or experimental. Different formulae are used for calculating the sample size for a study depending on the type of variables and study designs. Several software are available for calculating sample size.

Sample size is a crucial component of research design. More often researchers want sample size to be justified on non statistical grounds. For example, a researcher may say that I could get only these many cases in the specified time period so as a statistician do something to make the study valid in spite of whatever sample size I have used. Similarly, financial constraint is sometimes cited an excuse for not having attained a given sample size. Others view sample size as a biggest hurdle in their conduct of a methodologically correct research. So let us simplify the issue of sample size once forever.

Why do we need to calculate a sample size?

An ideal research is one where no sample is required, meaning thereby that all eligible persons are included in the study. More often than not, it is next to impossible due to time, money and other resource constraints. Therefore, the focus is on conducting the research on a smaller number of subjects in such a way that the findings are applicable on the entire population in question and not the selected sample. It may be best explained by an analogy of buying rice from a traditional Indian grocery store. The shopkeeper pulls out few grains of rice from a sack containing 40 kg of rice. By examining the few grains placed on the hand, one has to judge whether the rice in the sack is of good quality or not. Similarly, in research by conducting the experiment on a few subjects we want to judge whether the findings will be applicable to all subjects of the same type. In order to be as correct as possible in this endeavor, two criteria are to be met. One, the way sampling is done or sample is selected and second the appropriate number of subjects selected. In this article, we will focus on the sample size that is the number of subjects to be selected.

In ophthalmology, we have to be careful about few things in sample size estimation. First that each person has two eyes and that one person at a time may present one or both of his/her eyes for the purpose of research. So here, each eye is a sample and the sample size calculated is the number of eyes and not number of individuals. However, if right eye of person is different from the left eye and this has a bearing on the research, then we have to treat right eye as a separate entity from left eye and this will have a bearing on the process of sampling. Similarly, if a pathology in one eye of a person has the potential of spreading to the other eye of the same person, and we are looking at determinants of the illness, then we have to use individual persons as our sample and not individual eye as a sampling unit.

Every research study will have one outcome variable. This outcome variable may be qualitative, for example outcome of a treatment may be cure or death, occurrence of disease as a result of exposure to a risk factor or non occurrence of the same. Variables which have two or more categorical outcomes are called qualitative variables. In case of such variables, outcome is expressed as proportion. For example, what proportion of patients suffering from cataract will be cured after surgery.

On the other hand, the outcome variable may be a measurable quantity, like intra ocular pressure, focal length, body weight, hemoglobin level, such variables are called quantitative variables. Here the outcome is expressed as mean and Standard Deviation or Standard Error, provided the variable has a normal distribution.

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Finally to talk about sample size, it depends on type of research. Whether it is descriptive, analytical or experimental.

**A descriptive research study** is one in which we want to estimate the magnitude of a problem/disease or health condition or the distribution of the disease/health condition with respect to time place and person. For example, to know the prevalence of cataract in a given population requires a descriptive study design. Similarly, to estimate the incidence of retinopathy among diabetics, a descriptive study design is required. Thus to calculate incidence or prevalence or age and gender distribution of an ophthalmic condition we need to conduct a descriptive study. For descriptive studies, sample size is calculated using a following standard formula: 

\[
N = \frac{\frac{r + 1}{r} \frac{p(1-p)}{1}}{\left(\frac{Z_{\alpha/2}}{2}\right)^2 pq/l^2}
\]

Where,

- \(p\) denotes the prevalence of the condition expressed as absolute percentage
- \(q = 1-p\)
- \(z = \) the z-score for the confidence interval of the prevalence to be estimated. Conventionally, 95% confidence interval is used and the corresponding value of \(z\) is 1.96 and for 99% confidence interval (to get more precise value), it is 2.58
- \(I\) denotes the permissible error within which the prevalence estimate will be considered as valid.

Many a times question is asked, if the prevalence is already known \((p)\), why should I study it again? Or another question asked is, what if the prevalence is not known. If the prevalence for the given population is already known, obviously there is no need to carry out the study. But if prevalence in the given population is not known, we may use the prevalence value estimated by other researchers in other population groups which is similar to the population that the researcher wants to study. If no such prevalence value is available, there are two options. Either a scientific guess can be used or a pilot study may be conducted on a small fraction of the population to derive a quick estimate of the prevalence.

