"CLINICAL AND MICROBIOLOGICAL STUDY ON CORNEAL ULCER IN PATIENT ATTENDING EYE OPD AT TERTIARY CARE INSTITUTE,VIJAYAPURA, NORTH KARNATAKA"

By

Dr. GAUTAM N BELADIYA

Dissertation submitted to the

B.L.D.E. UNIVERSITY VIJAYAPURA, KARNATAKA

In partial fulfillment of the requirements for the degree of

MASTERS OF SURGERY

In

OPHTHALMOLOGY

Under the guidance of

Dr. VALLABHA.K M.S, DOMS.

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KARNATAKA

2015

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AMT	Amniotic Membrane Transplantation
ATP	Adenosine Triphosphate
CF	Counting finger
KOH	Potassium Hydroxide
NV	Near vision
SDA	Saboraud’s Dextrose agar
VA	Visual Acuity

ABSTRACT

Background and objectives:

To determine the microorganisms causing corneal ulcer as analyzed by clinical examination and laboratory diagnosis.

Methods:

A prospective study was carried out on all the patients presenting to the outpatient clinics in the Department of Ophthalmology in Shri B M Patil Medical College and research centre during the period October 2013 to April 2015.After diagnosing infective keratitis clinically, corneal scrapings, staining and cultures were performed along with routine laboratory investigations.

Results:

Over 18 month's period, 50 patients with infective keratitis were evaluated. Out of which 25 (50%) cases were of fungal etiology, 7 (25%) were bacterial. Corneal ulceration is a common problem in this part of Karnataka and most often occurs after a superficial corneal injury with organic material. Males were affected more than females and 41-65 age group was affected more. Among laboratory investigations gram stain, Blood and chocolate agar were found to be highly sensitive in identifying bacteria. KOH and SDA were found to be highly sensitive in detecting fungal elements.

Conclusion:

This study was developed primarily to determine the clinical features, specific pathogens responsible for corneal ulceration in Vijayapur, Karnataka. In this study majority of the cases were Fungal keratitis (50%), followed by bacterial. Streptococcus pneumonia and Staphylococcus aureus accounted for majority of bacterial ulcers and Fusarium and Aspergillus species were responsible for most of

fungal infections. These findings have important public health importance for the treatment, rapid referral, diagnosis, and prevention of corneal ulceration in the developing world.

Key words – Infective Keratitis.

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INTRODUCTION

Infective corneal diseases are major cause of blindness worldwide second only to cataract.[1] Corneal ulcer is defined as loss of corneal epithelium with underlying stromal infiltration associated with signs of inflammation with or without hypopyon. corneal ulceration is leading cause of monocular blindness in developing countries like India. Corneal blindness is responsible for 1.5 to 2 million new cases of monocular blindness every year in which ocular trauma and corneal ulceration are significant contributors.[2]

The reported incidence of corneal ulceration in India is 1130 per million population.[3]

Aetiologic and epidemiologic pattern of corneal ulceration varies with patient population, geographic location and climate.[4]

Corneal ulcerations are caused by bacteria, fungi, virus and parasites. Fungal corneal ulcers are common in countries like China, India, Bangladesh, Nepal.[5] Fusarium species and Aspergillus species account for 70% of cases .[6] most common causative agents of bacterial keratitis are Staphylococcus aureus, Streptococcus pneumoniae, and gram-negative bacilli, especially P. aeruginosa.[7]

Hence understanding of the aetiologic agents, epidemiologic features and risk factors that occur in specific region are important in rapid recognition, institution of therapy, management and prevention of disease.

The purpose of this study is to evaluate the clinical and microbiological profile of corneal ulcer in patient attending eye OPD in tertiary care hospital in Vijayapura, North Karnataka which has a dry climate and agriculture as a main occupation.

AIMS AND OBJECTIVES

(1) To evaluate the clinical and microbiological profile of corneal ulcer.

REVIEW OF LITERATURE

Amrutha Kumari B, D. Venkatesha have studied in the year 2014 showed that out Of the 100 patients, Bacteria were isolated from 45 (67.17%) ulcers and fungi in 17 patients (25.37%). The commonest bacterial isolates were Staphylococcus aureus in 29.41% and Fusarium spp in 61.91% was the predominant fungal isolate. [8]

S.Krishna et al. have studied in the year between January and June 2012 showed that out of the total 120 patients, 57 patients showed lesions resembling either fungal or bacterial keratitis. 36 cases (63.2%) were fungal keratitis, while 21 (35.1%) cases were bacterial keratitis and 1 case (1.7%) was co-infection of both fungus and bacterial pathogens. The principal fungal pathogen was Aspergillus species in 45%. The predominant bacterial pathogen was Staphylococcus aureus in 62.5%.[9]

Geethakumari PV et al. in 2007 to 2009 showed that out of 1503 patients, microorganisms were isolated in 321 cases (21.36%). 88 cases (27.41%) were bacterial and 224 cases (69.78%) were fungal. Among bacteria, Pneumococci and Pseudomonas were predominant (26.14% each). Among fungi Fusarium(37.05%) was the most frequent.[10]

Ibrahim MM et al. in 2003 to 2006 showed that among 190 patients with subsequent diagnosis of keratitis, 118 had a medical judgment of microbial keratitis, which was found to be of bacterial keratitis in 66 (55.4%) cases, fungal keratitis in 52 (43.7%) cases. The predominant bacterial species isolated was Staphylococcus epidermidis (13 of 56; 23.21%), The predominant fungal species isolated was Fusarium spp. (11 of 17; 65%).[11]

Anil Kumar et al. have studied in the year 2003 to 2005 showed cultures were positive in 110 (55%) of the 200 corneal ulcers. Pure bacterial growth was present in 53 (26.5%) and pure fungal growth in 45 (22.5%). Mixed microbial growth was

present in the cultures of 12 (6%) and there was one case of Acanthamoeba keratitis (0.5%). Of the 70 bacterial isolated, 44 (72.86%) were gram positive and 14 (27.15%) were gram-negative bacteria. Staphylococcus spp. was the most commonly isolated bacterial organism representing 33 (47.4%). Fusarium spp. (30%) was the most common fungus followed by Aspergillus spp. (21%).[12]

T. Shokohi, K et al. have studied in the year 2004 to 2005 showed that out Of 22 patients, fungal keratitis was identified as the principal etiologic agent of corneal ulceration in 7 (31.8%) patients (5 males and 2 females).[13]

Sirikul et al. have evaluated 127 patients in the year 2001 to 2004 showed that, the most frequent microbiological diagnosis was bacterial keratitis in 76 (60%) eyes, followed by fungal keratitis in 48 (38%) eyes, and Acanthamoeba keratitis in 3 (2%) eyes. Pseudomonas species were the most common bacterial isolate 55%. Fusarium species were the most common fungal isolate 27%.[14]

M. Jayahar Bharathi et al. have studied in the year 1999 to 2002 showed that out of the 12, 644 new cases of corneal ulcers, 3295 patients were clinically diagnosed as infective keratitis (non viral). Of these 1138 (34.5%) patients had fungal growth alone, 1066 (32.4%) had bacterial growth alone, 33 (1%) had Acanthamoeba growth alone, 83 (2.5%) had mixed microbial growth and the remaining 975 (29.6%) had no growth. A total of 1226 fungal isolates were recovered, most common fungal pathogen was Fusarium spp. (41.7%) and most common bacterial pathogen was Streptococcus pneumoniae (36%). Pseudomonas aeruginosa was more frequently isolated from corneal scrapes obtained from contact lens wears than other microorganisms in their study.[15]

ANATOMY OF THE CORNEA

Cornea is a transparent avascular tissue with a smooth convex surface and concave inner surface, which resembles a watch glass. The cornea must be transparent, refract light, contain the intraocular pressure and provide a protective interface with the environment. Each of these functions is provided by a highly specialized substructural organization, and in an absence of vessels.

Dimensions of Cornea

Anteriorly cornea appears elliptical, being 11.7 mm wide in the horizontal meridian and 10.6 mm in the vertical. Posterior surface of the cornea appears circular, about 11.7 mm in diameter. This difference is due to greater overlap of sclera and conjunctiva above and below than laterally.

The axial thickness of the cornea is 0.52 mm with a peripheral thickness of 0.67 mm.

In its central third, the optical zone the radius of curvature of the anterior surface is about 7.8 mm and that of the posterior 6.5 mm.[16]

The main function of the cornea is optical; it forms the principal refractive surface, accounting for some 70% of the total refractive power.

Structure of the Cornea

Behind the precorneal film are five tissue layers:

1. Epithelium
2. Bowman’s layer
3. Stroma
4. Descemet’s membrane
5. Endothelium

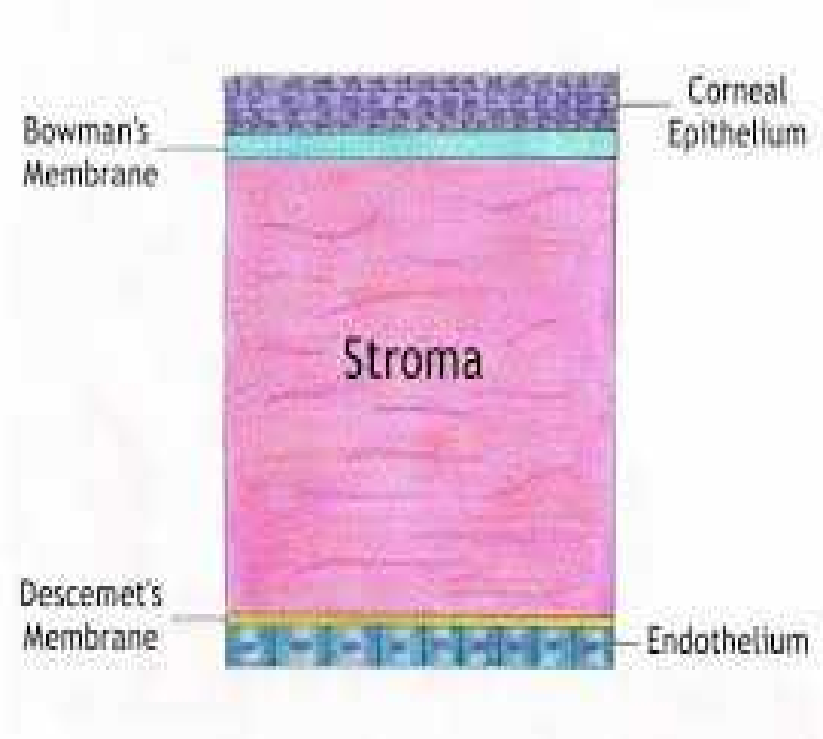


Fig 1: Histology of the cornea.

1. Epithelium:

It is stratified squamous and non-keratinized. It is continuous with that of the conjunctiva at the corneal limbus, but differs strikingly in possessing no goblet cells. The epithelium is 50-90 Fm and consists of five or six layers of nucleated cells.

Deepest layer of basal cells are columnar with rounded heads and flat bases. It forms the germinative layer of the epithelium, continuous peripherally with that of the limbus.

The second epithelial layer (wing or umbrella cells) consists of polyhedral cells, which cap the basal cells, and send processes between them.

The next two or three layers are also polyhedral and become wider and increasingly flattened towards the surface. Their flattened nuclei project backwards, leaving the surface perfectly smooth.

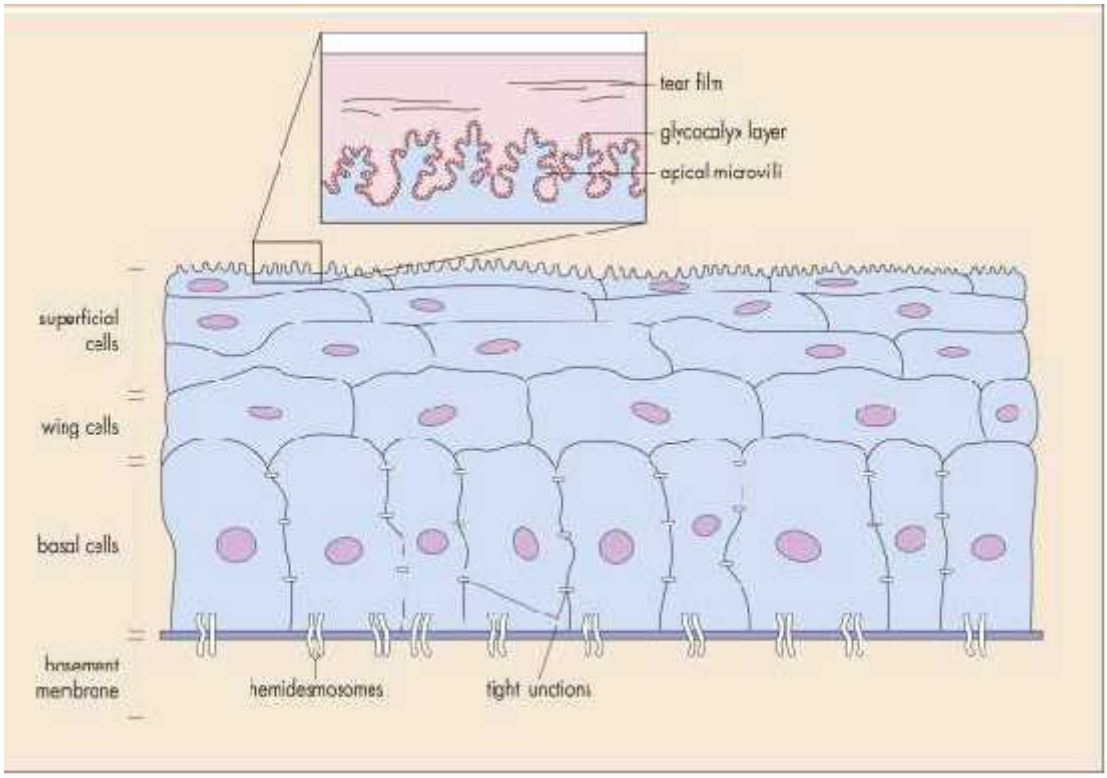


Fig 2: Diagram showing structure of corneal epithelium

Ultrastructure:

Epithelial cells contain the usual organelles of actively metabolizing cells. There is a high glycogen content in the form of large and small granules, especially in the wing and superficial cells. The basal cells are connected to each other by desmosomes and to the underlying basal lamina by hemidesmosomes. The most superficial cells of the epithelium exhibit surface microvilli about 0.5 µm high, serve a physical function in stabilizing the deep precorneal tear film.

Basal Lamina:

It consists of collagen and glycoprotein constituents integrated structurally with the underlying Bowman's layer to which it is firmly attached by an array of short anchoring filaments.

2. Bowman's Layer:

It is a narrow, acellular homogenous zone, 8-14 µm thick, immediately subjacent to the basal lamina of the corneal epithelium. The perimeter of bowman's layer, which has a rounded border, delineates the anterior junction between cornea and limbus and is marked clinically by summits of the marginal arcades of the limbal capillaries.[16]

It is perforated in many places by unmyelinated nerves in transit to the corneal epithelium.

3. Stroma (Substantia Propria):

It is about 500 µm thick, consists of regularly arranged lamellae of collagen bundles. These vary between 9 and 260 µm in width and 1.15 and 2 µm in height, lie in a proteoglycan ground substance together with a relatively small population of cells called keratocytes.

The lamellae are arranged in layers parallel to each other and with the corneal surfaces and become continuous with the sclera lamellae. They run generally from limbus to limbus. They have an oblique orientation in the anterior one-third of the stroma. In the posterior two-thirds the alternating layer of lamellae are at right angles to each other.

The cells among the lamellae are keratocytes, wandering macrophages, histiocytes and a few lymphocytes. Keratocytes occupy 2.5 - 5% volume of the corneal stroma and is responsible for the synthesis of the stromal collagen and proteoglycan during development and maintaining thereafter. Their stellate processes extend for great distances and frequent contacts are made with the other keratocytes in the same horizontal plane.

4. Descemet's Membrane:

It is the basal lamina of the corneal endothelium and first appears at the second month of gestation. Its synthesis continues throughout adult life. Its thickness varies with age, being 3 µm at birth and 10-12 µm in young adults. The major protein of this layer is type IV collagen.

On electron microscopy, anterior third of the adult Descemet's membrane having a vertically banded pattern is produced during the fetal life. The posterior twothirds of the membrane is formed after birth, and consists of a homogenous fibrogranular material.

The peripheral rim of this membrane is the internal landmark of the corneal limbus and marks the anterior limit of the drainage angle (Schwalbe's line).

After traumatic injury of this membrane and endothelium, the endothelium will resurface the defect by spread of its cells and synthesis of fresh basal lamina structurally similar to normal Descemet's layer.

5. Endothelium:

The endothelium is a single layer of hexagonal, cuboidal cells applied to the posterior aspect of descemet's membrane which on slit lamp biomicroscopy appear as a mosaic.

The cell density is around 6000 cells/mm2 at birth. In the human adults, these cells have hardly any ability to divide. Therefore, with increasing age the number of cells is reduced to about 2400- 3000 cells/mm2 in young adults. The defect left by dying cells is filled by enlargement of the remaining cells. There is considerable

functional reserve for the endothelium and corneal decompensation occurs only when the cell count becomes less than 500 cells /mm^2. [17]

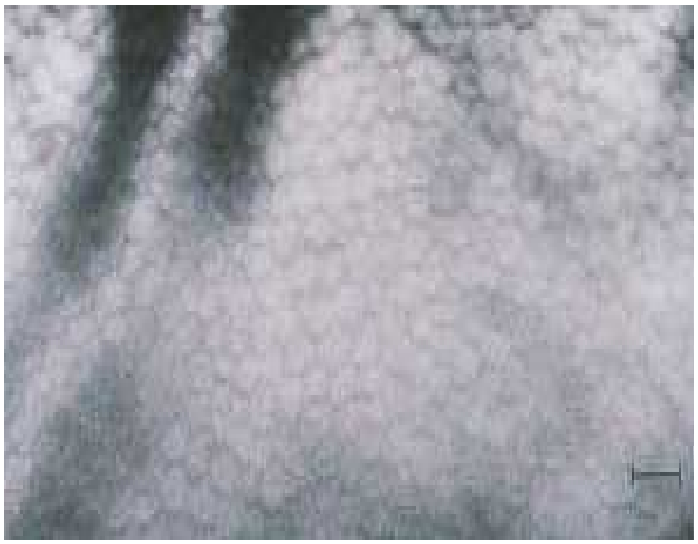


Fig 3: Specular Microscopy showing normal endothelial cells.

