Study Protocol

A Study Protocol for Assessing the Effect of Diode Green Photocoagulation with Rose Bengal in Progressive Infectious Keratitis

Abstract

Background: Infectious keratitis manifested as corneal ulcer can result in formation of pus in corneal tissue. The incidence has increased in recent years due to overuse of steroids. Infectious keratitis is the most common cause of blindness in India. Rose Bengal proves to be a promising option to treat this condition. Rose Bengal is topical ophthalmic dye which is mildly toxic. It is absorbed by damaged epithelial cells, mucus and fibrous cells. This stain is available in the form of 1% solution or impregnated filter strips. This study aims to assess the duration of resolution of corneal ulcer after Rose Bengal photodynamic therapy.

Methodology: This will be an experimental study carried out at ophthalmology department of AVBRH, Wardha. Total 55 cases will be enrolled and examined on daily basis for one week. Pachymetry and specular microscopy will be done before Rose Bengal Photodynamic Therapy and at the end of three months of treatment.

Results: Resistant infectious keratitis is expected to show complete healing of epithelial defect after receiving photodynamic therapy with green diode laser.

Keywords: Infectious keratitis, Green diode laser, Rose Bengal Photodynamic Therapy, Ophthalmic, Steroids.

Introduction:

Infectious keratitis is term used for corneal diseases caused by infective organism. Infectious keratitis seen in countries where medical care is not proper, drugs are not available for treating the keratitis and no other facilities for examination of the disease due to low socioeconomic condition. Corneal ulcer is manifestation of infectious keratitis due to organism which causes death of the tissue and formation of pus in corneal tissue. The incidence of suppurative corneal ulcers caused by fungi has increased due to use of steroids.

Corneal ulcer caused due to vegetative material associated injury like crop leaf, branch of tree, thorn, animal tail. Identification of ulcer causing organism is done by sensitivity and culture of the swab taken from corneal ulcer. Corneal ulcer may be caused by bacteria, virus or fungus. Bacterial corneal ulcer causing organisms are Pseudomonas, Staphylococcus aureus, Staphylococcus albus, Pneumococcus, Neisseria gonorrhoea, E. coli. Virus which are responsible for corneal ulcer are Herpes zoster, adenovirus, chlamydia. Fungus infection leads to ulcer formation are Aspergillus, Histoplasma, Candida etc. Secondary corneal ulcers are seen in immunocompromised individuals who have diseases like herpetic keratitis, dry eye, bullous keratopathy or postoperative case of keratoplasty(1). Candida albicans fungi is more commonly seen in immune- compromised cornea. Fungal corneal ulcer are more common as compare to bacterial and virus as most common mode of injury leads to corneal ulcer is by vegetative material. Fungal corneal ulcer is dry looking, greyish white in colour

with rolled out margins. Some species of fungus shows pigmented brown ulcer. In stroma of the cornea feathery finger like structure are visible in fungal ulcer. Yellow colour line of differentiation is appreciated at point where fungal antigen and host antibodies meet known as sterile immune ring. Small lesions are seen around main corneal ulcer called as satellite lesions. In fungal corneal ulcer as compare to the size of ulcer size of hypopyon is large.

According to The National Impairment Survey untreated non-infectious corneal opacities is one of the cause of blindness which needs keratoplasty as a treatment. In addition to that refractory cases of corneal ulcer which are not responding to medical management also need therapeutic keratoplasty. Keratoplasty is associated with many complications like rejection of graft, infection, keratitis etc. Despite the number of eye donations in India has increased, there is a huge gap between demand and supply. So to avoid the need of therapeutic keratoplasty we are augmenting this novel technique in treating such refractory cases of infectious keratitis.

Patient comes with complaint of pain and foreign body sensation, watering from eyes as increase sensitivity to light, reflex hyper lacrimation, visual blurring and redness of eyes. Topical drugs and cycloplegics are given as treatment and if the hypopyon is severe then systemic drug are also given(1). Now a days after medical therapy of one week in some cases intrastromal injection is also given to cure the progressive infectious keratitis. Such cases are then labelled as refractory cases of progressive infectious keratitis after one week. In such progressive infectious keratitis cases not responding to medical treatment despite of vigorous medical management Diode green photocoagulation with rose Bengal is done as curative measure before doing keratoplasty which will be the last option for the treatment of progressive infectious keratitis.