**Example:** Suppose the estimated prevalence of cataract is 30% as reported in a study conducted on a similar population. In order to find a true estimate of cataract prevalence within 5% of the known estimate (that is, the detected prevalence will be between 25% and 35%), the required sample size will be given as below:

\[
P=0.3, Q=0.7 (1-p), L=.05 (5%), Z_{(1-\alpha/2)}=1.96
\]

So, \(n =\frac{(1.96)^2 (0.3) (0.7)}{(0.05)^2} = (3.8416)(0.21)/(0.0025) = 322.72 \sim 323\)

Now, the question is 323 eyes or 323 persons. It depends on the premise of the study. If the research question is, “What proportion of eyes have cataract?”, we need to included 323 eyes. If the question is “What proportion of people have cataract, at least in one eye?”, we need to study 323 people. In the second case, even if the person has cataract in both eyes, it will be treated as one unit for sample size estimation. Answer to the first question is useful, if we want to estimate the number of eyes to be operated in a given area. The answer to second question is useful if we want to know the magnitude of cataract related blindness in the community.

Usually population based surveys use such estimation of sample size. This sample size estimation is valid when simple random sampling is used. If cluster sampling technique is used then sample size estimate has to be multiplied by a design effect. If stratified sampling technique is used then sample size should be estimated for each strata. For example, if there is a reason to believe that cataract prevalence rates are different among male and female population, then sample size should be separately calculated for male and female populations.

Note: Prevalence is a qualitative variable expressed as percentage. If the variable is quantitative and follows a normal distribution, the ‘pq’ of the above formula are replaced by Standard Deviation (SD) of the quantitative variable. For example, if intra ocular pressure is to be estimated in a population, then SD of mean intra ocular pressure is to be used, which again may be taken from a previously conducted study or from a pilot study or can be a scientifically guessed estimate.

**Analytical study:** Case control study and cohort study are the analytical studies. Case control studies are used to measure associations, commonly between risk factors and diseases. The association is measured by Odds Ratio in case control studies and Relative Risk in cohort studies. In statistical parlance, these measures are also known as effect size. Some statistical software calculate the sample size based on Odds Ratio or Relative Risk, others ask for effect size. In case control study, number of controls should be at least equal to that of cases. Controls may be up to 4 times the number of cases, any larger number of controls beyond this do not add value to the study.

In order to calculate the sample size for case control studies, the following formula is used

\[
N = \frac{r + 1}{r} \frac{(p)^2 (1-p)^2 (Z\beta - Z\alpha/2)^2}{(p1 - p2)^2}
\]
Where,

\[ r = \text{ratio of control to cases}, \]

\[ p^* = \text{average of proportion cases and controls exposed to the risk factor} \]

\[ Z_\beta = Z \text{ score for the power of the study. For 80% power (type II error=20%), Z score is 0.84 and for 90% power (type II error=10%), Z score is 1.28.} \]

\[ Z_{\alpha/2} = Z \text{ score for the type I error (conventionally Type I error is fixed at 95% and } \alpha/2 \text{ is used for two tailed significance.} \]

\[ p_1 = \text{proportion of cases exposed to the risk factor for which the study is being carried out} \]

\[ p_2 = \text{proportion of controls exposed to the risk factor for which the study is being carried out} \]

\[ p_1 - p_2 \text{ is a measure of effect size} \]

In case of a normally distributed quantitative variable, instead of \( p \) and \( q \), square of pooled Standard Deviation is used and \( p_1 - p_2 \) in the denominator is replaced by difference of mean between cases and controls.

Formula for calculating matched case control study is different.

**Example:** Suppose a study is to be conducted to find out the association between obesity and glaucoma. Previous studies have shown that among obese people risk of glaucoma is 3 times higher than that of non-obese people (OR=3.0) and prevalence of obesity in the control group (normal population) is 10% and in cases is 25%. (If OR is known and \( p_2 \) is known, \( p_1 \) can be estimated from a formula). In order to estimate the true Odds Ratio, by fixing Type I error at 5% and Type II error at 20% (power = 80%), fixed ratio of controls to cases is taken as 1:1 and sample size calculation using the above formula will be 62.72. Practically we need to take 32 cases and 32 controls. The calculated sample size is always the minimum sample size required.

Cohort studies are the other type of analytical studies, where point of selection of subjects in the study is prior to exposure to the factor(s) and occurrence of the event.