The endothelial cells are attached to the descemet's membrane by hemidesmosomes and laterally to each other by tight junctional complexes. The desmosomal linkages and zonulae occludentes are continuous around the entire cell, and thus close the intercellular space from anterior chamber. This linkage is calcium dependent and plays an important role in maintaining the barrier function of endothelium. The endothelium also contains an active secretion and protein synthesis. There is presence of abundant mitochondria, free ribosomes, rough and smooth endoplasmic reticulum and Golgi complexes in the cytoplasm of these cells.

Blood supply of the cornea

The cornea is an avascular structure. Small loops derived from the anterior ciliary vessels invade its periphery for about 1mm and provide nourishment. Actually, these loops are not in the cornea but in the subconjunctival tissue which overlaps the cornea.

Nerve supply of the cornea

The cornea has a rich nerve supply of sensory nerve endings derived mainly from the long ciliary nerves which are branches of the nasociliary nerve, a branch of ophthalmic division of trigeminal nerve.

The long ciliary nerves after arising from the nasociliary nerve enter the eyeball around the optic nerve along with the short ciliary nerves and run forwards in the suprachoroidal space. Short distance from the limbus, these nerves pierce the sclera to leave the eyeball, divide dichotomously and connect with each other and the conjunctival nerves to form a pericorneal plexus of nerves. About 60-80 myelinated trunks from the pericorneal plexus enter the cornea at various levels like sclera, episclera and conjunctiva. After having gone for 1-2 mm in the stroma the corneal nerves loose their myelin sheath, branch dichotomously and form a stromal plexus. The fibres from here penetrate the pores in the Bowman's membrane, loose their Schwann sheath, divide into filaments under the basal layer of epithelium which extends between the cells of all the layers of epithelium, and form intraepithelial plexus. The nerves end in the epithelim as fine beaded filaments.[17]

Thus cornea has an extensive innervational density which is highest near the centre and gradually decreases towards the periphery. However there are no nerves in the posterior part of the cornea, descemet's membrane and endothelium.[17]

Structural proteins of the cornea

Collagen:

The cornea is unusual in the variety of molecular collagen types which it contains.

The basal lamina of the epithelium contains type IV collagen in its periphery. The predominant collagen(about 90%) of the stroma is type I and in the Bowman's layer is type V. the occurrence of hybrid fibrils of type V and type I collagen may be a determinant of the narrow fibril size of stromal collagen. Descemet's membrane contains predominantly type IV collagen.

Stromal collagen shows a high order of specialization with its fine and uniform fibril size, uniform fibril separation and high level of fibrillar organization.

Table 1: Contribution of different biochemical components of cornea in percentages.

	Percentages
Water	78
Collagen	15
Type I	50-55
Type III	≤1
Type IV	8-10
Type VI	25-30
Other protein	5
Keratan sulphate	0.7
Chondroitin sulphate	0.3
Salts	1.0

Proteoglycan:

Proteoglycans are a family of glycosylated proteins that contain at least one glycosaminaglycan chain covalently bonded to a protein core. These acid mucopolysaccharides represent 4-4.5 % of dry weight of the cornea. Cornea contains three major GAG fractions namely:

Keratan sulphate (50%), Chondroitin sulfate A (25%) and Chondroitin 25%. [8]

The GAGS are present in the interfibrillar space of the corneal stroma and account for the stromal swelling pressure i.e., the tendency to imbibe water and thus plays an important role in the maintenance of the corneal hydration level and transparency.

PHYSIOLOGY OF THE CORNEA

Functions of the cornea

1. Cornea together with the sclera forms the outer shell of the eyeball, occupying 1/3rd of the ocular tunic and it also accounts for a substantial proportion of anterior ocular surface. It thus protects the inner components of the eye from physical injury and maintaining the ocular contour.
2. The cornea also contributes to the Ocular Biodefense System. The corneal epithelium thus forms an effective mechanical barrier as a result of the interdigitations of cell membranes and the formation of tight junctions and desmosomes between adjacent cells. Furthermore, together with the cellular and chemical components of the conjunctiva and tear film, the corneal surface protects against potential pathogenic agents.[18]
3. Maintenance of corneal shape and transparency is critical for refraction. The cornea accounts for more than two-thirds of the total refractive power of the eye.

Nutrition and Metabolism:

Cornea receives its nourishment from the peripheral vessels in the episclera and conjunctiva, aqueous humour and tear fluid. The peripheral vessels play a major role in the corneal nutrition and most of this corneal metabolites are capable of diffusing along the corneal stroma towards the centre of the cornea, but the rate at which they diffuse is slow and they cannot reach the centre of the cornea in a sufficient concentration to meet the requirement.[19]

Glucose and oxygen are the two substance of great metabolic importance. It is presumed that aqueous humour is the main source of glucose, tear film and peripheral vessels in small amount but it is unknown whether it enters the tissue by diffusion or by active process. The oxygen requirement of the cornea is supplied through the external surface of this tissue, from the atmosphere, through the tear fluid. Tears get some oxygen from the blood vessels of the palpebral conjunctiva, aqueous supply of oxygen to deeper layers of the cornea.

Cornea derives its energy mainly from the metabolism of glucose in the form of ATP. Respiratory quotient of the cornea is approximately one. The energy is mainly utilized for the maintenance of transparency and thickness of the cornea. The epithelium of the cornea contains lipids and it is possible that it may derive some energy from the oxidation of fatty acids.

There are two pathways involved in the metabolism of glucose. The anaerobic glycolysis(Embden- Mayerhoff pathway) and the direct oxidative pathway (Hexose Monophosphate shunt). With the breakdown of one molecule of glucose into pyruvic acid and with its conversion into two moles of lactic acid, two moles of ATP are generated the utilization of pyruvic acid through the Kreb's cycle with the utilization of the oxygen results in formation of 36 moles of ATP per molecule of glucose.

Transparency of cornea

The main physiologic function of the cornea is to act as a major refracting medium, so that clear retinal image is formed. Maintenance of corneal transparency of high degree, is a perquisite to perform his function. The normal transparency is the result of anatomical and physiological factors.

Factors affecting corneal transparency:

1. Corneal epithelium and tear film: Normal epithelium is transparent due to homogenecity of its refractive index. The basal cells are firmly joined laterally to the other basal cells and anteriorly to the wing cells by desmosomes and maculae occludentes. These tight junctions account for the epithelium's transparency as well as its resistance to the flow of water, electrolytes and glucose.
2. Arrangement of stromal lamellae: Maurice proposed that corneal transparency is a consequence of a crystalline lattice arrangement of collagen fibrils within stromal lamellae and that light scattered by individual fibrils of uniform diameter is cancelled by destructive interference with scattered light from adjacent fibres; therefore light is scattered only in the forward direction.[19]

   Goldman et al 1968 postulated that the cornea is transparent because the fibrils are smaller in diameter in relationship to the wavelength of light and do not interfere with light transmission unless they are larger than one half a wavelength of light.[19]
3. Corneal vascularization: Cornea is avascular except for small loops which invade the periphery for about 1 mm.
4. Corneal hydration: The normal cornea maintains itself in a state of relative dehydration, which is essential for the corneal transparency. The water content of normal cornea is 80 %, which is the highest water content of any connective tissue in the body. It is kept constant by a balance of factors which draw water in the cornea (swelling pressure of the stromal matrix and intraocular pressure) and the factors which prevent the flow of water in the cornea (mechanical barrier action of epithelium and endothelium which constitutes a relatively

impermeable membrane) and those which draw water out of the cornea (active pumping action of the endothelium).

5. Cellular factors affecting transparency: Keratocytes are important in maintaining transparency, as they are the source of stromal collagens and proteoglycans.

CORNEAL WOUND HEALING

1. Epithelial Injury:

Within minutes of a small corneal epithelial wound, cells at the edge of the abrasion begin to cover the defect as rapidly as possible by a combination of cell migration and cell spreading. A longer delay of upto 4- 5 hours is seen in larger defects. This lag phase is necessary for the preparatory cellular changes of an anatomical, physiological and biochemical nature to occur before rapid cell movement.

Various cell membrane extensions such as lamellipodia, filopodia and ruffles develop at the leading edge of the wound. Anchoring hemidesmosomes disappear from the basal cells. This early nonmitotic wound covering phase is remarkable for its speed; the cells have been measured to migrate at a rate of 60-80 μm/h.[20] The migrating sheet of epithelial cell is attached most firmly to the underlying substrate at the leading margin. The relatively firmer adhesion at the leading margin suggests that the epithelial sheet movement may have "front wheeled drive" with the less well anchored cells behind the leading margin being pulled forward, possibly by intracellular contractile mechanisms that involve actin.[21]

Fibronectin, a ubiquitous extracellular matrix protein present in plasma and fresh wounds, is thought to be one of the key elements in the mediation of cell to substrate adhesion cell migration.

At about 24-30 hours after medium sized epithelial injuries, mitosis or cell proliferation begins and restores the rarified epithelial cell population. After large epithelial injuries, significant increases in cellular division occur as late as 96 hours.[22] Only the basal cells, transient amplifying cells, and the limbal stem cells partake in this reconstitutive process.

Various pathological conditions may delay or prevent the normal epithelial healing process. These include the following:

Damage to the cellular substrate (caused by herpetic or other infectious disease, diabetes mellitus, chemical burns, or basement membrane injuries or dystrophies), ocular surface inflammation or atopic disease, medicamentosa, dry eyes, neurotrophic and exposure keratopathies, conjunctival disease (pemphigoid, radiation keratoconjunctivitis and Stevens- Johnson syndrome), extensive damage to the limbal stem cells and eyelid abnormalities.[23]

2. Stromal Injury:

Stromal wound healing consists of repair, regeneration and remodeling involving complex interplay of cytokines, growth factors and chemokines.[24] Importantly stromal repair differs from dermatological healing in that it occurs avascularly and ideally maintains corneal clarity. The reparative cascade begins with activation of stromal keratocytes, which enlarge in size within the first six hours after injury and migrate into the injured area within 24 hours, becoming more fibroblast-like in appearance and behavior.[25]

Activation of the keratocytes may be triggered by epithelial factors, and the reparative cascade that follows typically results in corneal opacity in the affected area. The keratocytes within the area of injury undergo apoptosis, peaking 4 hours after the initial insult.[26]

Within 1-2 weeks, myofibroblasts with contractile properties enter the area under the epithelium and become involved in the remodeling of the stroma, with increased expression of matrix metalloproteases. These cells may be responsible for the “haze” after corneal injury or surgery.

3. Endothelial Injury:

Non perforating puncture injuries of the anterior cornea that do not directly involve the endothelium may cause concentric lesions of the endothelium from rapid focal distortion of the cell layer. The damaged cells are rapidly replaced by enlargement of the surrounding cells (polymegathism) and their centripetal migration into the injured region. When injured, Descemet's membrane curls in towards the stroma and surrounding endothelial slide in to cover the defect and produce new Descemet's membrane. Although the endothelium does not appear to replicate in vivo, recent evidence suggests that endothelial cells retain a degree of latent proliferative potential even into adulthood.[27]

EPIDEMIOLOGY OF CORNEAL ULCERS :

Suppurative keratitis is the most common cause for corneal blindness in India. Incidence of cornel blindness 15.4% the corneal ulcer contributing 9.34%[28] of this. Corneal infections are 2nd most common cause of monocular blindness in developing countries. [18] The reported incidence of corneal ulceration in India is 1130 per million population.[3] The incidence varies from 11/1,00,000 persons /year in the United States to 799/1,00,000 persons/year in Nepal.[29]

Epidemiological pattern and causative agents for suppurative corneal ulcers varies significantly from country to country. Fungal corneal ulcers are common in countries like China, India, Bangladesh, Nepal. Fusarium species and Aspergillus species account for 70% of cases . most common causative agents of bacterial keratitis are Staphylococcus aureus, Streptococcus pneumoniae, and gram-negative bacilli, especially P. aeruginosa.

ETIOPATHOGENESIS OF CORNEAL ULCERS

Classification of Bacteria :

Bacteria.

i. Gram- positive organisms-

A. Aerobes

a. Micrococci - Staphylococcus aureus

- Staphylococcus epidermidis

b. Streptococci - Alpha, Beta- hemolytic streptococci

- Nonhemolytic streptococci

- Streptococcus pneumonia

c. Bacilli-

- Spore forming- Bacillus and Clostridium
- Non- spore- forming- Cornebacterium and Listeria

B. Anaerobes

a. Cocci- Peptostreptococcus, Peptococcus

b. Bacilli-Clostridium, Actinomyces

C. Acid- fast bacilli- Mycobacterium and Nocardia

ii. Gram- negative organisms-

A. Aerobesa.

a. Diplococcic- Neisseria

b. Rods- E.coli, Klebsiella, Enterobacter, Proteus, Pseudomonas

c. Diplobacillus- Moraxella

d. Coccobacillus- Heamophilus

B. Anaerobes- Rods- Fusobacterium and Bacteriodes

Classification of Fungi:

Fungi- broadly classified into filamentous and non-filamentous depending on presence or absence of hyphae.

I. Filamentous-	Septate	Non septate
	Blastomyces	Rhizopus/Mucor
	Non pigmented	Pigmented
	Fusarium	Curvularia
	Aspergillus	Alternaria
	Pencillium	
II. Non- filamentous-	Yeasts	Dimorphic fungi
	Candida	Cryptococcus
		Sporothrix

Corneal response to infection:

Infection of the ocular surface involves 4 processes:

1. Access of the microbe to the ocular surface
2. Attachment of the microbe to the ocular surface.
3. Penetration of the microbe through the corneal epithelium.
4. Growth of the organism.

Humans have evolved a robust immune system to prevent and respond to infection. The immune system can be broadly divided into 2 types. These are the innate immune system and the active immune system.

Innate immune system can again be divided into anatomical, tissue, cellular and biochemical.

Anatomical components:

1. **Bony orbit** – limits access of foreign material to the ocular surface.
2. **Eyelids** – limits access of foreign material to the ocular surface, redistribute tear film, and pumps tears into the nasolacrimal sac.
3. **Lashes** – limit access of foreign material to the ocular surface and trigger the blink reflex.
4. **Blink reflex** – stimulates eyelid closure to limit access of foreign material to ocular surface and helps remove foreign material that contacts ocular surface.
5. **Tear reflex** – flushes foreign material from the ocular surface.
6. **Tear film** –

Aqueous: dilutes foreign materials and creates flow of tears to remove foreign material that contacts the ocular surface.

Mucin: limits adhesion of microbes and encapsulates microbes to facilitate their clearance in aqueous tear flow.

Lipid: limits adhesion of microbes.

7. **Commensal bacteria** – limits adhesion of microbes and deplete the tear film of nutrients important for microbial growth.

8**. Corneal epithelium** – mechanical barrier for microbial penetration.

Tissue factors

Corneal collagen lamellae - ultrastructure resists enzymatic degradation and thus microbial migration.

Cellular factors

1. **Tear film neutrophils** – phagocytic cells that kill and remove microbes from the cornea and the ocular surface.

2. **Phagocytic cells** – conujunctival phagocytic cells kill and remove microbes that penetrate the epithelium.

Biochemical factors[30]

Table 2: Biochemical factors in tear film.

Lipocalins	**Inactivates bacterial proteases**
sIgA	Bind microbial surface proteins preventing adherence and facilitating microbial clearanceby phagocytic cells.
Lactoferrin	Binds divalent cations important for microbial growth and has antibacterial, antiviral and antifungal activities.
Lysozyme	Degrades polysaccharide cell walls of bacteria.
Complement	Antibacterial activity and some comopents opsinogenic.

Active immune system in cornea

It is dependent on humoral and cellular events to eradicate infectious microbes. This system differs from the innate immunity and inflammation in that it responds to specific antigens, and memory for the particular antigen is established with the initial presentation. Three phase characterize the active immune response. First foreign antigens are recognized and presented to T lymphocytes by antigen presenting cells which are the Langerhans cells. The antigen is then processed and both B and T lymphocytes are activated. During the third phase, activated lymphocytes destroy the offending agent.[30]

Microbial mechanisms of host defense avoidance

Most cases of bacterial, fungal and atypical bacterial infections result from mechanical defects in the corneal barriers to infection induced by inadvertent trauma, contact lens wear or iatrogenic causes. Viral infections commonly occur in the absence of antecedent trauma.

Staphylococcus aureus secretes several proteins that increase its resistance to phagocytosis. Coagulase reduces access of circulating leukocytes to the area of infection. Catalase decreases oxidative killing by neutrophils. Production of exotoxins appears to be an additional virulence factor.

Pseudomonas species produce a glycocalyx and possess pili and flagella that facilitate their adherence to contact lenses and host epithelium.

Fungal keratitis is an opportunistic infection. Fusarium elaborates extracellular proteases that may facilitate its pathogenicity and ability to penetrate intact Descemet's membrane. Candida produces several surface proteins that bind integrins such as the complement receptors CR3, CR4 and fibronectin which play a role in adhesion to epithelium as well as migration through tissues.[31]

Latent infection and ability to replicate in intact epithelium are important virulence factors.

NATURAL HISTORY OF CORNEAL ULCERS

The clinical evolution can be divided into 4 stages:

1. **Stage of Progressive Infiltration:** There is an infiltration of the epithelium by polymorphonuclear cells or lymphocytes from the peripheral circulation supplemented by similar cells from the underlying stroma, followed by necrosis.[32]

2. **Stage of Active Ulceration**: The necrotic epithelium is desquamated and a dense infiltration of pus cells gathers. The necrotic tissue is cast off leaving a saucer shaped defect, the walls of which project owing to swelling of lamellae by imbibitions of fluid and packing of leukocytes between them. This zone of infiltration may extend a considerable distance round and beneath the ulcer. At this actively progressive stage, the sides and floor are grey, infiltrated, uneven and sloughing, and the surrounding area is also grey, infiltrated and swollen.

This stage may progress either superficially to involve new areas of cornea or deeply through the thickness of the stroma until descemet's membrane may be reached and a keratocele is formed.[32]

3. **Stage of Regression:** While the pus cells in the central necrotic area are killed or paralysed by toxins, in the surrounding infiltrating zone the leukocytes survive and digest the necrotic tissue which is eventually thrown off. At this stage the defect becomes larger, but the infiltration of the edges and base has disappeared. They become smooth and transparent instead of grey, swollen and transparent.