Rose Bengal is topical ophthalmic dye. It is bright red stain which is mildly toxic. It is absorbed to and absorbed by damaged epithelial cells, mucus and fibrous cells. This stain is available in the form of 1% solution or impregnated filter strips. Some studies show good result with rose Bengal dye if it is used in solution form as compare to the filter strips. Rose Bengal dye is able to stain the surface epithelial cells where tear film is not protecting the cornea as rose Bengal dye is not be able to stain cells because of the preocular tear films which act as protective barrier (2). Because of this quality of rose Bengal dye, it is often used as a stain in diagnosing certain medical issues such as corneal ulcer, epithelial defect. In cases like dry eye, where tear film is absent completely rose Bengal dye stain normal epithelium cells of cornea.

Laser are the monochromatic parallel beam of light which has ability to concentrate in short time interval and produces nonlinear effect. In 1963 first ophthalmic laser was used on human being. Laser are of two type i.e pulsed laser and continuous laser. In pulsed laser output energy is concentrated at regular duration with no beam of laser in between. In continuous laser fixed, stable continuous energy is delivered at given point without any interval. Diode laser is continuous laser in which energy is delivered for more time with less power. Diode green laser produces light that absorbed well which is absent in blue light therefore for treatment green laser is replaced the blue green laser.

In few studies it is observed that photo-crosslinking involving activation of Rose Bengal (RB) with green light (3) where there were approximately equivalent increases in corneal stiffness. (3) these studies did not show any toxic side effect to stromal keratocytes. RB

associates with collagen strongly therefore it limits its penetration to 100m m in stroma where it strongly absorbs the green light and initiates crosslinking. This superficial penetration of RB into the stroma removes the possibility of a photodynamic effect of this procedure on the endothelium. Hence can be used safely while treating thin corneas, even less than 400 micro m (4). During this treatment a small amount of the green light which is not absorbed by RB is transmitted through the cornea where it impinges on the iris or pass through the constricted pupil and get absorbed in the retina by melanin and hemoglobin. There are some reports on the potential effects of this RBPDT studies on the potential effects on the retina or iris of the green light levels used in the treatment and most of these studies are carried on ex vivo and very few studies are conducted on human with infectious keratitis o. To our knowledge this is the first ever Indian study with the diode green laser and RB in infectious keratitis.

Now to study the defiance to degradation by collagenase A of corneas which have been crosslinked with Rose Bengal dye and green light (RGX)(5). Treating corneas in case of infectious keratitis with Rose Bengal stain (RB) locally and treating the tissue to green light (RGX) has been given as a choice for collagen crosslinking technology to RF-UVA crosslinking. Goal of this study is to evaluate RBPDT in infectious keratitis and make it a standard operational protocol in refractory cases of corneal ulcer.

In a study conducted at Bascon Palmer Eye Institute by Naranjo et al (6), noticed the average stromal depth hyperreflectivity measured with anterior segment optical coherence tomography was 269 $\pm$ 75 micro meter with an average period to clinical cure i.e. to decreased pain and inflammation is 46.9 $\pm$ 26.4 days after the RB-PDAT treatment. (7, 8)

Aim-

To study the Effect of Diode Green Photocoagulation with Rose Bengal in progressive Infectious Keratitis.

Objective-

1. To study the duration of resolution of corneal ulcer after rose Bengal photodynamic therapy.
2. To study the thickness of cornea before and after resolution of ulcer.
3. To study the effect of rose Bengal photodynamic therapy on corneal endothelium after resolution of ulcer.

MATERIAL AND METHODS

The study will be according to the tenets of the declaration of Helsinki and will be approved by the Institutional Ethical Committee (IEC) of DMIMS (DU).

Informed consent will be taken from all the patients who are participating in the study after fulfilling inclusion criteria.

SETTING: All procedures will be conducted at the department of ophthalmology, AVBRH Sawangi, under standard conditions by single ophthalmologist.

RESEARCH DESIGN: It is an experimental type of study design

DURATION OF STUDY: 2 years (September 2020 to August 2022)

PARTICIPANTS: All the patients with progressive infectious keratitis coming to ophthalmology OPD at AVBRH will be selected sequentially for the study after taking inclusion and exclusion criteria into consideration.