For example, if it is to be tested whether exposure to volatile organic compounds (voc) increase the risk of corneal opacity; a cohort study can be conducted by recruiting subjects before exposure to voc has started and the exposed and unexposed subjects are followed up till a predetermined end point (development of corneal opacity) is reached or study is completed (censored). For such cohort studies, sample size is calculated using following formula:

\[ n = \frac{[Z_{\alpha/2} + Z_\beta]^2 [p_1(1-p_1) + p_2(1-p_2)]}{(p_1-p_2)^2} \]

Where, \( p^* = \frac{p_1 + mp_0}{m + 1} \)

Please remember, \( n \) is the total number of subjects to be recruited in the study

\( Z_{\alpha} = \text{ Type I error (usually set at 5%), corresponding z score is 1.96} \)

\( Z_{\beta} = \text{ Type II error (usually set at 20%), corresponding z score is 0.84} \)

\( p_0 = \text{probability of corneal opacity among the subjects not exposed to voc} \)

\( p_1 = \text{probability of corneal opacity among the subjects exposed to voc} \)

\( m = \text{ratio of unexposed to exposed subjects} \)

Sample size formula for paired cohort study is different.

In this example, suppose we want to determine the relative risk of corneal opacity among subjects exposed to voc as against those not exposed. Let \( m=1, \ p_0=0.2, \ p_1=0.7, \ Z_{\alpha}=1.96, \ Z_{\beta}=0.84, \ p^*=0.45 \)

Then the required sample size will be: 45.03, hence 45 subjects are to be recruited in this cohort study.

If the outcome variable is quantitative, \( p_0 \) and \( p_1 \) are replaced by mean values in the denominator and the expression \( p^*(1-p^*) \) are replaced by pooled SD, \( p_0(1-p_0) \) and \( p_1(1-p_1) \) are replaced by SD for unexposed group and SD for exposed group respectively.

**Experimental studies:** Experimental studies are similar to cohort studies except that in clinical trials exposure is under control of the researcher. In cohort studies, the researcher passively observes the exposure. In clinical trials, researcher creates exposure by giving interventions. All drug, vaccine trials, and non-pharmacological interventions like health education etc. can be evaluated using a clinical trial design. The formula for calculating sample size for such studies is:

\[ n = \frac{[Z_{\alpha/2} + Z_\beta]^2 [p_1(1-p_1) + p_2(1-p_2)]}{(p_1-p_2)^2} \]

You may note that the equation is very similar to that of a cohort study sample size formula. This formula is used when outcome variable is a qualitative variable and its value is expressed as proportion (p). In this case p1 denotes outcome in control group and p2 denotes outcome in experimental/intervention group. \( Z_{\alpha/2} \) is used when the significance is two tailed.
Example: Suppose the researcher wants to find out if Vitamin A intake (intervention) prevents night blindness in school going children as compared to intake of vitamin A rich food (control). The role of vitamin A intake will be considered significant if occurrence of night blindness in the intervention group is at least 50% less than that of the control group. If the incidence of night blindness in the control group is 30%, it should be no more than 15% in intervention group. After fixing alpha error at 5% and power of study at 80%, $p1$ 30% and $p2$ 15%, the number required to be recruited in each group is $N=117.6$ thus by rounding off to the nearest integer, 118 subjects are to be recruited in each group.

If the outcome variable is quantitative, the formula will be:

$$n = \frac{(Z\alpha + Z\beta)^2(\sigma^2)}{(\delta)^2}$$

Where $\sigma$ is the pooled SD of the outcome variable and $\delta$ is the difference of the two means.

In the previous example, if impact of intervention is measured in terms of serum vitamin A level as outcome, the later formula should be used for sample size calculation.

A few checks that should be performed while calculating the sample size:

1. Outcome variable should be clearly defined.
2. It should be measurable.
3. Check whether outcome variable is qualitative (expressed as proportion) or quantitative (expressed as mean and Standard Deviation).
4. For quantitative variable, it is important to ensure that the variable follows normal distribution. For non normally distributed data, the above formulae do not apply.
5. For descriptive studies and surveys, only $Z\alpha$ is relevant, that is only type I error is taken into consideration.
6. For analytical and experimental studies, both $Z\alpha$ and $Z\beta$ are required.
7. These formulae are based on the presumption that simple random sampling procedure will be adapted.
8. In case simple random sample is not achievable, alternative probability sampling techniques can be put to use, for example stratified random sampling and cluster sampling. In such cases the sample size calculated by any of the above formula will require to be corrected using a correction factor called design effect.
9. In studies involving human beings, there is always a chance of subjects withdrawing from the study, a phenomenon called attrition. This attrition should be factored into at the time of deciding the sample size. Suppose the required sample size for a cohort study is 200 using simple random sampling method. But it is not feasible and cluster sampling technique is to be used, and experts suggest that it requires a design effect of 2.0, then the sample size will become, $200 \times 2=400$. Now if you fear that 20% subjects may drop out or may be lost to follow up in course of the study, the effective sample size will become $400+20\%\times400$. Hence 480 subjects should be recruited in this study.
10. There are other study designs and analysis methods also. As for example, survival analysis, Cox proportional hazard analysis, longitudinal data analysis etc. In such scenario different formulae are prescribed for calculating the sample size and it is better to be advised by a statistician with expertise in such analysis.

