INTRODUCTION

Bullous Keratopathy is a condition in which the cornea becomes permanently swollen. This occurs because the inner layer of the cornea, the endothelium, has been damaged and is not pumping fluid properly. The cause of the endothelial damage could be from trauma, glaucoma, or inflammation after eye surgery ***(Research Foundation of America, 2013)***.

Corneal edema occurs for many reasons, but it is often a sequel of intraocular surgery. Corneal edema resulting from cataract extraction is called either pseudophakic bullous Keratopathy (PBK) or aphakic bullous Keratopathy (ABK) ***(Gicquel et al., 2007)***.

Corneal ulcer (infectious keratitis) as one of the causes of corneal edema is a major cause of blindness throughout the world. Bacterial infection in the cornea is invariably an alteration of the defense mechanism of the outer eye. Fungal infection (mycotic keratitis), Pseudomonas aeruginosa, Proteus spp, viral infections are also causes of keratitis and cause corneal damages ***(Katara et al., 2013)***.

Melting keratitis is a serious condition presenting a high risk of permanent blindness and is caused by infectious or noninfectious factors. In humans, the clinical efficacy of collagen cross-linking (CXL) has been described in the

treatment of refractory infectious keratitis by arresting keratomalacia ***(Famose, 2013)***.

Corneal collagen cross-linking (CXL) was first described over a decade ago and is now considered to be one of the most important surgical innovations of modern ophthalmology. Prior to its introduction, no interventions were available to arrest, or slow down ectatic disease progression, with corneal transplantation required in the majority of cases. The emerging combination of CXL with other interventions (termed 'CXL plus') optimizes the visual and topographic outcomes. With the expansion of the techniques' indications for other clinical conditions, such as microbial keratitis and bullous Keratopathy, this highlights the continuous improvement of the initial technique and confirms its wide acceptance representing a clear example of recent advances in ocular therapy ***(Kymionis et al., 2013)***.

There are two techniques of CXL, Epithelial-on (Epi-ON) transepithelial CXL, and epithelial-off (Epi-OFF) or standard CXL ***(Waring and Chicago, 2013)***.

Photooxidative cross-linking of corneal collagen is based on the combined use of the photosensitizer riboflavin (vitamin B2) of a concentration of 0.1% and UV light of a wavelength of 370 nm ***(Spörl et al., 2008)***.

CXL results in an increase in intra- and interfibrillar covalent bonds by photosensitized oxidation, and causes a biomechanical stabilization of the cornea ***(Wollensak, 2006)***.

CXL should be considered as a potential adjuvant therapeutic tool in patients with combined bullous Keratopathy and infectious keratitis, who are resistant to traditional topical therapy ***(Wollensak et al., 2010)***.

Corneal cross-linking showed to be a safe procedure and potential therapeutic alternative for the treatment of corneal edema ***(Barbosa et al., 2010)*.**

UVA-riboflavin CXL treatment could be performed when the patient was still on medical therapy in the management of corneal ulcers or edema unresponsive to medical management ***(Sağlk et al., 2013)*.**

Botto´s et al. supposed that their study showed an immediate effect of CXL with a limited long-term sustainability. Cross-linked corneas had a pronounced anterior zone of organized collagen fibers. Even the treated corneas with advanced bullous Keratopathy and stromal fibrosis had histological evidence of collagen fibers organization, but this effect seems to be decreased compared with corneas in initial stages of the disease ***(*Botto´s *et al., 2010)*.**

Several long-term and short-term complications of CXL have been studied and documented. AlthoughCross-linking is a

low-invasive procedure with low complication and failure rate, the possibility of a secondary infection after the procedure exists because the patient is subjected to epithelial debridement and the application of a soft contact lens. Formation of temporary corneal haze, permanent scars, endothelial damage, treatment failure, sterile infiltrates, and herpes reactivation are the other reported complications of this procedure ***(Caporossi et al., 2010)*.**

AIM OF THE ESSAY

To evaluate the role and efficacy of UVA and riboflavin crosslinking in cases of bullous Keratopathy and other causes of corneal edema.