4. **Stage of Cicatrization:** Superficial vascularization grows in from the limbus, epithelium grows over the edges to form a permanent covering and this stage commences. It occurs from the division of fixed corneal cells to form spindle shaped cells and form new collagen fibres which are irregularly arranged to form an opaque scar of varying density depending on the thickness of cornea involved.[32]

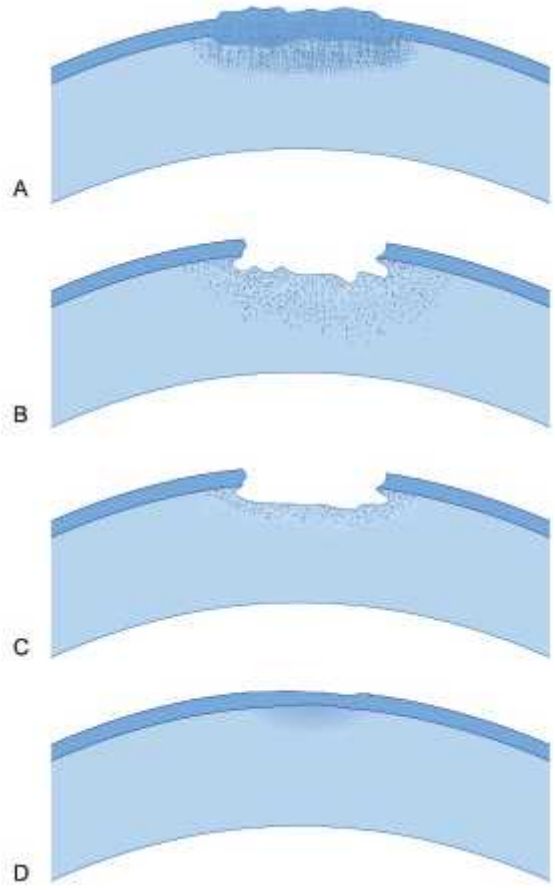


Fig. 4: Natural history of corneal ulcer.

A- Stage of progressive infiltration; B- Stage of active ulceration; C- Stage of regression; D- Stage of cicatrization.

CLINICAL FEATURES

A. Bacterial keratitis:

The symptoms of corneal ulcers are similar in most patients; decreased visual acuity, photophobia, pain, redness, swelling and discharge. The severity depends on the organism, the condition of the host and the duration of the symptoms before the patient is examined.

Slit lamp examination reveals a non- specific conjunctival response (mainly papillary response) and conjunctival chemosis, if a significant iritis is present, a circumlimbal ciliary flush may be seen. Frank purulent suspended white blood cells and debris. The corneal epithelium become ulcerated and the stroma exhibiting variable degree of gray white infiltration. The cornea will be edematous; with visible fold in Descemet membrane. Fine keratic precipitates are found on the endothelium. Anterior chamber reaction exhibits variable degrees of cells and flare. A hypopyon may be present which in bacterial keratitis is typically sterile. It is relatively more common in ulcers caused by Streptococcus pneumonia and Pseudomonas.

Specific clinical features:

Staphylococci: Typically localised round or oval ulcers with distinct borders surrounded by grayish white stromal infiltration. The ulcer develops more in depth than in width may cause intrastromal abscess that may lead to perforation. Corneal ulcers produced by non-aureus strain tend to be more indolent with less infiltration and anterior chamber reaction.

Fig. 5 Central corneal ulcer with hypopyon -Staph aureus

Streptococcal pneumonia: Pneumococcal ulcers tend to remain localized or may have a tendency to spread in one direction, usually centrally in a serpenginous fashion (ulcus Serpens). It frequently occurs in chronic dacryocystitis. There is a marked anterior chamber reaction with hypopyon formation, and perforation is more common.

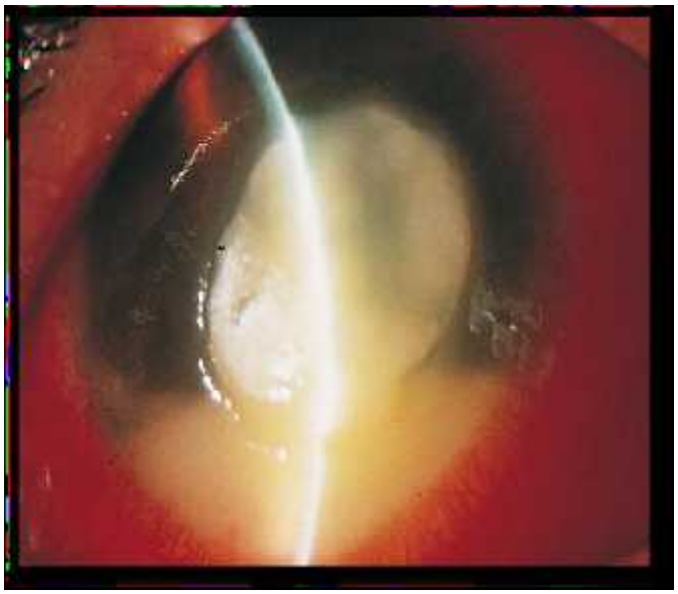


Fig.6 Central corneal ulcer with hypopyon- margins - ulcer serpens

Pseudomonas: Largely associated with the use of soft contact lenses. It is the most common gram negative and highest virulent organism causing corneal ulcers clinically. It causes a rapidly spreading central or paracentral ulcer and associated with dense stromal infiltration. The surrounding cornea is edematous which gives a characteristic “ground glass" appearance, untreated the ulcer spreads circumferentially to form a ring ulcer within 48-96 hours.Despite appropriate treatment, the keratitis may progress rapidly into a deep stromal abscess There may be melting of the cornea with eventual perforation within 2-5 days of the onset of infection.

Neisseria: These ulcers are extremely dangerous because they can lead to rapid corneal perforation, treated aggressively with systemic ceftriaxone because of its destructive nature and ability to penetrate intact corneal epithelium.

Gram- negative rods (Klebsiela, E.coli and Proteus): Cause indolent ulcer and is commonly seen in cases of trauma, malnourished debilated patients and alcoholic patients.

Bacillus: Rapid and devastating keratitis following trauma or wound
contamination. B. cereus keratitis is characterized by a distinctive stromal ring infiltrate remote from the site of injury with rapid progression to stromal abscess, corneal perforation, and intraocular extension with destruction mediated by specific exotoxins.

Anaerobes and higher order bacteria cause ulcers following corneal injury with contaminated soil. Actinomyces species is a filamentous bacteria it is an important cause of canaliculitis, but a rare cause of keratitis. Nocardia and

mycobacterium fortuitum causes indolent indolent ulcers with “cracked windshield” appearance.

B. Fungal keratitis:

The symptoms of fungal keratitis may not present as acutely as with other forms of microbial keratitis, especially bacterial.

Patients may report the initial symptom of a foreign body sensation for several days with a slow onset of increasing pain. In some cases days or weeks may elapse before the patient seeks medical care.

The most frequently encountered external and slit lamp signs of fungal keratitis are commonly seen in other forms of bacterial keratitis which include suppuration, conjunctival injection, epithelial defect, stromal infiltration and anterior chamber reaction and thick immobile hypopyon. History of trauma and some findings such as hyphate branching ulcers, irregular feathery margins extending to the adjacent stroma, a dry rough texture elevated above the surface of uninvolved cornea and satellite lesions[33] can be helpful in suggesting the diagnosis. The appearance of macroscopic brown pigmentation in fungal keratitis may be due to the presence of dematiaceous fungi.[34,35] Satellite lesions are foci of infiltration seen several millimeters away from the main area of involvement. The epithelium can be intact over the infiltrate.

An endothelial plaque may be seen parallel to the ulcer. A ring infiltrate may surround the primary lesion, most likely representing an antibody response to fungal antigen.

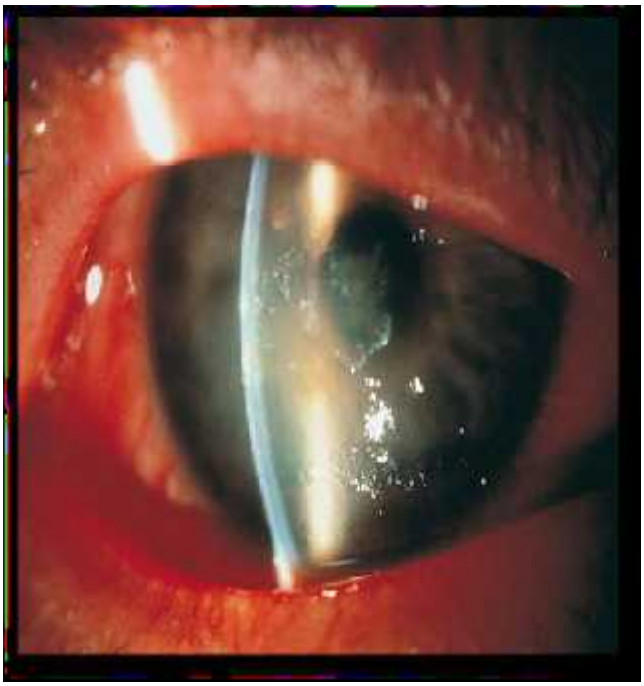


Fig.7 Feathery stromal infiltrates typical of Fungal keratitis

Table 3 : Difference between filamentous fungi and yeast fungal keratitis

Filamentous fungi	Yeasts
Occurs more frequently in young people (occupational and outdoor activity), usually no predisposing factor.	Usually occurs in an immunocompromized host, pre-existing corneal disease or steroid treatment.
Involved area can be localized and is often elevated; epithelial defect may or may not be present;	Usually more focal, elevated and suppurative, resembling bacterial keratitis
Often has feathery edges and satellite lesions.	Edges not feathery and satellitism not usually seen.

In a study to assess whether the presence of characteristic clinical features can be used as a diagnostic aid, it was found that serrated margins, raised slough, dry texture, satellite lesions and discolourations other than yellow occurred more frequently in cases of filamentous fungal ulcers than bacteria. The probability of fungal infection if one clinical feature was present was 63% and 83% if all three were present.[36]

COMPLICATIONS OF CORNEAL ULCERS

Toxic iridocyclitis – occurs due to diffusion of toxins in the anterior chamber.

Secondary glaucoma – It occurs due to fibrinous exudates blocking the angle of anterior chamber. It can be objectively assessed using non- contact tonometers.

In some cases when the whole of the thickness of the cornea except Descemet's membrane may be destroyed. Descemet's membrane is unable to support the intraocular pressure by itself and therefore herniates through the ulcer as a transparent membrane called a keratocele or descemetocoele. This may persist, surrounded by a white cicatrical ring or it may eventually rupture.

Perforation of the ulcer is caused by sudden exertion by the patient, such as coughing, sneezing, or spasm of the orbicularis muscle. Any such activity causes a rise in blood pressure which at once manifests itself by a rise in the intraocular pressure and the weak floor of the ulcer, unable to support the sudden strain, gives way. When an ulcer perforates, the aqueous suddenly escapes and the intraocular pressure falls to the atmospheric level, the iris and the lens being driven forwards into contact with the back of the cornea.

Usually the perforation takes place opposite some part of the iris which is drawn into the aperture when the aqueous escapes. If the perforation is small the iris becomes gummed down to the opening forming a layer of scar tissue over the adherent iris which is referred to as PSEUDOCORNEA. If the perforation is large, a portion of the iris is carried not only into the opening but through it causing a PROLAPSE OF IRIS.

An ectatic cicatrix in which the iris is incarcerated is called an anterior staphyloma, depending on its extent may be partial or total.

If the perforation happens to be opposite the pupil, the pupillary margin of the iris often becomes adherent to the edges and the aperture becomes filled with exudates. The anterior chamber is then reformed very slowly; if the lens remains in contact with the ulcer for a long time a permanent opacity may occur forming an anterior capsular cataract.

As the anterior chamber reforms, the exudate filling the opening is submitted to strain and frequently ruptures. This process may be repeated, so that the opening may become permanent forming a corneal fistula.

Sometimes the whole cornea sloughs with the exception of a narrow rim at the margin leading to total prolapse of iris.

If however the perforation takes place suddenly the suspensory ligament of the lens is stretched or ruptured, causing subluxation of the lens, or even anterior dislocation and spontaneous expulsion of the lens and vitreous through the perforation.

Superficial ulcerations commonly heal with varying degrees of scarring but if the ulcer is deep, the loss of tissue may lead to marked thinning of the entire cornea at the site of the ulcer so that it bulges under the influence of normal intraocular pressure. As the cicatrix becomes consolidated the bulging may disappear, or it may remain permanently as secondary keratectasia, an ectatic cicatrix.

The sudden reduction of intraocular pressure when perforation occurs dilates the intraocular blood vessels, which may rupture causing an intraocular haemorrhage. It may be so profuse that the contents of the globe are extruded along with the outflowing blood, i.e. expulsive haemorrhage.[37]

MANAGEMENT OF CORNEAL ULCERS

A. Laboratory diagnosis of corneal ulcer.

B. Nonspecific ocular treatment.

C. Specific ocular treatment

1) Medical

2) Surgical

D. Physical and General systemic treatment.

A. Laboratory Diagnosis of Corneal Ulcers:

The laboratory work up of a corneal ulcer, regardless of suspected etiology, must be performed in a deliberate and meticulous manner. Failure to do so often accounts for the failure to identify the causative organisms and to initiate prompt, specific antimicrobial therapy.

The following techniques should be followed in a corneal ulcer.

i. The eye must be anaesthetized with 2% or 4% Lignocaine or 0.5% proparacaine hydrochloride.

ii. All exudates and necrotic material should be removed from the ulcer surface using sterile moist cotton swab. Multiple samples from the advancing borders of representative areas of the ulcer are often required to achieve maximal yield of organisms. Cultures of contact lenses, lens case, and associated solutions may be useful in situations where Acanthamoeba is suspected or corneal cultures are negative.

iii. Using a sterile Kimura‟ s spatula or a 26G disposable needle, multiple scrapings are collected from the base and margin of the ulcer under magnification of operating microscope. Care must be taken not to allow the

spatula or needle to touch the lid margins or conjunctiva. Vigorous scrapping is not done in Descemetocele for fear of perforation.

iv. The material obtained is spread on glass slides and stained with Gram" s and/or Giemsa stain for immediate microscopic examination. An additional slide prepared by placing the material in a drop of 10% KOH. A cover slip in placed on this preparation and the slide is examined for fungal filaments under low and high power of microscope. Gram and Giemsa stained smears yield information regarding bacteria, filamentous fungi and Acanthamoeba as well as yeasts. Special fungal stains Gomori methenamine- silver nitrate, periodic acid Schiff and Hematoxocylin and eosin to identify Acanthamoeba.

v. Scrapping materials must be inoculated immediately into various media for culture studies. Culture from routine corneal scrapings are positive in only 60% of patients. Commonly used solid media for culture of bacteria and fungus are blood agar, chocolate agar and Saboraud's agar. Liquid media in common are thioglycollate broth and brain heart infusion, the former allowing the growth of anaerobic bacteria in addition to aerobic bacteria. Acanthamoeba grow best on horse blood agar and E.coli seeded non- nutrient agar.

vi. Cornea specific sensitivity method could be routinely employed, the clinical decision and choice of antibiotics would be greatly facilitated.

vii. Corneal biopsy: For histopathology and culture and sensitivity. Indicated in partially treated or unresponsive corneal ulcer. A 2-3mm circular trephine can be used to outline the area to be biopsied. The biopsy specimen should be at least 1-2mm.

viii. Newer methods: Polymerase chain reaction. Non specific fluorescent stains- Calcoflour white, Blankophor, Uvitex 2B stains fungi and Acanthamoeba require fluorescent microscope. Acridine orange stains bacteria, fungi. Impression cytology, confocal microscopy is a new and non invasive in vivo diagnostic method for microbial keratitis used to distinguish some unusual pathogens such as Acanthamoeba cysts or fungal hyphae. Special illumination system (viewing hematoxylin and eosin stained material under UV light) and fluorescein conjugated lectins identify fungi.

Interpretation of smear and culture:

Smear:

a. Gram stain:

Stains both bacteria and fungi. Classifies the bacteria into two major groups based on the cell wall of the bacteria. Gram- positive bacteria retain the gentian violet-iodine complex and appears blue- purple, whereas the gram- negative bacteria lose their gentian violet –iodine complex with decolorization step and appear pink when counterstained with safranin. Fungal filaments exhibit variability in their staining pattern, with cell wall and septae remaining unstained and only the protoplasm being stained. Yeast on the other hand stain typically blue. It has been reported to yield an accuracy of 66-75%.

b. Giemsa staining:

It is used to determine the type of inflammatory cells present. It differentiates bacteria from fungi, and also identifies trophozoites of Acanthamoeba cells. With Giemsa technique the bacteria appear dark blue in color. The yeast cells and fungal hyphae absorb the stain and appear purple or blue while the cell walls and the septations do not stain.

c. Ziehl- Neilsen Acid-fast stain:

Used for identification of Mycobacteria, Actinomyces or Nocardia.

d. KOH wet preparation:

A 10-20% solution of KOH has been used to visualize fungal elements in corneal scrapings. Owing to chitin in their cell wall; fungal elements are clearly delineated in a homogenous background of corneal tissue digested by KOH. Yeast cells are oval or round and colorless and may sometimes produce psuedohyphae. In a study conducted by Sharma et al., the specificity of KOH is 83.8% and sensitivity is 81.2% as compared to that of Calcoflour white being 83.8% and 93.75% respectively[38]

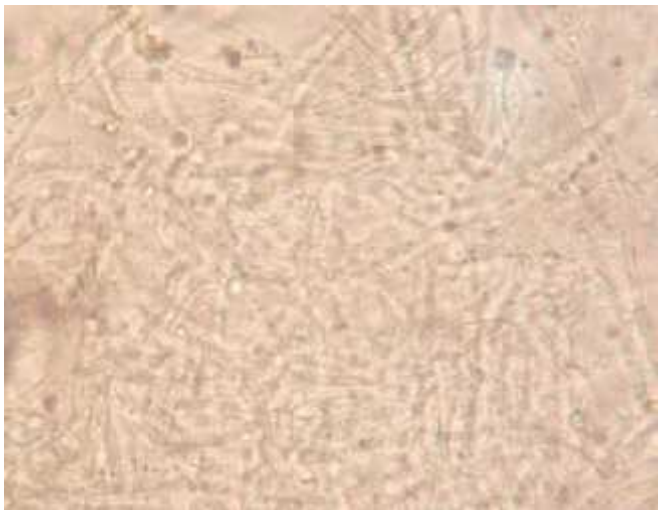


Fig.8 KOH preparation showing fungal filaments in low power.

e. Calcofluor white:

Calcofluor white binds to chitin and cellulose of the cell walls of yeast and filamentous fungi. These organisms stain bright green with Calcofluor white under epiflorescent microscope. The cysts of Acanthamoeba also stain bright green, while the trophozoites stain reddish-orange.

f. Acridine orange:

It is a chemoflorescent dye, which stains fungi and bacteria yellow-orange against a green background when pH is acidic and an epiflourescent microscope is used to visualize these organisms. It identifies gram-positive and gram-negative bacteria, yeast and hyphal forms of fungi and both the trophozoite and cyst form of Acanthamoeba.

g. Lacto phenol cotton blue stain is used for microscopic examination of fungal elements.

h. Modified Grocott-Gomori Methenamine Silver Nitrate stain:

The specimens should be spread onto gelatin- coated slides. With the methenamine silver nitrate stain, fungus cell walls and septa stain black and can be easily seen against the background, which is fairly transparent green.

i.Periodic Acid Schiff stain: Hyphae stains as pinkish red.