Life for researchers have been made easier by the computing powers of the machines, which can handle large data sets and carry out complex mathematical operations in no time. Even sample size calculation has been made easy by computers. There are several software and freeware available which compute the sample size for the researcher. Most of these are menu operated and therefore easy to handle; but the researcher must know the parameters that are to be fed into the software to get the appropriate sample size calculation.

There are many online programs also which allow you to calculate the sample size and power of the study. Some of the freeware are given below:

1. Statcalc module of Epi Info, a software distributed frf of cost by CDC, Atlanta. The most recent Version is Epi Info™ 7.1.4. Can be accessed at http://www.cdc.gov/epiinfo/7/
History of Ophthalmology

History Of Endeavour: Ophthalmology In India

Simantini Bhattacharya

Abstract

This article presents the history of endeavour in ophthalmology in India, taking up some interesting and offbeat references. An ophthalmologist treating patients does not sound unusual but if a king, a prince or even a religious preacher is found indulged in treating ocular diseases, taking care of intricate ocular surgeries, its implication upon the mass becomes manifold. Grafting few of such references the paper wants to ensure the need of a wide ranged awareness to contribute to the persistent glory of India in this field. Eye-care still needs to be propagated among the masses to substantiate the endeavour put forward by ophthalmologists, technologists and concerned persons.

Keywords: medicine, endeavour, awareness, ophthalmology

Medical knowledge, especially Ophthalmology, in this part of the globe has a fascinating antiquity. As an 'object' of medical concern, human eye, gained importance at an early stage of civility. The Indian endeavour in the field of ophthalmology interestingly evolved from ancient Indian philosophy. Unlike any other part of body, 'Eye' was considered important. So as a field of study, Ophthalmology, where object of vision and object of knowledge coincides with the mode of vision and mode of knowledge, developed under special care. For centuries the mainstream knowledge-system of this sub-continent was being nourished under the concept of 'Darashana'. The term literally denotes the act of seeing but its connotation goes far deeper and far wider. It is not the mere corporeal seeing rather it is the perception of the truth critical behind the existence. So the act of 'looking' at things not only through eyes but also through other sensory organs was taken quite seriously. And likewise, visual inability was very often been metaphorized as the symbol of ignorance. Interestingly, the modern medicine is in tune with the ancient metaphysical concept of 'Darashana'; the all-pervasive seeing. The 'clinical eye' encompasses a wide range of experience; it not only sees a disease but also it understands it, feels it, and even listens to it. With advanced technology it monitors the changes caused by it.

History of Indian ophthalmology is replete with vibrant activities. Voluminous works on Susruta and his 'Samhita' unanimously attribute him as the 'Father of Indian Ophthalmology'. Exercising ECCE in ca. 500 B.C.E is till date thought a rarity. But such cases are not beyond expectation as at the day's end we expect such 'magic-healings' if not expertise from physicians. An ophthalmologist treating patients does not sound unusual but if a king, a prince or even a religious preacher is found indulged in treating ocular diseases, taking care of intricate ocular surgeries, then the implication of it up on the masses becomes manifold. Susruta, Charaka, Jivaka, Cakradatta et al were physicians by profession. They even reared disciples. But Gautama Buddha was not a physician by profession. Yet some literary texts call him 'master physician' who is expert in operating cataract. Comparing the Buddha with an ophthalmic surgeon is steeped with allegory. But if it were an actuality then its impact on the masses had been enormous. Equally interesting are these records which tell the story of princely figures who in spite of not being physicians contributed significantly to their contemporary ophthalmic endeavours; both treatment and awareness.