INCLUSION CRITERIA

1 Patient with progressive infectious keratitis not responding to the medical management.

2 Patient will be selected irrespective of sex.

3 Patient will be selected after giving written consent in the local language

EXCLUSION CRITERIA

1 Patient having other corneal pathology like corneal dystrophies perforated cornea,

2.Thin corneas

3.Preexisting bullous keratopathy.

4. Not willing to participate

VARIABLES: Size of corneal ulcer

Time of resolution

Duration of diode laser application.

Sample size:

Using sample size formula with desired error of margin

$$\frac{n=Z^2\ \alpha/2\ *P*(1-P)}{d^2}$$

Where

Zα/2 is the level of significance at 5 % i.e: 95 %

confidence interval =1.96

P=Prevalence =7.3%=0.073

D=Desired error of margin=7%=0.07

$$n = \frac{1.96^2 x\ 0.205\ x\ (1-0.073)}{0.07^2}$$

=53.05

=55 patients needed in the study

Methodology:

Microbiological culture and sensitivity will be sent in each case by scraping and specific treatment will be started. Regular visits everyday for one week will be done. If patient improves then only medical treatment is continued and the patient is excluded from the study. If there is no improvement in the ulcer then the subject is included in the study as ulcer would be considered as resistant. All the patients participating in the study will be informed in details about the procedure. The patient who are fulfilling the inclusion criteria will be recruited for the study. Clinical history will be taken and ophthalmic examination will be done which includes best possible visual acuity (Snellen's chart), slit lamp examination, fundus examination (if possible in affected eye) will be performed on all patients considering the inclusion – exclusion criteria. Detailed slit lamp examination of corneal ulcer is done which includes size in mm in both h diameters (horizontal and vertical), site, shape of corneal ulcer , colour, edge, epithelial defect, anterior chamber depth and content, presence of satellite lesion or Wesseley ring. Corneal thickness will be measured in each case pre and post rose Bengal photodynamic treatment. Corneal endothelium will be examined pre and post rose Bengal photodynamic treatment.

Procedure of RBPDT:

Surface anaesthesia will be given by putting topical anaesthetic agent (proparacaine 0.5%) two drops. Wait for 2 min after staining the ulcer with rose Bengal dye. Patients is asked to sit comfortably and laser therapy was done using diode green laser 532 nano meter wavelength. A size of the spot 500 µm, duration of pulse 0.2 s, and power of 900 mW are taken as standard. Number of shots varies in each case depending upon the size of corneal ulcer. This rose Bengal stained epithelial defect allow diode laser beam of energy to be absorbed by the corneal cells or else this diode laser beam may traverse the cornea which not stained without absorption as in pan-retinal photocoagulation and may affect retina. Diode laser therapy will be done using diode green wavelength (Nidek Green laser photocoagulator Model GYC – 1000). Depending upon the size of ulcer the number of shot will vary in each cases where we target the ulcer edge and bed during the laser treatment and number of laser shot will be counted.

Follow up:

Each case will be followed up until total healing is attained. In each case we will examined on the basis of the size of the epithelial damage, density of infiltration, edema of cornea, depth of hypopyon level and depth of corneal ulcer. After that every subject will be followed up for 3 months to know any recurrence of the ulcer. Each case will be examined every day for one week. Pachymetry and specular microscopy will be done before RBPDT and at the end of three months of treatment. Statistical analysis will be done by using Chi square test and values are considered significant when $p < 0.005$.

Expected Results:

We expect from our result that resistant infectious keratitis after receiving photodynamic therapy with green diode laser will show complete healing of epithelial defect and we may avoid a need of therapeutic keratoplasty.

Discussion:

We expect total healing of the epithelial injury and recovery of stromal infiltration without any side effect. So there is no need of keratoplasty is the resistant infectious keratitis after receiving diode green photocoagulation. A number on studies related to different aspects of eye health are available (9-12). Studies by Muley et. al(13), Sheikh et al (14), Singh and Pathak(15) and Bhutada (16) were reviewed. Articles on phacoemulsification and related treatment modalities in different eye conditions were reported (17-26).

Conclusion

Conclusion will be drawn from the resulted outcome.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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