Morphology of corneal pathogens:

A. Bacteria:

I. Staphylococcus: It consists of gram-positive cocci arranged in grape like clusters. It is distinguished from Streptococcus by itsbiochemical properties.

II. Streptococcus: It consists of gram-positive cocci arranged in chains or pairs. Different species are classified according to biochemical and serological characteristics. Based on serological characteristics, Streptococcus can be classified into “Lancefield groups” group A,group B, and so on.

III. Haemophilus: It consists of gram-negative, rod-shaped cells of the Proteobacteria group.

IV. Pseudomonas: It consists of nonfermentative gram- negative rods. It is a member of the Proteobacteria. It does not possess a fermentation pathway; instead, it metabolizes carbohydrates exclusively through the pathway of respiration.

V. Enterococcus: It consists of gram-negative cocci in chains, similar to those of Streptococcus. It is distinguished by biochemical and serological testing.

B. Fungi:

I. Fusarium: widespread saprophytic fungi growing in decaying vegetation roots, stems, leaves and fruits. It has special affinity for the cornea. Main identifying morphological features being large banana shaped macro conidia that are produced on short lateral hyphae.

II. Aspergillus: Found in most parts of the world thriving in a variety of substrates decaying vegetation and soil .Morphologically the conidospore have swollen terminal end, surrounded by flask shape or stergimate. Each of which produces long chain of coccoid conidia that out from the terminal end. Hyphae of Aspergillus are septate and characteristically branch dichotomously, the small hyphae at 45deg angle with a distinct cross septa (V for fungus victory) is characteristic of Aspergillus.

III. Dermatiacious fungi (Curvularia): These fungi are distinguished by the brown pigmentation of their colonies.

IV.

V. Candida: It is ubiquitous yeast. It is not linked to environment factors. Commonly seen in immunocompromised individual. Reproduces by budding and forms pseudohyphae, which is the most invasive and virulent phase.

Culture:

The culture media used to culture the organisms should be stored in a refrigerator and should be re-stocked frequently to avoid any contamination. In order to enhance the recovery of the organisms, the media should be warmed to the room temperature to prevent lethal cold shock to the organism. The selective media agar plates are inoculated by streaking the platinum spatula lightly over the surface to produce a row of separate inoculation marks in a C shaped configuration. Liquid thioglycollate broth is inoculated by transferring the material to cotton tipped applicator which has been moistened with trypticase soy broth or calcium alginate swab. The swab is inserted to the bottom of the tube to enhance the growth of anaerobic organisms.

a. **Blood agar:** Enriched media such as blood and chocolate agar help to isolate the fastidious organisms. Blood agar is the standard medium for the isolation of aerobic bacteria at 35 degree Celsius and helps support the growth of most saprophytic fungi at room temperature. The agar is derived from the seaweed and produces optimal surface moisture, and addition of 5-10% red blood cells provides nutrients and an index of hemolysis.
b. **Chocolate agar:** It is prepared by the heat denaturation of blood and provides hemin (X factor) and diphosphyridine nucleotide (V factor) essential for the growth of Heamophilus. It should be incubated at 35 degree Celsius with 10% carbon dioxide. It also supports the growth of Neisseria and Moraxella.
c. **Sabouraud's agar:** It consists of glucose and peptone agar. Yeast extract is added to improve nutritional characteristics and an antibiotic such as Gentamycin or chloramphenicol is added to inhibit bacterial contamination.

The Sabouraud‟ s agar should not contain any additives such as cycloheximide as this inhibits saprophytic fungi.

d. **Thioglycollate Broth:** It promotes the growth of aerobic bacteria, as well as obligate and facultative anaerobic organisms. It consists of the basic nutrients required to support the growth of aerobic bacteria and also has sulf-hydryl compound that act as an oxygen- reducing agent to facilitate the recovery of the anaerobic bacteria. It also supports a number of saprophytic fungi.
e. **Thayer- Martin media:** This is enriched chocolate media that suppresses the growth of the inhibitory components and selectively allows the growth of the N. gonorrhoeae.
f. **Lowenstein Jensen media**: This medium allows the growth of Mycobacteria. It contains glycerol and egg mixture which provides fatty acids and proteins.
g. **For isolation of Acanthamoeba:** The scarped material should be directly inoculated on a confluent lawn of Escherichia coli (monoaxonic culture) plated on non nutrient agar. The laboratories have recommended a temperature of 35 degrees C, possibly with a second plate at 30 or 25 degrees C. the culture plates should be sealed with adhesive tape to prevent evaporation and loss of Acanthamoeba organisms from dying. Acanthamoeba trophozoites track through the lawn of the bacteria. The bacteria do not fill in these paths as there is absence of nutrition for bacteria in the non nutrient agar. The path depicts the ingestion of bacteria by trophozoites; the bacteria are unable to reproduce fast enough to fill in the defect in the nutrient poor medium. The cultures may require more than 9 days to recover the organism and should be maintained for more than two weeks.

Duration of isolation of organism:

Most aerobic organisms responsible for keratitis are seen on standard culture media within 48hours. In some cases the pathogen may be recognized in 12 to 15 hours. All plates should be examined daily with the help of dissecting microscope and liquid media should be evaluated for the presence of turbidity. Growth outside the C streak should be disregarded as it implies contamination and circled with wax pencil. Indigenous organisms in the tear film may appear on the inoculation marks but may be distinguished on the basis of their sparse growth and isolation of the same organisms from the ipsilateral lids or the conjunctiva, if these specimens have been taken. Aerobic cultures of the corneal specimens should be held for 7 days, anaerobic cultures for 7 to 14 days and mycobacterial and fungal cultures for 4 to 6 weeks before being reported as no growth.

The majority of fungi causing keratitis can be detected on SDA within 48-72 hours. Initial growth occurs within 72 hours in 83% and within 1 week in 97% of culture. Culture media should be observed for at least 2 weeks before they are considered negative.

Colony characteristics of various corneal pathogens

A. Bacteria:

i. Staphylococcus aureus:

Colonies of Staphylococcus are typically small, often less than 1mm in diameter.

ii. Streptococcus pneumonia:

Streptococcus is frequently cultured on blood agar, a medium that contains whole erythrocytes. Some species can break down the erythrocytes to produce a clear zone, or “halo” around the colonies. This is called hemolysis, and it is a property that is useful in helping to identify species of Streptococcus.

iii. Hemophilus influenza:

Microbiologists grow this organism on an enriched medium called chocolate agar. Chocolate agar does not contain cocoa- it is actually blood agar that has been heated to release the necessary nutrients from the red blood cells.

iv. Pseudomonas aeruginosa:

Several species of Pseudomonas secrete soluble pigments, some of which are only fluorescent and may be seen with the aid of an ultraviolet lamp.

v. Enterococci:

The colonies of Enterococcus are similar to Streptococcus. It belongs to the Lancefield serological group D.

B. Fungi:

i. Fusarium:

- White in early stage, acquires buff coloration in later stage.
- Colour pigments of colonies vary from yellow to red to purple.
- Reverse pigmentation – pigmentation that are secreted on the agar are best seen on the under surface of the colony.

ii. Aspergillus:

- Aspergillus fumigates colonies are white at first but become velvety green owing to the pigmentation of the conidia on potato dextrose agar.
- Aspergillus flavus colonies are granular, flat, yellow at first but quickly becoming bright to dark yellow- green with age.
- Niger colonies are also white at first but turn completely black as they undergo sporilation.

iii. Dermatiaceous fungi:

- Are distinguished by the brown pigmentation of their colonies.

iv. Candida :

- Smooth grey white colonies

Interpretation of culture results:

It should be made with regard to the clinical situation, the adequacy of the sample and the possibility of contamination by organisms present on the skin, eyelids and conjunctiva.

Positive culture:

Reported culture positive rates in presumed infectious keratitis varies from 40 to 73 percent. Criteria for a significant positive culture by some investigators include the clinical signs of keratitis plus one of the following: 1) growth of the organism in

two or more media 2) confluent growth of a known ocular pathogen in one solid medium or 3) growth in one medium of an organism with positive smear results or growth of same organism in liquid media. Jones criteria for positive culture include: clinical signs of infection plus isolation of bacteria (10 or more colonies) on one solid medium or one additional medium. Although liquid media provide a highly sensitive method for demonstrating a pathogen, a positive culture from broth is less specific than a positive culture from a solid media as it is difficult to quantify the broth cultures. Anaerobic bacteria are suspected in the following situations: Pleomorphic, slender or fusiform morphology seen on gram stain of corneal smear or culture, growth in the anaerobic zone of liquid medium or within the depth of solid agar, production of gas on liquid media and failure to grow organism in aerobic media despite organism detection in gram stain.

Negative cultures:

Negative cultures maybe present truly in cases of sterile or non infectious ulcers or due to prior partial antibiotic treatment, inadequate sampling methods, improper selection of the media and incubation conditions and false interpretation of the data. When the culture results are negative, antibiotic treatment can be suspended temporarily for 24 hours and rescraping is done following which repeat cultures are sent and examined.

B. Nonspecific Ocular Treatment [39]

i. Cycloplegic Drugs: Preferably 1% atropine eye ointment or eye drops should be used to reduce pain from ciliary spasm and to prevent the formation of posterior synechia from secondary iridocyclitis. Atropine also increases the blood supply to the anterior uvea by relieving pressure on the anterior ciliary

arteries and so brings more antibodies in the aqueous humour. It also reduces exudation by decreasing hyperaemia and vascular permeability. Other cycloplegics which can be used are 2% homatropine or 1% cyclopentolate.

ii. Lowering of intraocular pressure by simultaneous use of acetazolamide 250 mg QID orally , intravenous mannitol (20%) drip stat, oral glycerol twice a day, 0.5% timolol eye drops twice a day and even paracentesis with slow evacuation of aqueous from the anterior chamber may be performed if required.

iii. Cauterization of the ulcer may also be considered in non- responding cases.Cauterization may be performed with pure carbolic acid or 10-20% trichloroacetic acid.

iv. Local causes of nonhealing ulcer like concretions, trichiasis, impacted foreign body, dacrocystitis, lagophthalmos, wrong diagnosis should be identified and treated.

C. Specific Treatment of Corneal Ulcers

Medical management

Bacterial corneal ulcer

Topical broad spectrum antibiotic eye drops are used initially and later modified according to culture reports and clinical response. For severe or non responsive cases, combination therapy with topical fortified antibiotics can be given. Antibiotic eye ointment can be used as adjunctive therapy at bedtime in less severe cases [32]. Subcunjunctival injections may not have therapeutic advantage over topical solution but may be indicated in certain clinical situation such as imminent perforation or spread of infection to adjacent sclera, especially when patient compliance is an issue .

Fluoroquinolones has been effective as a single agent as well as in combination therapy in treating bacterial corneal ulcer. Besides fortified penicillin, aminoglycosides provides an adequate coverage of both gram positive and negative organisms . Vancomycin is the drug of choice in case of penicillin allergy or resistance to Staphylococcus species [40].

A loading dose is achieved with a drop every 5 minutes for 5 applications . Antibiotic is then continued every 30 minutes to 1hour around the clock .clinical improvement may not be seen during first two days due to increased inflammation and suppuration from bacterial exotoxins. Although a lack of improvement or clinical worsening after 48 hours may warrant repeat cultures. Topical therapy can be tapered as the clinical picture improves [40].

In case of post LASIK corneal ulcer , the flap should be lifted for smears and culture as well as for soaking of the stromal bed and flap with antibiotics. Fortified amikacin ,clarithromycin or azithromycin are the drugs of choice . Flap amputation may also be necessary to allow increased antibiotic penetration.

Recently use of intra-stromal gentamycin, ciprofloxacin, tobramycin and ofloxacin for the treatment of corneal ulcer due to Pseudomonas have been tried but no clinical proven benefits have been demonstrated [41].

Fungal corneal ulcer

Before 1969, most cases of fungal keratitis were treated with topical. Amphotericin B. Subsequently the related polyene natamycin was used. In 1979, the Food and Drug administration approved natamycin for topical ocular use. In a study conducted by O'day and co-workers, it was found that Amphotericin B in concentrations of 0.050 to 0.075% were superior to all other antimycotics tested.

Table 4 : Classification of Antifungals [42]

	Fungistatic/ Fungicidal	**Topical (%)**
Polyenes		
• Amphotericin B	Fungistatic	0.05-0.15%
• Natamycin	Fungicidal	5%
Imidazoles		
• Clotrimazole	Fungicidal	1%
• Miconazole	Fungicidal	1%
• Econazole	Fungistatic	1%
• Ketaconazole	Fungicidal	1-2%
• Fluconazole	Fungistatic	0.3%
• Itraconazole	Fungistatic	1%
• Voriconazole	Fungistatic	1%
Pyrimidines		
• 5-flouorocytosine	Fungistatic	1%
Others		
• Pradicimicins		
• Cispentacin		
• Jasplakinolide		
• Terbinafine		
• Nystatin		

1. Polyene Antifungals:

Polyenes are the first major group of antifungals to be discovered by Hazen and Brown in 1959. Among the drugs discovered later only Amphotericin B, Natamycin and Nystatin are of practical interest as far as ocular fungal infection is concerned.

The basic structure of polyene antibiotics is a large conjugated double bond system linked to an amino acid sugar, Mycosamine. Polyenes bind to sterols in the cell membrane of eukaryotic cells. They have no effects on cell menbranes in which sterols are absent. They tend to have a greater affinity for ergosterol(fungal membrane sterol). This apparently accounts for their invivo selective antifungal effect in the treatment of mycotic disease in animals. However , their affinity for cholesterol is considerable which tends to make them toxic.

Polyene antimycotics penetrate poorly if the epithelium is intact but do penetrate into atleast the superficial stroma in the denuded cornea.

Functionally the polyenes are divided into two classes:

LARGE POLYENES: with 35 or more carbon atoms amphotericin B and Nystatin.

SMALLER POLYENES: with 30 or fewer carbon atoms – Natamycin.

Both the groups bind to sterols. Large polyenes act by binding to membrane phospholipids and by grouping together to form narrow circular channels for the passage of K+, Cl- and other ions and by doing so they alter the permeability of the membrane. Subsequently, a loss of intracellular cations particularly K+ occurs and cell death occurs. Smaller polyenes accumulate in the membrane and form blisters that disrupt it and cause the sterol phospholipid to breakdown.

a. Amphotericin B: It is a heptaene polyene. It is consistently active against Candida, Cryptococcus and Aspergillus spp., but is variably active against Fusarium spp. It was the most frequently used agent before the development of natamycin and was effective in many cases but resistance was common and toxicity limited its use.

It is fungistatic, insoluble and relatively toxic and it cannot penetrate intact epithelium. Stromal penetration of denuded, inflamed stroma is better, but only a small percent of drug present is active. It is unstable in light, water, heat and pH extremes.

The adverse effects of topical amphotericin include chemosis, epithelial clouding, burning, greenish discolouration and punctuate keratopathy. These effects are partly due to bile salts used to stabilize the drug in solution. Dilute preparations (0.05% to 0.15%) are less toxic and appear to be as effective, systemic administration is toxic and ineffective for keratitis. The toxicity is due to membrane damage in erythrocytes and renal tubular cells.

It is recommended to use a 0.05%, with an initial application every 5 minutes for one hour and then one drop hourly. Amphotericin B should be prepared in distilled water for eye drops because it precipitates in sodium chloride solution. It should not be given subconjunctivally because it is painful and can lead to local necrosis.

Preparation of amphotericin B ophthalmic solution: Take 50 mg vial and 10 ml of distilled water, take 9 ml of the above solution and add 21 ml of distilled water. Amphotericin B (0.15 mg/ml) should be refrigerated and discarded after 7 days.

b. Natamycin: It is the only antifungal agent commercially available for ophthalmic use. Like amphotericin B, it alters the permeability of fungal cell membranes by binding to sterols. Instead of forming ionic channels, natamycin accumulates in the membranes, disrupting their integrity.

Like amphotericin B, natamycin is insoluble and lacks stability, but it is less toxic. It penetrates the deepithelized cornea well, and to a much lesser extent also penetrates intact epithelium. A small percentage of the intrastomal drug is bioactive. It appears to be active against relatively superficial infections. Natamycin has a broad spectrum of activity against filamentous fungi and is the drug of choice for these organisms. It is also effective against yeasts including Candida albicans. In South florida , Forster and Rebell found natamycin to be effective in 85% of Fusarium solani infections, 60% of infections caused by other non-pigmented filamentous fungi, 90% of those caused by pigmented filamentous fungi 75% of yeast infections.

A 5% suspension is applied topically and it usually adheres to the ulcer site. It also forms a rope like strand in the inferior fornix, which may act as a reservoir of drug. For the first 3-4 days, drops should be given every half an hour and then decreased to 6-8 times per day. With frequent or prolonged use it can cause epithelial toxicity.

c. Nystatin: It is a polyene antifungal agent that is used commonly as a dermatologic ointment for yeast infections. A solution of 50,000 to 100,000 U/ml can be formulated for treatment of superficial Candida keratitis. The dermatologic cream can also be used for ocular infections. Nystatin is administered every 2 hours initially. If the ointment form is used any excess should be wiped from the lid margin after each instillation.

Imidazole group of antifungal agents

They exert their antifungal effect by two effects:

- By interfering with the synthesis of ergosterol, the fungal membrane sterol.
- A direct action on the cell membrane causing them to become leaky probably by the binding of the imidazole to fatty acids.

1. Miconazole: Topically used as a 1% solution in arachis oil every hourly during the day, every 2nd hourly at night.

Systemically 300-600 mg/day intravenously has been used for ocular infection.

It is active against a larger spectrum of filamentous fungi and Candida organisms, but less effective against Aspergillus and Fusarium species than other agents. It also has some antibacterial activity against gram positive organisms.

2. Clotrimazole: Topically used as 1% solution in arachis oil every one hour until response occurs, then 4 times a day for 8 to 12 weeks.

Systemically 60-100mg /kg/day for 2 weeks and it is highly effective against Aspergillus, Candida species and some filamentous fungi including Paecilomyces, Dreschlera, Alternaria and Cladosporium species. It has poor effect against Fusarium.

3. Econazole: Topically used as 1% solution in arachis every hour during day and every 2 hrs during night and systemically 200mg/day.