An 11th Century Tale: A reference of ca. 11th century healing house or 'Atursalai' of Tamil Nadu can be sited. This hospital with 15 beds was constructed by the Chola rulers within the temple complex of Venkatesa Perumal. Apart from an outstanding team work of supervision under Asvatthama Bhattaraka, it used to keep a stock of medicine required for any given year. This stock included 'Sunetri' of which it earned specialization. 'Sunetri' was an ophthalmic medicine especially effective for glaucoma. Other ocular problems also found a remedy in this. Sunetri attained specificity along with generality, the combination which is intrinsic feature of modern medicine. Medicines for cataract were also prepared with great distinction. The fact of keeping a good stock of an ophthalmic solution along with other medicines testifies their serious concern. This can be an inspiration for modern healing houses regarding the medicine-stock.

The Sultanate: Historical documents on medicine belonging to sultanate period tell us of another royal figure,
Firuz Shah Tughlaq (1309-1388 C.E) as a physician. His speciality was in bone-setting and ophthalmic treatment. He is credited in preparing an effective collyrium, known as ‘Kuhl-e-Firuz Shahi’ from selected drugs and snake skin. It is pertinent to mention that collyrium had been regarded as a mean to good vision from the time of Susruta. It was employed “to stimulate the growth of eye-lashes, brighten the lusture (lustre) of the eye-balls and clean the pupil.” Specific materials and metals were allotted to specific kinds of collyrium; like - gold pot for sweet collyrium, silver and lapis lazuli for acidic, horn for salty, copper and iron for the astringent, bell-metal for bitter and the lot. Firuz Shah further carried on the legacy. Under his dictation a medical treatise, Tibb-e-Firuz Shahi, was composed but “is not so far traceable”. Another ruler, Muhammad Quli Qutub Shah V of Golkonda Qutub Shahi had interests in oculist studies. He ordered famous oculist of his time Shamsuddin Ali Husain al-Jurjani to translate the famous ‘Tazkirat-ul-kahhalin’ or ‘Notebook of oculists’ of Ali bin ‘Isat. The purpose behind these translations and composition of medical treatises was to diffuse the knowledge among the masses. And the initiator wisely chose an ophthalmologist to do the same for the sake of perfection. Such initiations were employed many a times due to its effectiveness. It is pertinent here to refer that the first Bengali ophthalmologist, Rai Bahadur Lal Madhab Mookerjee also did a translation work in 1902. He translated ‘A Manual of the Diseases of the Eye’ of C. Macnamara, into Bengali to help the native students in learning.

Mughal story: Emperor Shah Jahan patronized Persian men of medicine among who was the famous oculist Haikm Ain-ul-Mulk Shiraji. He was appointed as the personal physician to prince Dara. It is thought that the medical work named ‘Tibbe-e-Dara-Shikohi’ was his work though it acknowledged the prince for the purpose. According to it cataract was treated with medicine.

Case-studies of Tanjore: The endeavour put forward by the king Serfoji II (ruled 1798-1832 C.E) of Thanjavur Maratha dynasty is of signal importance in this field. He himself was an expert ophthalmic surgeon. The hospital he founded had British ophthalmologists. The endeavour he put forward has at least two points of interests. First to mention is the ophthalmic records of patients which he ordered to keep for further and future references. Researchers have found case studies of at least 44 patients whose age limit varied from 5 to 60. Along with these 18 paintings of patients were found7. These paintings can be accounted as (probably) the first conscious effort, in ophthalmology in India, to maintain a record that can help in practical-study; the research and teaching. The frequent use of complex medical terms like lens capsule, posterior chamber, cornea etc and imaging diseases like spring catarh, lenticular cataract, proptosis, leucomas etc hint at the depth of study. Treatment was done applying both oriental and occidental medicine. And second; in eye-hospital patients were given cash reward after their recovery7. This is probably done to advertise their activities and for mass-awareness.

These references unmistakably present the fact that these authority shouldered with the renowned physicians in uplifting the status of respective medical works. Their initiation might have enriched ophthalmology but their engagement surely aggravated the mass-awareness. But the problem of blindness and impaired vision is still looming large. Today’s India is approaching, in a very systematic way, to meet the target of “Vision 2020: the Right to Sight” through various schemes and programmes by NPCB and leading institutions of Ophthalmology. Following the past, the leaders of India, the emblematic personalities of India must come forward to help NPCB and institutions of Ophthalmology. If such personalities are engaged as ambassadors, as propagators in various awareness programmes, in eye-donation propagations the result would yield better fruit. Manipulating the hero-worshiping, the awareness projects can bring qualitative change to this field. Such engagement worked effectively in the past and promises are there to be worthy in present. History records the endeavours but Present counts them.

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References:
AUTHOR GUIDELINES

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