It is more effective than Miconazole against Aspergillus, Fusarium and Penicillium species but less effective against Candida species.

4. Ketaconazole: It is more water soluble and is better absorbed after systemic administration than other imidazoles. Good corneal levels are obtained after topical, subconjunctival or oral administration. It has a slightly broader spectrum of activity with clinical case reports of efficacy against Aspergillus, Fusarium and Curvularia

ocular infections. Its principal ophthalmic use was systemically to augment topical therapy but its side effect is hepatotoxicity.

Topically given as 1%-2% (10-20 mg/ml) and systemically 200-400mg/day orally in one dose.

Triazole group of antifungal agents

Based on the experience with imidazoles a new of compound, the triazoles has been developed. The triazoles have three nitrogens in the azole ring. Triazoles, like the imidazoles inhibit the sterol 14-Q- demethylase. This impairs ergosterol synthesis, leading to accumulation of methyl sterols that impair the function of membranebound enzyme systems, thus inhibiting fungal growth. As a class, the triazoles considerably improved antifungal activity virtually replaced the imidazoles.

a. Fluconazole: Is the first antifungal agent with a good pharmacokinetic profile and a low incidence of systemic side effects. It is water soluble and exhibits low protein binding. Uptake and persistence in all ocular tissues is excellent when administered systemically. The systemic preparation can be administered topically as a 2 mg/ml drops. It is most active against yeasts.

b. Itraconazole: it is poorly water soluble and exhibiting high protein binding. Nevertheless, it has proven efficacy not only against yeast but also against aspergillus sp. for corneal infections, its principal use has been systemically to augment topical therapy.

c. Voriconazole: It is a triazole antifungal agent and a synthetic derivative of fluconazole, it is effective against yeast and filamentous fungi. Its mode of action is inhibition of cytochrome P-450 mediated 14- Q- lanosterol demethylase causing fungal cell wall destruction. Reports of topical and oral voriconazole have illustrated its efficacy in the management of fungal keratitis caused by Candida, Fusarium,

Alternaria, Saedosporium that was refractory to standard antifungal treatment. Epithelial debridement may not be necessary for penetration because it is a small lipophillic molecule. For optimal intraocular drug penetration, both oral and topical administration of voriconazole is recommended.

Orally 400-800 mg/day and topically 1% drops.

1% drops are prepared by dissolving 200 mg powder in 19 ml water for injection to 20 ml.

Because of its broad spectrum of coverage, good tolerability and excellent bioavailability with oral administration, it may be a good alternative to fungi resistant to standard antifungal agents. However the expenditure involved in its use pose a constraint to its more frequent usage.

Flucytosine: It is a fluorinated pyrimidine and is fungistatic. Flucytosine is selectively taken up by susceptible fungi and deaminated to fluorouracil which blocks thymidine synthesis. It is not metabolized by human cells, upto 95% of the dose is excreted. However gastrointestinal flora can convert flucytosine to fluorouracil which is then absorbed. Flucytosine is effective against Fusarium or Cephalosporium. In general, the results are disappointing when flucytosine is used alone. Combined treatment, particularly with amphotericin B, is probably best because the combination appears to be more effective and the chance for resistance is reduced.

Topically it is used as a drops in a 1% solution (10mg/ml) every hour and appears to be well tolerated. It penetrates the cornea well when administered topically and penetrates the cornea and anterior chamber well when administered orally. The oral dose is 50-150 mg/kg/day in four divided doses. Systemic administration is relatively risk free, but bone marrow and liver toxicity can occur. These complications

are dose related and reversible if the drug is withdrawn. Therefore regular hematologic and liver function evaluation should be performed.

Indications of systemic treatment:

The use of systemic antifungal agents is generally not indicated in the management of fungal keratitis, especially toxic agents like such as amphotericin B. Several clinical and experimental studies have reported favourable results in the treatment of fungal keratitis with systemic ketoconazole, miconazole and fluconazole. The most frequently used oral antifungal is ketoconazole. Fluconazole may penetrate better into the cornea after systemic administration and associated fewer side effects. The indications are:

a. Severe deep keratitis

b. Scleritis

c. Endophthalmitis

d. Keratitis extending to the limbus

e. Prophylactic treatment after penetrating keratoplasty for fungal keratitis.

Role of topical corticosteroids

Previously steroids were contraindicated in the treatment of fungal keratitis. Now topical steroids are considered to decrease corneal inflammation and scarring after atleast 2 weeks of antifungal treatment and clear clinical evidence of control of infection. Careful follow-up is required to ensure that improvement is taking place. The steroid drops are used in conjunction with the topical antifungal and never without. Usually steroid treatment is carried on for 2-3 weeks.[43]

Other newer modalities of treatment

1. Collagen shields soaked in amphotericin B to increase the concentration of drug in the initial treatment periods.
2. Tissue glue has fungistatic capabilities.
3. Intracameral amphotericin B in the management of deep keratomycoses.[46]

Surgical treatment of corneal ulcer

The different surgical treatments are as follows:

1. Debridement
2. Conjunctival flap
3. Therapeutic keratoplasty
4. Cryotherapy
5. Amniotic membrane transplantation.

1. Debridement: Daily debridement with a spatula or a blade is the simplest form of surgical intervention and is usually performed every 24-48 hrs and works by debulking organisms and necrotic material and by enhancing the penetration of topical antibiotic and antifungal. A biopsy may not be used only for diagnosis but also as a therapeutic intervention.

2. Conjunctival Flap: It is indicated for indolent fungal infections not responding to aggressive antifungal therapy. It helps in healing of the ulcer but has a disadvantage that viable fungal organisms have been recovered under the conjunctival flap. It can be done as thin total conjunctival flap (Gunderson flap) or a partial conjunctival flap.

3. Therapeutic Penetrating Keratoplasty: The main goals are to control the infection and maintain the integrity of the globe. An optical keratoplasty can then be performed at a later date. The timing of the therapeutic keratoplasty is important.

Most retrospective series indicate that keratoplasty was performed within 4 weeks of presentation primarily because of medical treatment failures; in some cases may be required because of recurrence of infection. When progression of the keratitis is noted, penetrating keratoplasty should be performed. If it is allowed to progress until it involves the limbus or sclera, unfavourable outcomes secondary to scleritis, endophthalmitis and recurrences are more common.

The success of keratoplasty has been reported as better than medical therapy by several authors. A lamellar keratoplasty is generally contraindicated in the treatment of active fungal keratitis. The fungal organisms can be trapped in the intralamellar space, keeping them isolated from the antifungal therapy and the host immune response, thereby leading to the potential for persistence or recurrence of the infection.

4. Cryotherapy: It may be used in conjunction with topical antifungal agents and/or a corneoscleral graft in cases of fungal scleritis and keratoscleritis. Retrobulbar anaesthesia is usually required and a conjunctival recession is performed to expose the infected sclera. Using a retinal cryoprobe, two freeze thaw cycles of several seconds are applied primarily to the borders of the infection where the organisms are presumably replicating and invading. The area is left exposed and subconjunctival antifungals injected. These patients are usually continued on both topical and systemic antifungal agents.

5. Role of Amniotic Membrane Transplantation:

The inhibitory effect on inflammation, proteolysis, angiogenesis, fibrosis and the promoting effect on epithelialization following amniotic membrane transplantation have been well recognized, as well as the potential advantage of AM over traditional penetrating keratoplasty in terms of graft rejection.

If perforation is small, if there is some residual stroma around the perforation site, AMT especially multilayered AMT, can still be performed, as the residual stroma together with the AM can prevent leakage of aqueous. However if the perforation is large and the lesion edge is blunt, it is better to perform patch grafting using cryopreserved or fresh cornea to enhance the tectonic strength. For extensive ulcers, AMT could still be performed as an emergent procedure until a donor cornea is available.

D. Physical and general treatment

i) Pad and bandage helps to heal the ulcer. However if there is associated acute conjunctivitis and copious discharge, it should be avoided. Alternatively, green shade or dark goggles should be used to protect the eye from irritating effects of strong light. In case of impending perforation pressure bandage should be applied.

ii) Vitamins (A, B complex and C) help in early healing of the ulcer.

iii) Systemic conditions like diabetes mellitus, severe anaemia, malnutrition, chronic debilitating diseases and patients on systemic steroids and other causes of immunosuppression like HIV and hepatitis B should be ruled out.

MATERIALS & METHODS

Source of Data:

The materials for the study were drawn from corneal ulcer patients attending the Outpatient Department and those refered to Department of Ophthalmology, B.L.D.E.U's Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapura from October 2013 to April 2015.

Method of collection of data :

Fifty cases of infective corneal ulcer attending the outpatient department of Ophthalmology were selected for the study.

Inclusion Criteria:

All cases of clinically diagnosed suppurative keratitis, who attended our institution during the study period after taking informed consent.

Exclusion Criteria:

(1) All suspected viral, interstitial and autoimmune keratitis.

(2) All Atheromatous ulcers.

Each patient was subjected to a detailed history taking followed by detailed ocular examination as per the enclosed proforma. Patients were advised to get admitted to the hospital for observation and better follow up. If not they were advised to attend follow up in OPD without fail.

1. Examination of anterior segment and corneal ulcer was done in detail with the help of a slit lamp biomicroscope.
2. Recording of visual acuity using Snellen's chart.
3. Fundus Examination.

4. Lacrimal sac syringing.
5. Fluorescein staining.
6. Routine laboratory investigations:

    a) Complete hemogram

    b) RBS

    c) Urine - sugar, microscopy and albumin

    d) HIV, HBsAg 2.

7. Microbiological investigations :

The sample for microbiological investigations was obtained by corneal scraping. The cornea was anaesthetized using 0.5% proparacaine solution and scraping was done using sterile No. 15 Bard Parker blade or 26gauge ½ inch needle under aseptic conditions from the margins of the corneal ulcer.

The following microbiological investigations were done immediately:

a. Gram's stain

b. 10% KOH preparation

c. Bacterial culture using blood agar and chocolate agar.

d. Fungal culture was done using Saboraud's dextrose agar medium. Culture reports were declared negative at the end of 21 days.

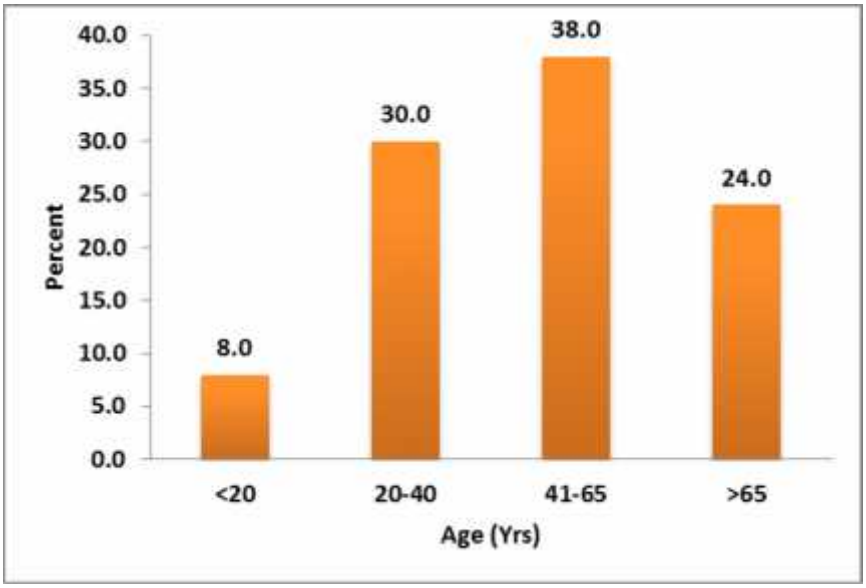
RESULTS

The following observations were made from the present clinical study and the results are tabulated.

Table 5 : Age distribution of patients

Age (Yrs)	N	Percent
<20	4	8
20-40	15	30
41-65	19	38
>65	12	24
Total	50	100

Graph – 1 showing Age distribution of patients

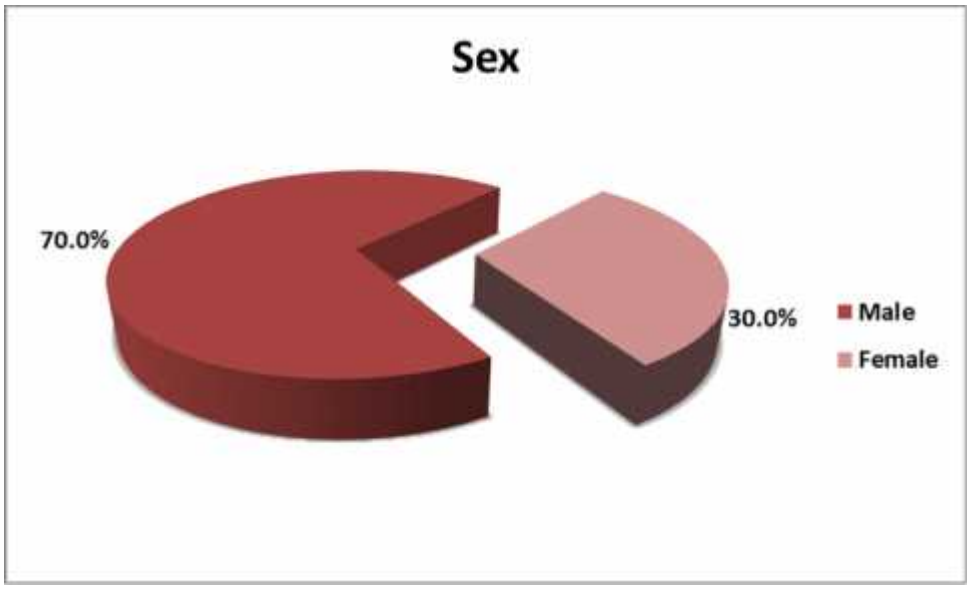


This table shows that majority of patients were in the age group of working population i.e., from 21 -65 years.

Table 6 : Sex of the patients

Sex	N	Percent
Male	35	70
Female	15	30
Total	50	100

Graph – 2 showing Sex of the patients

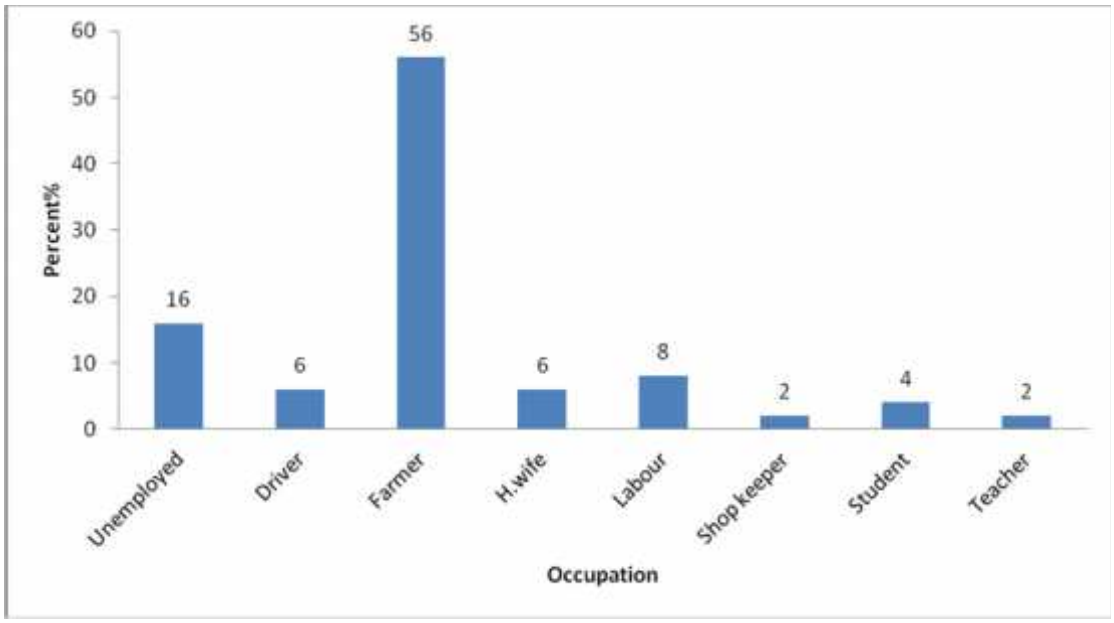


This table shows that majority of the patients in this study were male (70 %) and the rest of the patients were females (30%).

Table 7 : Occupation of patients.

Occupation	N	Percent
Unemployed	8	16
Driver	3	6
Farmer	28	56
H.wife	3	6
Labour	4	8
Shop keeper	1	2
Student	2	4
Teacher	1	2
Total	50	100

Graph – 3 Showing Occupation of patients.

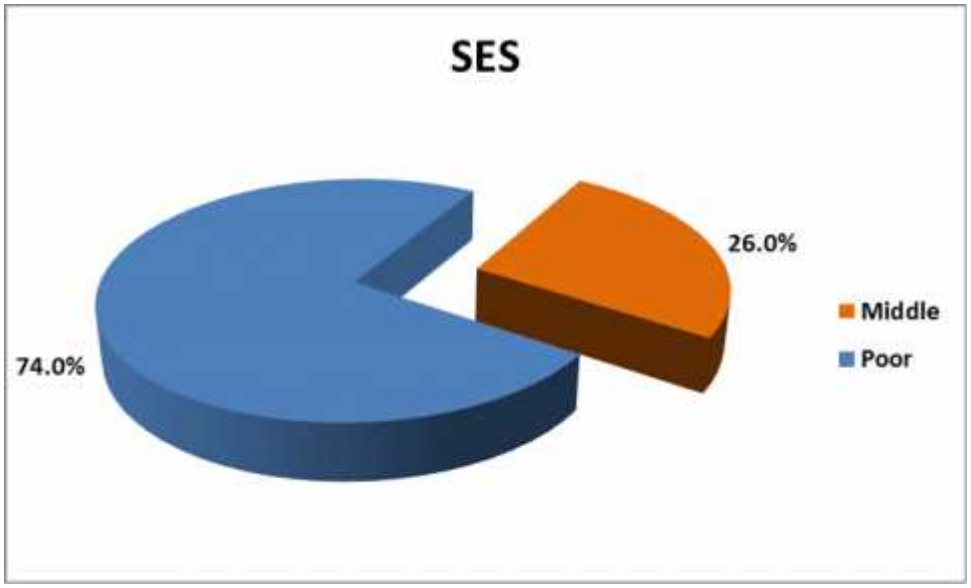


The above table shows that most of the patients were farmers (56 %), followed by others (28 %) category which included other professions like Labour (8%),Driver (6%), Housewife (6%), Students (4%),Teacher (2%), Shopkeepers (2%).

Table 8 : Socioeconomic Status Of Patients

SES	N	Percent
Middle	13	26
Poor	37	74
Total	50	100

Graph – 4 Showing Socioeconomic Status Of Patients

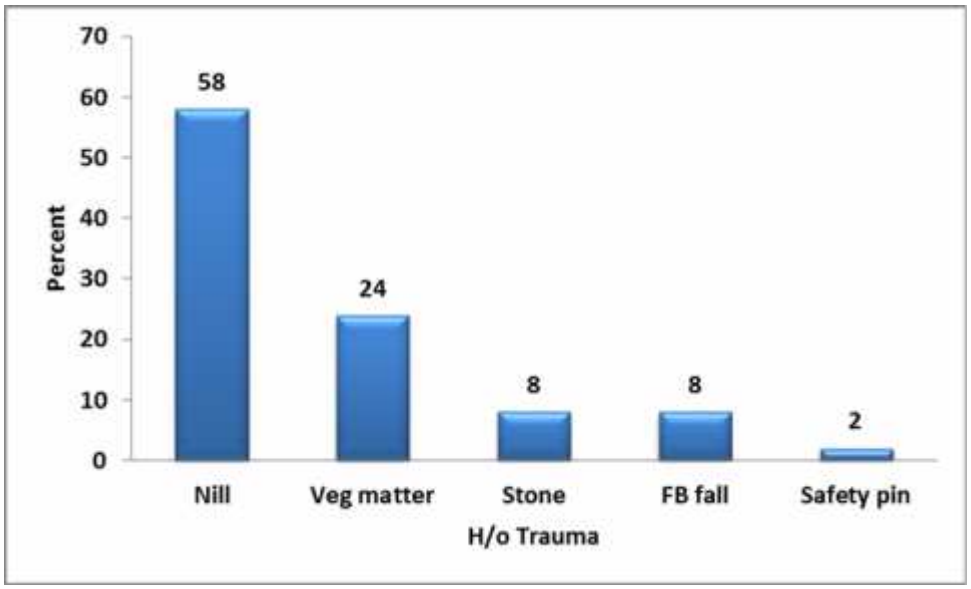


The above table shows that most of the patients were came from lower socioeconomic class (74%) and followed by middle class (26%).

Table 9 : History of trauma to the eye

H/o Trauma	N	Percent
Nill	29	58
Veg matter	12	24
Stone	4	8
FB fall	4	8
Safety pin	1	2
Total	50	100

Graph – 5 Showing History of trauma to the eye

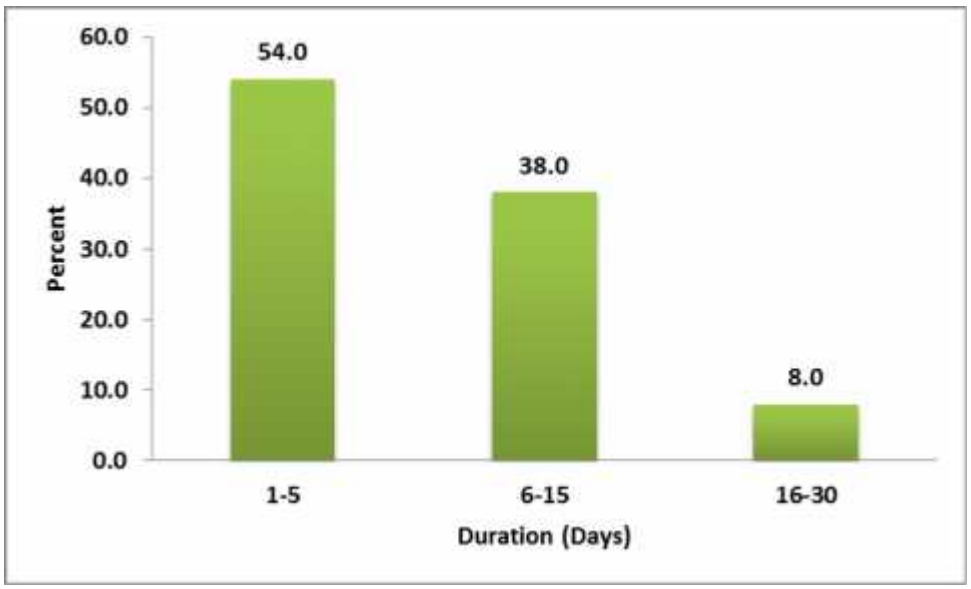


Out of the 50 cases, 42% gave history of injury of which 24% had injuries with vegetable matter.8% had injuries with stone piece and 8% gave history of foreign body fall, 2% had injuries with safety pin, while the remaining 58% did not give any history of injury.

Table 10 : Duration of symptoms at presentation

Duration (Days)	N	Percent
1-5	27	54
6-15	19	38
16-30	4	8
Total	50	100

Graph – 6 Showing Duration of symptoms at presentation.

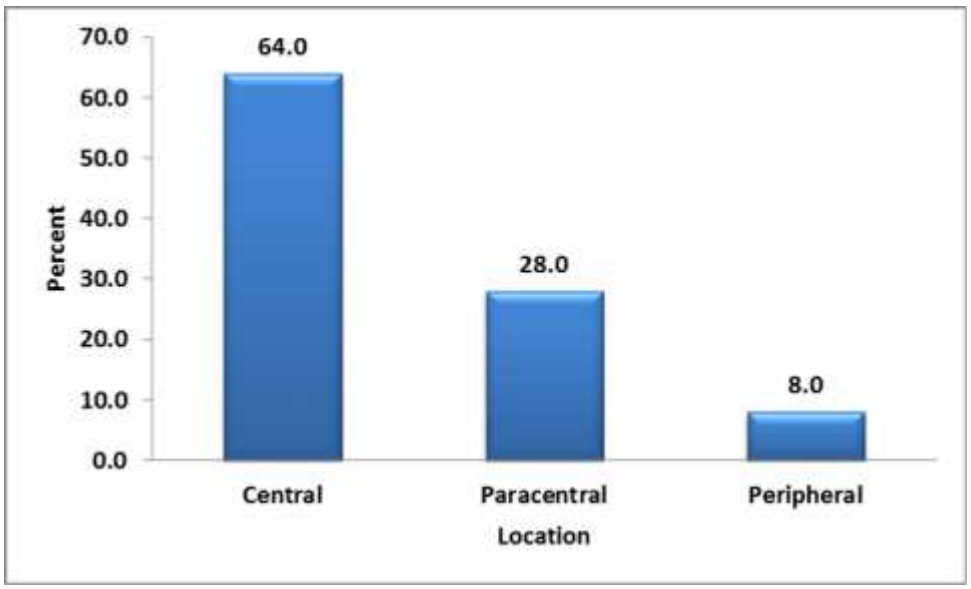


In this study, 27 (54 %) patients presented after 1-5 days of having symptoms like pain, redness, diminution of vision and watering of eyes. 19 (38 %) patients presented after 6 to 15 days of symptoms and 4 (8%) patients after 16 to 30 days of symptoms.

Table 11: Location of corneal ulcers

Location	N	Percent
Central	32	64
Paracentral	14	28
Peripheral	4	8
Total	50	100

Graph – 7 Showing Location of corneal ulcers

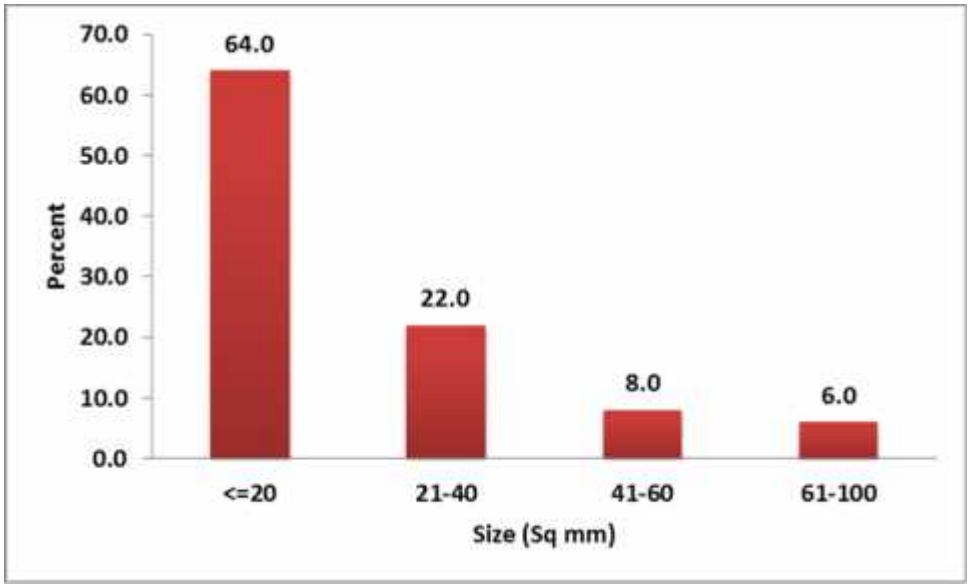


In this study, most 32 (64 %) of the corneal ulcers were located centrally, 14 (28%) were located paracentrally and 4 (8 %) were peripheral in location.

Table 12 : Size of the ulcers

Size (sq mm)	N	Percent
<=20	32	64
21-40	11	22
41-60	4	8
61-100	3	6
Total	50	100

Graph – 8 Showing **Size of the ulcers**

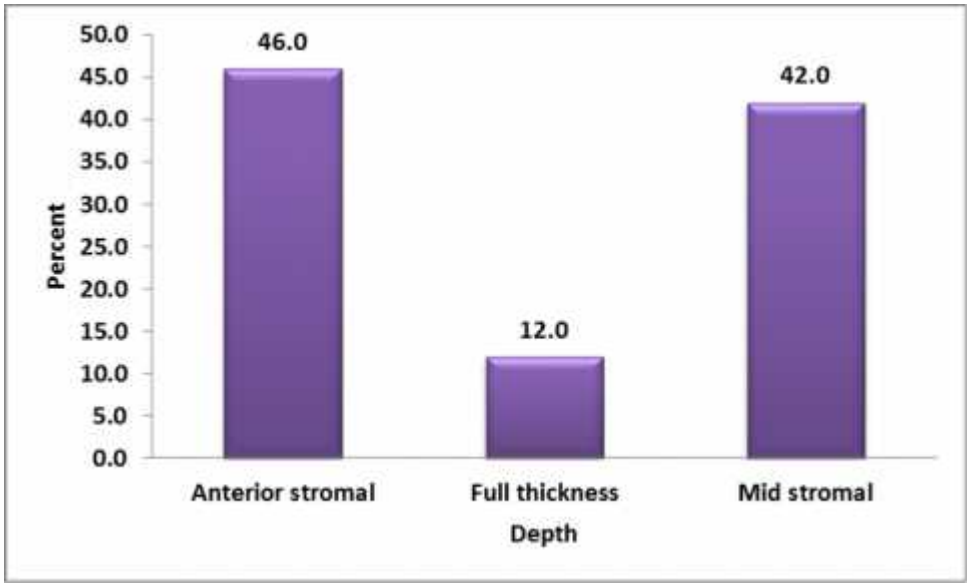


In this study, 64 % of the ulcers belonged to the category of <20 sq. mm, 22 % in 21-40 sq. mm 8 % in 41-60 sq. mm category and 6 % to 61-100 sq. mm.

Table 13 : Depth of Infiltration

Depth	N	Percent
Anterior stromal	23	46
Full thickness	6	12
Mid stromal	21	42
Total	50	100

Graph – 9 Showing Depth of Infiltration

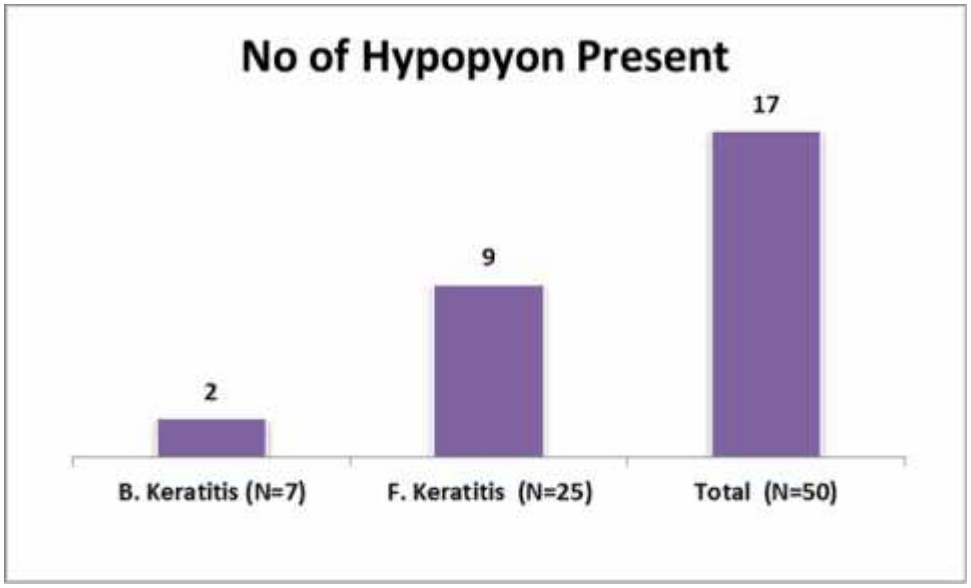


In this study 46% of the patients involved anterior stromal of the cornea, 42 % patients had mid stromal involvement and 12 % patients had full thickness involvement.

Table 14: Percent Distribution of Hypopyon(mm)

Hypopyon Present	Total		B. keratitis		F. keratitis		p value
	N=50	Percent	N=7	Percent	N=25	Percent	0.711
	17	34	2	28.5	9	36	

Graph – 10 Showing Percent Distribution of Hypopyon(mm)

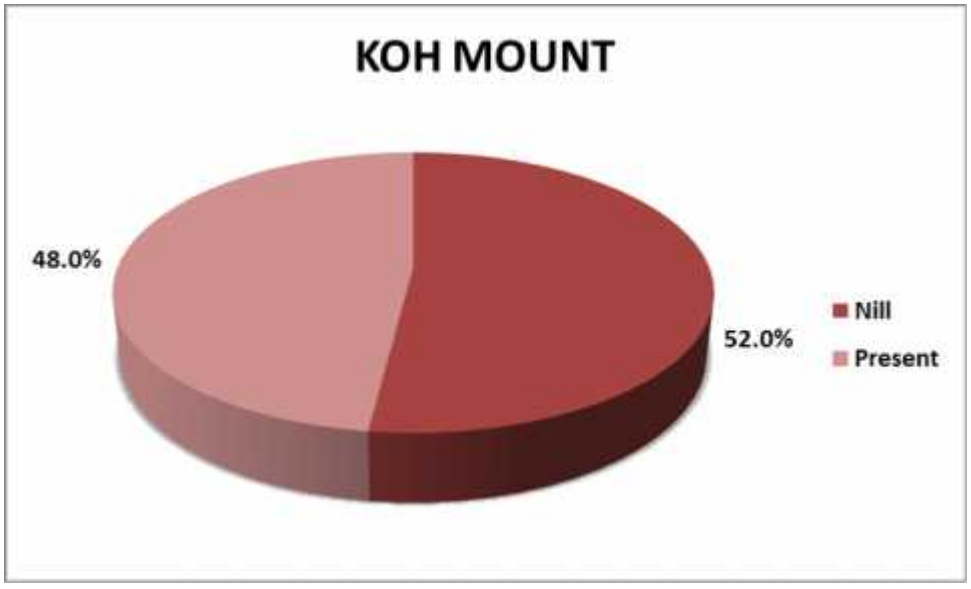


Hypopyon was present in 9 (36%) cases of fungal, 2 (28.5%) of bacterial cases of the total 17 cases.

Table 15 : Percent Distribution of KOH MOUNT

KOH MOUNT	N	Percent
Nill	26	52
Present	24	48
Total	50	100

Graph – 11 Showing Percent Distribution of KOH MOUNT

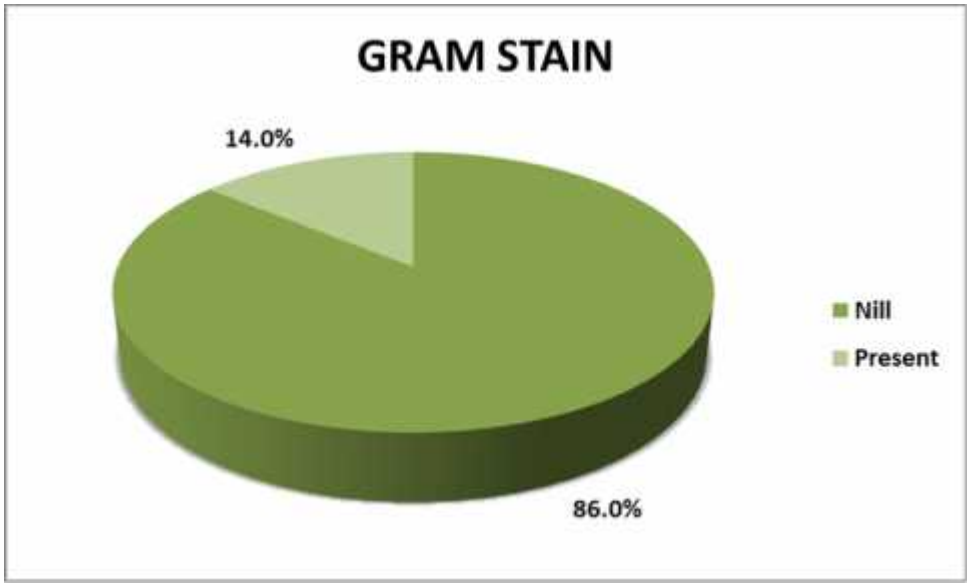


In this study 24 (48 %) patients had fungal elements positive in KOH Stain.

Table 16 : Percent Distribution of GRAM STAIN

GRANSTAIN	N	Percent
Nill	43	86
Present	7	14
Total	50	100

Graph – 12 Showing **Percent Distribution of GRAM STAIN**

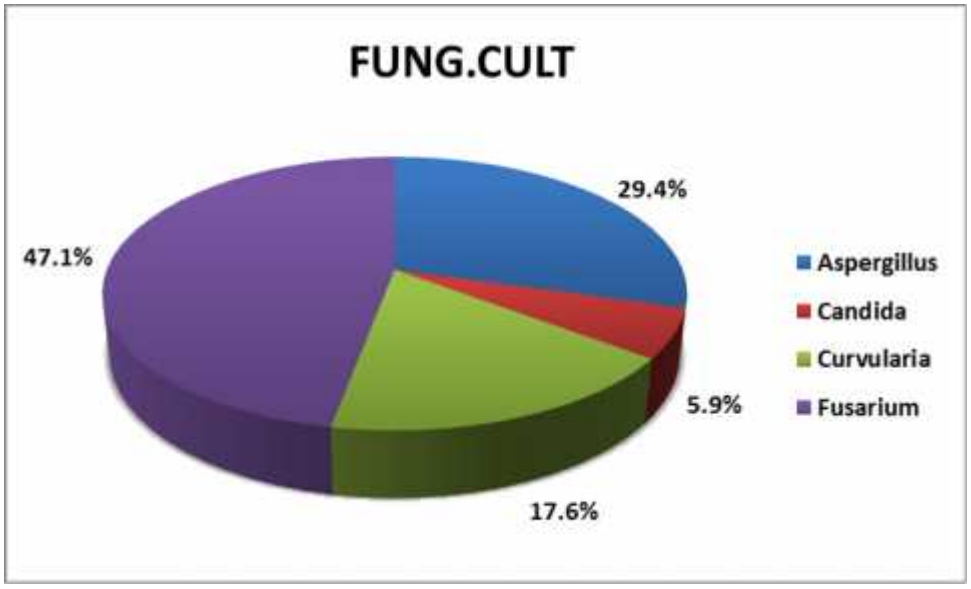


In this study 7 (14 %) patients had Bacteria positive in GRAM Stain.

Table 17 : Fungal Culture results

FUNG.CULT	N	Percent
Fusarium	8	47.1
Aspergillus	5	29.4
Curvularia	3	17.6
Candida	1	5.9
Total	17	100.0

Graph – 13 Showing Fungal Culture results

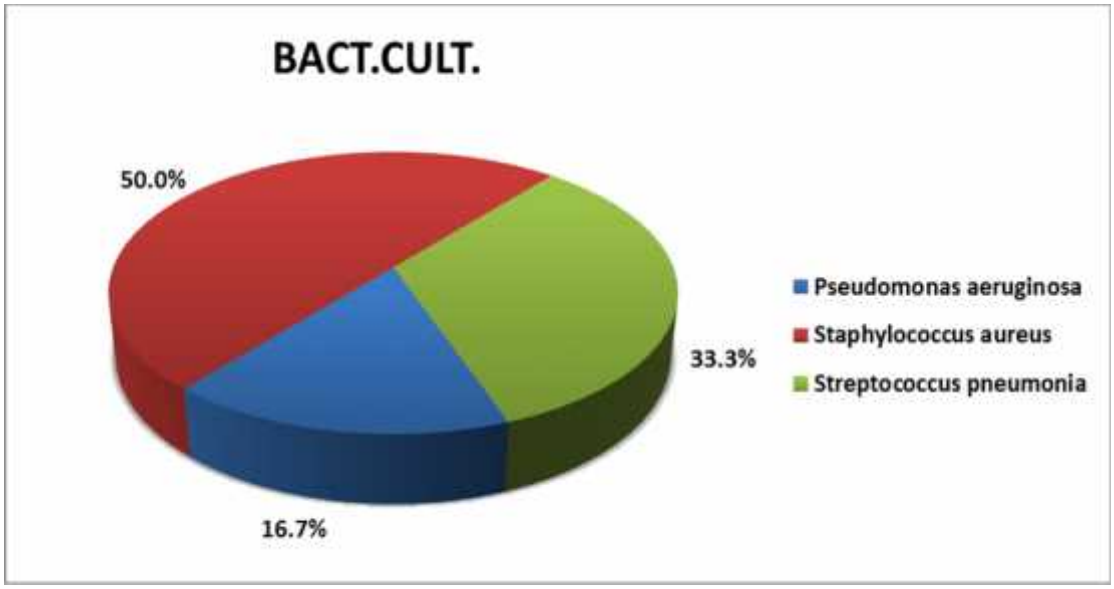


Fungal culture was positive in 17 patients. After 21 days of inoculation of the SDA medium in 8 (47.1%) patients Fusarium was isolated. In 5 (29.4 %) patients Aspergillus was isolated and 3 (17.6 %) patients Curvularia was the species. Candida was isolated in one patient(5.9%) . In 33 patients no growth occurred.

Table 18 : Bacterial Culture results

BACT.CULT.	N	Percent
Staphylococcus aureus	3	50.0
Streptococcus pneumonia	2	33.3
Pseudomonas aeruginosa	1	16.7
Total	6	100.0

Graph – 14 Showing Bacterial Culture results

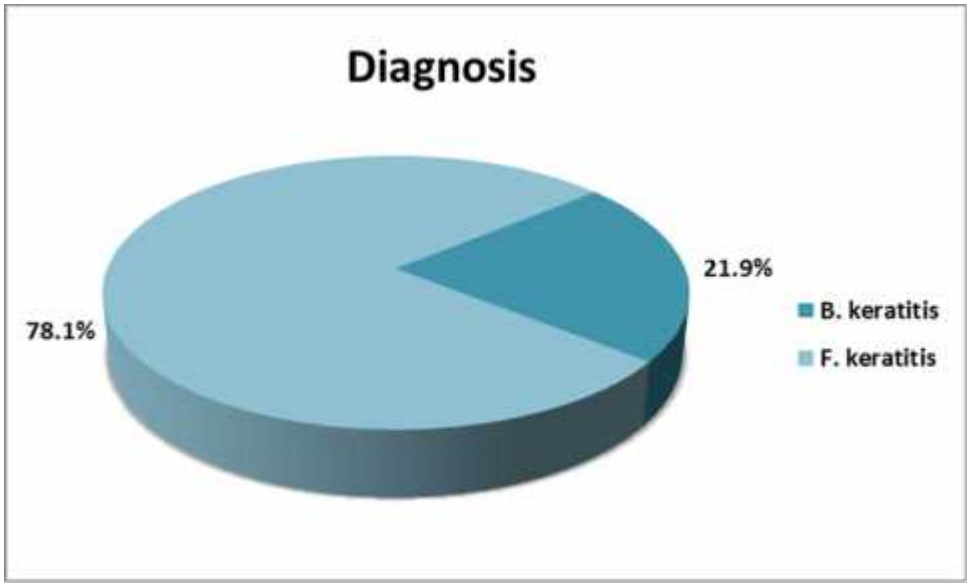


Bacterial culture was positive in 6 patients. In 3 (50%) patients Staphylococcus aureus was isolated, 2 (33.3%) patients Streptococcus pneumonia and 1 (16.7%) patient Pseudomonas aeruginosa was isolated.

Table 19 : Percent Distribution of Diagnosis

Diagnosis	N	Percent
B. keratitis	7	21.9
F. keratitis	25	78.1
Total	32	100.0

Graph – 15 Showing Percent Distribution of Diagnosis



In this study 25 (78.1 %) patients had Fungal keratitis and 7 (21.9%) patients had Bacterial keratitis out of 32 patients.

DISCUSSION

Among the 50 Corneal ulcer patients studied the commonest age group affected was between 41-65 yrs, followed by 21-40 years, >65 yrs and <20 yrs. In Nath et al study the commonest age group was between 41-50 years which was in agreement with the present study.[45] This has a considerable socioeconomic impact because this age group people are bread winners of the family. In similar other studies the commonest age group was 31-40 years.[46]

Regarding the gender of the patients, in this study males (70%) were affected more than females (27.5%). In other similar studies male preponderance ranged between 65 to 68 %.[37] By the nature of their work profile men are more exposed to outdoor activities thereby increasing their vulnerability to the disease.

In this study most of the patients were farmers (56 %), followed by others (28 %) category which included other professions like Labour (8%),Driver (6%), Housewife (6%), Students (4%),Teacher (2%), Shopkeepers (2%). In Bharathi et al study, farmers contributed to 64.75%, homemaker 5.11%, students 8.11%, tradesman 6.48%, unemployed 1% and labourer 14.52 %.[39] This occupational preponderance was justified because trauma with vegetative matter was an important risk factor for the occurrence of fungal keratitis. Out of the 50 cases 74 % belonged to the low class and 26 % to middle class.

In this study, 27 (54 %) patients presented after 1-5 days of having symptoms like pain, redness, diminution of vision and watering of eyes. 19 (38 %) patients presented after 6 to 15 days of symptoms and 4 (8%) patients after 16 to 30 days of symptoms. Majority of patients (54%) presented after 1-5 days of having symptoms. In Bharathi et al study, 46.66 % patients reported within 7 days.[47]

Out of the 50 cases, 42% gave history of injury of which 24% had injuries with vegetable matter. 8% had injuries with stone piece and 8% gave history of foreign body fall, 2% had injuries with safety pin, while the remaining 58% did not give any history of injury.The agents responsible for trauma were mainly organic materials to which farmers are exposed including paddy stalk, plant twigs, grass and animal products.

In South India paddy and rice stalk in the field was the most common cause of superficial corneal trauma. In this study the most common cause of superficial corneal trauma was by vegetable matter (24%), followed by stone piece (8%), and foreign body fall (8%).

In this study most (64%) of the Corneal ulcers were located centrally, 28% located paracentrally and 8 % were peripheral in location. 64 % of the ulcers belonged to the category of <20 sq. mm, 22 % in 21-40 sq. mm 8 % in 41-60 sq. mm category and 6 % to 61-100 sq. mm.

In this study 46% of the patients involved anterior stromal of the cornea, 42 % patients had mid stromal involvement and 12 % patients had full thickness involvement.

In this study 34 % patients had hypopyon at presentation with its height ranging from 2 to 4 mm. 66 % patients did not have hypopyon. In Srinivasan et al study hypopyon was present in 66% of cases.[48] In Chowdhary et al study hypopyon was present in 45 % cases.[49]

In this study Out of the 50 patients, 24 (48 %) patients had fungal elements positive in 10% KOH Stain and 7 (14 %) patients had Bacteria positive in GRAM Stain.

In the present study, KOH stain correlated with culture report in all cases, suggesting the wet mount is rapid reliable and superior technique for diagnosing mycotic keratitis. In a similar study conducted by Vajpajee RB in Delhi [50], it was observed that KOH sensitivity was found to be 94%. Therefore KOH is particularly important for the early initiations of antifungal treatment in mycotic keratitis.

In the present study, the gram stain report correlated with culture report in all of the cases, where as in the study conducted by Galentine[52] and Williams[51] it was found to be about 63%. Therefore, the gram stain report can be used as a guide for initial therapy that can be modified later according to the culture reports.

Microorganisms were isolated from 23 (46%) of the 50 cases that were cultured. This figure compares favourably with a recent study in Ghana [53] where (57.8%) of all cultures were positive. Another study by M. Srinivasan [3] in South India showed 68.4% of growth.

Out of 23 patients 6 were pure bacterial growth, 17 were fungal growth. In this study the most common bacterial pathogens isolated were staphylococcus 3 and Streptococcus pneumonia 2 followed by Pseudomonas aeruginosa 1. In the developing world streptococcus pneumonia should always be considered as the most likely cause of bacterial corneal ulceration until proved otherwise,[53, 3]

Of the 17 fungal isolates cultures from 50 corneal ulcers 8 were Fusarium species, 5 were Aspergillus species, 3 patients Curvularia species and Candida was isolated in one patient. This pattern of fungal organisms, dominated by Fusarium species is similar to the spectrum of microbial keratitis reported from south Florida by Leisegang [54] and Forster [55] and Hegan from Ghana [53.]

CONCLUSION

This study was conducted primarily to determine the clinical features, specific pathogens responsible for corneal ulceration in Vijayapura, Karnataka. Corneal ulceration is a common problem in this part of Karnataka and most often occurs after a superficial corneal injury with organic material.

In this study majority of the cases were Fungal Keratitis (78.1%),followed by bacterial Keratitis (21.9%). Streptococcus pneumonia and Staphylococcus aureus accounted for majority of bacterial ulcers and Fusarium and Aspergillus species were responsible for most of fungal infections.

Most commonly acquired suppurative ulcers resolve with appropriate treatment. Delay in diagnosis probably contributes to poorer outcome from therapeutic measures.

These findings have important public health importance for the treatment, rapid referral, diagnosis, and prevention of corneal ulceration in the developing world.

SUMMARY

50 cases of infective corneal ulcers were studied. Out of which 25 (50%) cases were of fungal etiology, 7 (14%) were bacterial. Males (70%) were affected more than females (30%) and 41-65 age group (38%) was affected more. Agricultural activity and superficial corneal trauma were the most significant predisposing factors, majority belonging to low socio- economic group. The earliest time the patient visited the hospital after the onset of the disease was 1 day and the largest duration was one month.

Common clinical characteristics of fungal keratitis was long duration of onset of symptoms, dry with raised slough ulcer, satellite lesions and hypopyon in 36 % of cases. Bacterial keratitis was short duration of onset of symptoms, greyish white with purulent slough, 28.5 % cases presented with hypopyon.

Among laboratory investigations gram stain, Blood and chocolate agar were found to be highly sensitive in identifying bacteria. KOH and SDA were found to be highly sensitive in detecting fungal elements.

PHOTOGRAPHS:

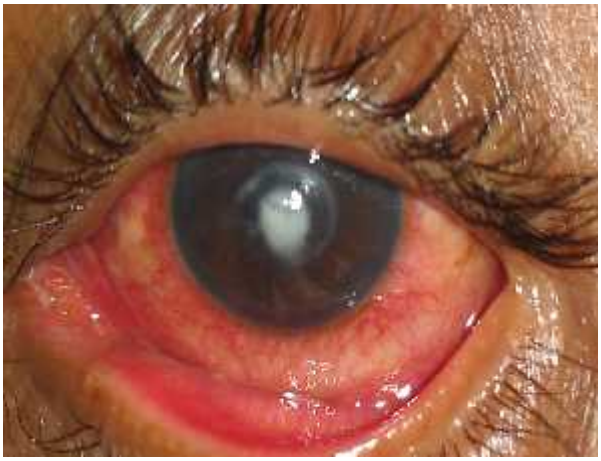


Fig. 9 Central Corneal Ulcer – Fungal Keratitis

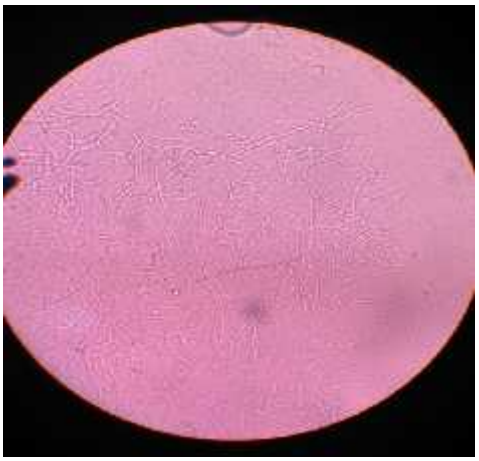


Fig. 10 KOH preparation showing fungal filaments.

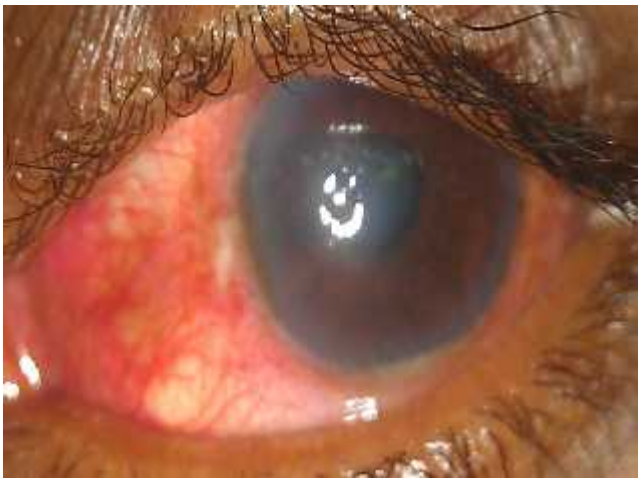


Fig. 11 ParaCentral Corneal Ulcer with hypopyon – Fusarium

Fig.12 SDA medium showing Fusarium Colonies

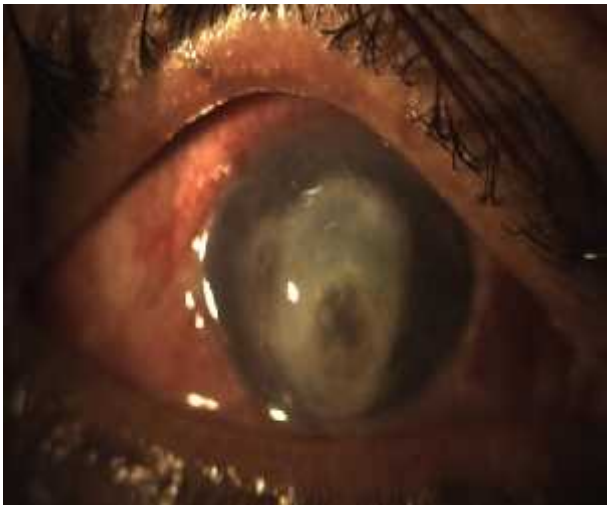


Fig. 13 Central corneal ulcer with pigmentation - Aspergillus

Fig. 14 SDA medium showing Aspergillus colonies

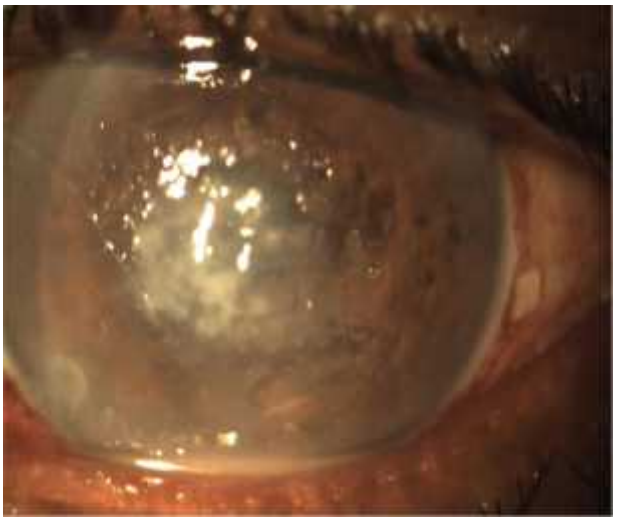


Fig. 15 Central corneal ulcer with hypopyon – staphylococcus aureus.

Fig. 16 Staphylococcus aureus colonies on blood agar.

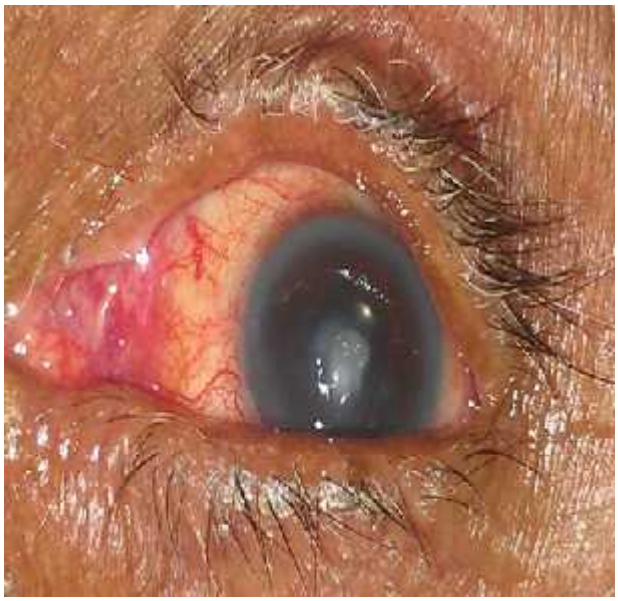


Fig.17 Central corneal ulcer – Streptococcus

Fig. 18 Streptococcus colonies on blood agar.

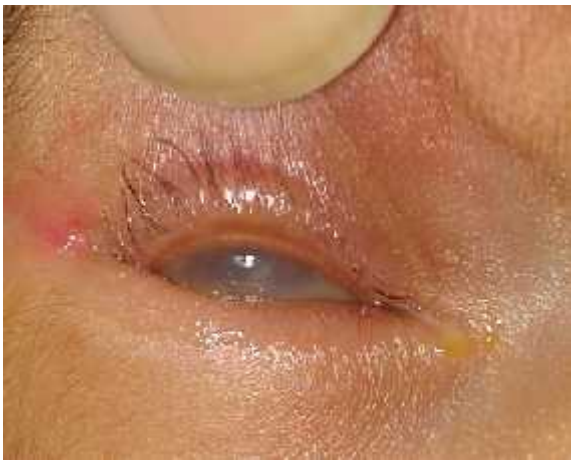


Fig. 19 Corneal ulcer with greenish yellow discharge – Pseudomonas aeruginosa

Fig. 20 Pseudomonas aeruginosa colonies on blood agar

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ANNEXURES

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "clinical and microbiological study on corneal ulcer in patient attending eye OPD at tertiary care institute, Bijapur, North Karnataka"

Name of P.G. student Dr. Gautam N. Beladiya.
Department of Opthalmology

Name of Guide/Co-investigator Dr Vallabha. K. Prof & HOD.
Department of Opthalmology.

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

B. L. D. E. U'S SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA,KARNATAKA.

RESEARCH INFORMED CONSENT FORM

Title of the Project："CLINICAL AND MICROBIOLOGICAL STUDY ON CORNEAL ULCER IN PATIENT ATTENDING EYE OPD AT TERTIARY CARE INSTITUTE,VIJAYAPURA,NORTH KARNATAKA"

Principal Investigator : **DR.GAUTAM NEMUBHAI BELADIYA**
DEPARTMENT OF OPHTHALMOLOGY
Email – gnb1230@gmail.com

P.g Guide Name : **DR.VALLABHA.K**$_{\text{M.S, DOMS}}$
PROFESSOR AND HOD
DEPARTMENT OF OPHTHALMOLOGY
B.L.D.E.U'S,
SHRI B.M. PATILMEDICAL COLLEGE,
HOSPITAL AND RESEARCH CENTRE,
VIJAYAPURA,KARNATAKA

1: PURPOSE OF RESEARCH:

I have been informed that this study will determine the causative organism of bacterial and fungal Keratitis and evaluate the sensitivity and treatment of microbial keratitis. I have been explained about the reason for doing this study and selecting me/my ward as a subject for this study. I have also been given free choice for either being included or not in the study.

2: PROCEDURE:

I/My ward will be subjected to detailed history and ocular examination. I/My ward will then be subjected to investigation (Gram stain,KOH,Culture) and management.

3: RISK AND DISCOMFORTS:

I understand this study which determines the causative organism in microbial keratitis will not cause any discomfort to me and do not involve any risk to my health.

4: BENEFITS: I understand that I/my wards participate in this study will help to identify causative organism of bacterial and fungal Keratitis and evaluate the sensitivity and treatment of microbial keratitis.

5: CONFIDENTIALITY:

I understand that medical information produced by this study will become part of institutional records and will be subject to the confidentiality and privacy regulation of the said institute. Information of a sensitive personal nature will not be a part of medical record, but willbe stored in investigator's research file and identified only by a code

number. The code key connecting name two numbers will be kept in a separate secured location.

If the data to be used for publication in the medical literature and for teaching purpose no names will be used and other identities such as photographs, audio and video tapes will be used

only with my special written permission. I understand I may see the photographs and the video tapes and have the audio tapes before giving this permission.

6: REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time. Dr. Gautam N Beladiya is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study which might influence my continued participation. If during the study or later, I wish to discuss my participation in all concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital is available to talk with me. A copy of this consent form will be given to me to keep for careful re-reading.

7: REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and may refuse to participate or may withdraw my consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that researcher may terminate my participation in this study at any time after she/he has explained the reasons for doing so and had helped arrange for my continued care by my physician or physical therapist if this is appropriate

8: INJURY STATEMENT

I understand that in unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, then medical treatment will be available to me, but no further compensation would be provided. I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

I have explained to _____(Name of subject) the purpose of the research, the procedure required and the possible risk and benefits to the best of my ability.

DrGautambeladiya/ PG student **Date**

9:STUDY SUBJECT CONSENT STATEMENT

I confirm thatDr. Gautam N.Beladiyahas explained to me the purpose of research, the study procedure that I will undergo, and the possible risk and discomforts as well as benefits that I may experience. Alternative to my participation in the study, I have also been to give my consent form. Therefore, I agree to give consent to participate as a subject and this research project.

Participant **Date:**

Signature of witness **Date:**

PROFORMA

PATIENT DETAILS

Name: **Address:**

Age: **Income:**

Sex: **O.p. / I.P. No:**

Occupation **D.O.A:**

Phone No: **D.O.D:**

PRESENTING COMPLAINTS:

SYMPTOMS	YES / NO	DURATION AND OTHER DETAILS
1.Pain		
2.Redness		
3.Watering / Discharge		
4.Photophobia		
5.Reduced vision		
6.FB sensation		
7.Any other		

DETAILS OF H/O TRAUMA

Where	
When	
With what material	Vegetable matter, Jower, Paddy, Sugarcane, Soil, Dust, Stone, Plant twig, any other..
Onset of Symptoms and Duration	

TREATMENT HISTORY

Previous history of Treatment	**Yes / No**
If yes, then which drug	**Antibiotics/Antifungal/Steroids/ any others**
Duration of Treatment	
Treated by whom	

OCULAR AND SYSTEMIC PREDISPOSING FACTORS

Contact lens wear	**Yes / No**
Use of topical medication	**Yes / No. If yes specify..**
Presence of any other eye problem	
Surgery	
Diabetes	**Yes / No. If yes duration..**
Mucocutaneous Fungal infection	
Systemic steroid use	
Any other factor	

CLINICAL EXAMINATION :

1.GENERAL PHYSICAL EXAMINATION

2.SYSTEMIC EXAMINATION

3.OCULAR EXAMINATION

	RIGHT EYE	LEFT EYE
EYELIDS		
LACRIMAL APPARTUS		
CONJUNCTIVA		
CORNEA		
ANTERIOR CHAMBER		
IRIS		
PUPIL		
LENS		
FUNDUS		
VISION		
INTRAOCULAR PRESSURE		
ANY OTHER		

DETAILS OF THE CORNEAL LESION:

SITE	
SIZE	
SHAPE	
BASE	
COLOUR	
EDGE	**Regular / Irregular / Feathry**
SURFACE	**Elevated / Depressed / Necrotic / Dry**
DEPTH OF INFILTRATION	**Epithelial / Stromal Superficial / Mid**

	stromal / Deep
SATELLITE LESION	
IMMUNE RING	
KERATIC PRECIPITATES	
ENDOTHELIAL PLAQUES	
DESCEMATOCELE	
VASCULARISATION	
SENSATION	
REST OF THE CORNEA	

FIGURE :

TESTS:

Fluorescein staining	
Lacrimal Syringing	

MICROBIOLOGICAL INVESTIGATIONS:

10% KOH Preparation	
Gram's Staining	
Fungal culture on Sabouraud Dextrose Agar	
Bacterial culture on Blood Agar / Chocolate Agar / Mac Conkey Agar.	

OTHER INVESTIGATION

Urine examination.	
Blood examination.	
Any other investigations.	

Signature of PG student **Signature**

KEY TO MASTER CHART

Asp	–	Aspergillus
AS	–	Anterior stromal
B. keratitis	-	Bacterial Keratitis
Can	–	Candida
C	–	Central
Cur	–	Curvularia
Fb	–	Foreign body
FT	–	Full thickness
F. keratitis	-	Fungal Keratitis
Fus	-	Fusarium
HM	-	Hand Movement
Inf. Keratitis	–	Infective Keratitis
Ms	–	Mid stromal
N	–	Negative.
No Org	–	No Organism.
P	–	Positive
PC	–	Paracentral
PH	–	Peripheral
PL	–	Perception of Light
PR	–	Projection of Rays
Pse	-	Pseudomonas aeruginosa
Staph	-	Staphylococcus aureus
Strep	-	Streptococcus pneumonia

MASTER CHART

SI.NO.	Name	Age	Sex	IP/ op No	occupation	SES	Laterality	Duration (day)	H/o Trauma	Visual Acuity	Size (mm)	Location	Depth	Hypopyon(mm)	KOH MOUNT	GRANSTAIN	FUNG.CULT	BACT.CULT.	Diagnosis
1	Sugalabai Y	68	F	3785	Farmer	Poor	LE	8	Nill	HM	6 × 6	C	MS	Nill	P	N	Fus	No Org	F. keratitis
2	Bhagirati H	50	F	4133	Farmer	Poor	RE	6	Veg matter	CF 1/2m	8 × 7	PC	FT	2	N	N	NoOrg	No Org	Inf. Keratitis
3	Vaisnavi H	5	F	27620	Nill	Middle	LE	2	Safety pin	6/60	3 × 3	C	MS	1	N	N	No Org	No Org	Inf. Keratitis
4	Gurusangappa	72	M	23032	labour	Poor	LE	3	Nill	6/12	3 × 2	C	MS	1	N	P	No Org	Staph	B. keratitis
5	Balappa T	38	M	414	Driver	Poor	LE	2	Nill	6/9	3 × 1	PC	AS	Nill	P	N	No Org	No Org	F. keratitis
6	Siddaram P	60	M	12970	Farmer	Middle	RE	15	Nill	6/12	4 × 2	PH	AS	Nill	P	N	No Org	No Org	F. keratitis
7	Bouramma K	50	F	5183	H. Wife	Middle	LE	15	Wooden stick	6/36	4 × 4	PC	AS	1	P	N	Fus	No Org	F. keratitis
8	Bouramma	46	F	89361	labour	Poor	LE	3	Nill	6/9	2 × 2	PH	AS	Nill	N	N	No Org	No Org	Inf. Keratitis
9	Harshvardhan B	32	M	8436	Farmer	Poor	RE	6	Stone	CF CF	6 × 6	C	MS	Nill	P	N	Cur	No Org	F. keratitis
10	Appasha S	25	M	11130	Driver	Poor	RE	5	Sugarcane leaf	6/60	4 × 4	PC	AS	Nill	P	N	No Org	No Org	F. keratitis
11	Imamshab	50	M	75509	Farmer	Poor	LE	3	Nill	6/36	2 × 2	PC	AS	Nill	N	N	No Org	No Org	Inf. Keratitis
12	Veeranna Dhari	66	M	13388	Farmer	Poor	RE	30	Soil particle	PL+PR+	6 × 6	C	FT	4	N	N	No Org	No Org	Inf. Keratitis
13	Shivayagi Akalkot	75	M	13356	Nill	Poor	RE	15	Nill	CF 2m	4 × 4	C	AS	Nill	N	N	No Org	No Org	Inf. Keratitis
14	Mohan	26	M	158022	Shop keeper	Middle	RE	5	Nill	CF 3m	3 × 3	C	AS	Nill	P	N	Cur	No Org	F. keratitis
15	Somanath	32	M	15247	Farmer	Poor	LE	2	Nill	6/60	3 × 3	PC	AS	Nill	N	N	No Org	No Org	Inf. Keratitis
16	Hanamanth B	16	M	15488	Student	Middle	RE	10	Nill	CF 1m	5 × 5	C	MS	1	P	N	No Org	No Org	F. keratitis
17	Balu	66	M	15306	Farmer	Poor	RE	3	Nill	CF 1m	5 × 5	C	FT	2	N	N	Fus	No Org	F. keratitis
18	Naveen Kumbar	17	M	17341	Student	Middle	RE	4	Nill	6/60	4 × 4	PC	AS	Nill	P	N	Fus	No Org	F. keratitis
19	Ameerama	40	F	224637	Farmer	Poor	RE	3	FB fall	6/36	3 × 2	PC	AS	Nill	P	N	No Org	No Org	F. keratitis
20	Gourishankar	20	M	252503	Labour	Poor	RE	2	Stone	6/36	2 × 2	PC	AS	Nill	P	N	Can	No Org	F. keratitis
21	Roopsingh	55	M	384621	Farmer	Poor	LE	4	Sugar cane leaf	CF CF	5 × 4	C	MS	2	P	N	No Org	No Org	F. keratitis
22	Raju	43	M	272053	Farmer	Poor	RE	2	FB fall	6/12	2 × 1	PH	AS	Nill	N	N	No Org	No Org	Inf. Keratitis
23	Gangavva Biradar	70	F	23841	Nill	Middle	LE	7	Nill	HM	6 × 5	C	MS	Nill	N	P	No Org	Staph	B. keratitis
24	Shrishail Malabadi	65	M	26569	Farmer	Poor	LE	20	Coconut	PL+ PR+	9 × 8	C	FT	Nill	N	N	No Org	No Org	Inf. Keratitis
25	Parsappa Kanakki	55	M	27433	Farmer	Poor	LE	15	Nill	CF 2m	4 × 4	C	MS	3	P	N	Asp	No Org	F. keratitis
26	Laxmibai Yalamelli	50	F	25759	Nill	Middle	LE	2	Nill	6/60	3 × 3	PC	AS	Nill	N	N	No Org	No Org	Inf. Keratitis
27	Modinsab	42	M	316285	Farmer	Poor	RE	4	Veg matter	6/9	2 × 2	PH	AS	Nill	P	N	Asp	No Org	F. keratitis
28	Mallanagouda S	70	M	30294	Nill	Middle	RE	3	Nill	HM	7 × 7	C	MS	Nill	N	N	No Org	No Org	Inf. Keratitis
29	Gangappa K	75	M	30325	Farmer	Poor	RE	15	Nill	HM	6 × 5	C	MS	Nill	N	N	No Org	No Org	Inf. Keratitis
30	Arati Biradar	32	F	31484	H.wife	Middle	LE	4	Wooden particle	CF CF	5 × 5	C	MS	Nill	N	N	No Org	No Org	Inf. Keratitis

31	Sharnamma patil	73	F	32793	Nill	Middle	LE	4	Nill	CF 3m	3 × 3	C	AS	2	P	N	No Org	No Org	F. keratitis
32	Chandrashekhar D	54	M	34263	Farmer	Poor	RE	15	Sugarcane leaf	CF CF	6 × 5	C	AS	Nill	P	N	Fus	No Org	F. keratitis
33	Basappa	60	M	384581	Farmer	Poor	RE	4	Nill	6/60	2 × 2	PC	AS	Nill	N	P	No Org	Strep	B. keratitis
34	Chandrawwa	90	F	388072	Nill	Poor	LE	3	FB fall	6/36	2 × 1	PC	AS	1	N	P	No Org	No Org	B. keratitis
35	Sundrabai Kumbar	55	F	274754	H.wife	Middle	RE	4	Nill	CF CF	5 × 4	C	MS	3	P	N	Fus	No Org	F. keratitis
36	Veeresh	16d	M	4761	Nill	Poor	RE	3	Nill	CF 1m	4 × 3	C	MS	Nill	N	P	No Org	Pse	B. keratitis
37	Hanamanth jevoor	56	M	2397	Farmer	Poor	RE	10	Nill	PL+ PR+	8 × 8	C	MS	3	N	N	No Org	No Org	Inf. Keratitis
38	Bhimangouda B	27	M	99212	Driver	Poor	RE	8	Nill	6/36	2 × 2	PC	AS	Nill	P	N	Fus	No Org	F. keratitis
39	Mahaningappa G	53	M	37978	Farmer	Poor	LE	7	Veg matter	6/60	3 × 3	C	MS	2	N	N	No Org	No Org	Inf. Keratitis
40	Gurupad S	25	M	9814	Farmer	Poor	LE	30	Nill	HM	7 × 7	C	MS	Nill	P	N	Asp	No Org	F. keratitis
41	Shivamma kanoli	36	F	112887	Farmer	Poor	RE	7	Nill	6/24	2 × 2	PC	AS	Nill	N	P	No Org	Staph	B. keratitis
42	Mohamad chikkali	45	M	11641	Farmer	Poor	RE	3	Nill	HM	7 × 7	C	MS	2	P	N	Asp	No Org	F. keratitis
43	Danappa Ligade	68	M	12993	Teacher	Middle	RE	15	Nill	PL+ PR+	9 × 9	C	FT	4	N	N	No Org	No Org	Inf. Keratitis
44	Parsappa S	24	M	14757	Farmer	Poor	LE	10	Sugarcane leaf	CF 2m	3 × 3	C	FT	Nill	N	N	No Org	No Org	Inf. Keratitis
45	Mallamma lokari	50	F	14969	Farmer	Poor	LE	4	FB fall	CF CF	6 × 6	C	MS	Nill	N	P	No Org	Strep	B. keratitis
46	Yallappa Bandivadar	50	M	305259	Labour	Poor	RE	8	Wooden Particle	HM	7 × 5	C	MS	3	P	N	Asp	No Org	F. keratitis
47	Sahebgouda B	24	M	234244	Farmer	Poor	RE	2	Nill	6/60	4 × 4	C	AS	Nill	P	N	No Org	No Org	F. keratitis
48	Sivabai siddappa	75	F	18748	Farmer	Poor	LE	30	Nill	6/60	4 × 4	C	MS	Nill	P	N	Fus	No Org	F. keratitis
49	Raju Madar	25	M	252049	Farmer	Poor	RE	6	Veg matter	CF 3m	4 × 4	C	MS	Nill	N	N	No Org	No Org	Inf. Keratitis
50	Sharnayya B	35	M	22802	Farmer	Poor	LE	4	Stone Particle	CF 3m	5 × 4	C	AS	Nill	P	N	Cur	No Org	F. keratitis