Chapter (1):

CORNEAL ANATOMY AND PHYSIOLOGY

Anatomy of the cornea:

The cornea is a transparent avascular tissue that is exposed to the external environment. The anterior corneal surface is covered by the tear film, and the posterior surface is bathed directly by aqueous humor (Fig1). The avascular cornea forms, together with the sclera, the outer shell of the eye-ball. The highly vascularized limbus constitutes the transition zone between the cornea and sclera, and contains a reservoir of pluripotential stem cells (palisade of Vogt) ***(Nishida, 2005).***

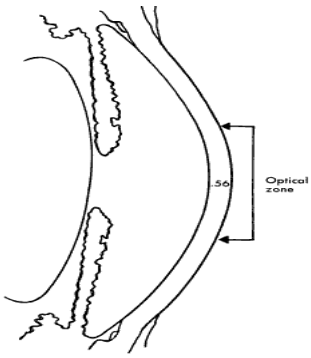

Figure (1): The cornea is thinner centrally (0.56 mm) and measures approximately (1.0 mm) in the periphery

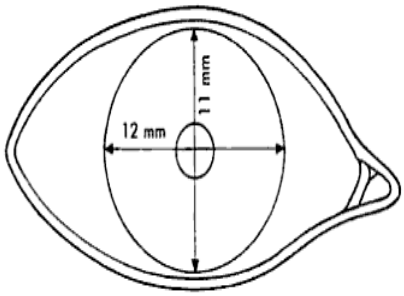

Figure (2): Anterior dimensions of the cornea ***(Stein et al., 2001).***

The shape of the anterior corneal surface is convex and aspheric. The anterior surface is transversely oval, the adult human cornea measures 11 to 12 mm horizontally and 9 to 11 mm vertically (Fig2), it is approximately 0.5 mm thick at the

center, and its thickness increases gradually toward the periphery, where it is about 0.7 mm thick. The curvature of the corneal surface is not constant, being greatest at the center (optical zone) (Fig 1) and smallest at the periphery. The radius of curvature is between 7.5 and 8.0 mm at the central 3 mm optical zone of the cornea where the surface is almost spherical ***(Nishida, 2005)*.**

Vision depends on the cornea and lens as refractive components, the refractive power of the cornea is 40 to 44 diopters and constitutes about two thirds of the total refractive power of the eye ***(Nishida, 2005)***.

The cornea is a highly specialized structure which possesses the following vital functions:

- Clear refractive interface,
- Tensile strength,
- Protection of the intraocular contents from the external environment **(Weng Sehu & Lee, 2005).**

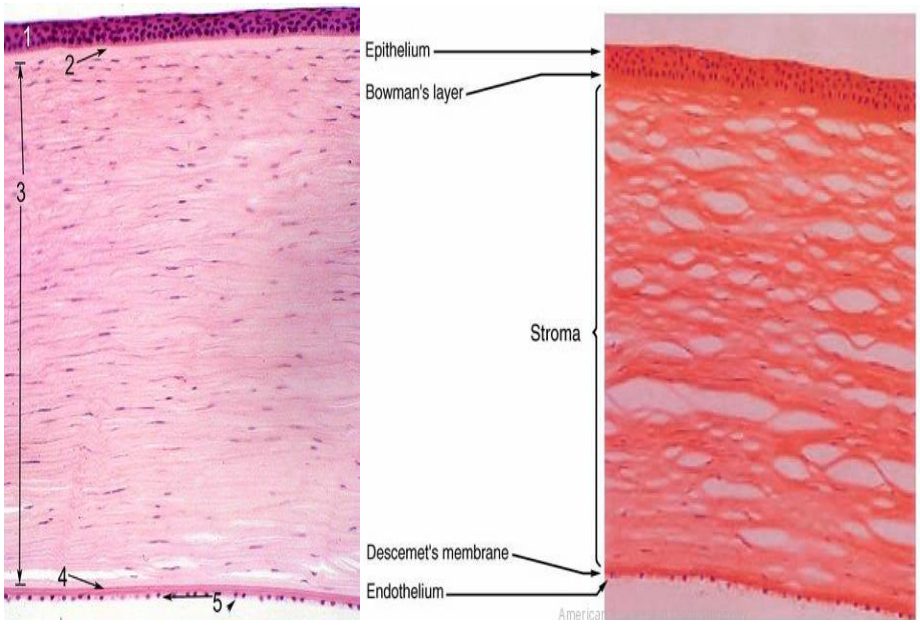

Figure (3): A full thickness histology section of the cornea demonstrates the relative thinness of the epithelium and endothelium in relation to the stroma ***(Weng Sehu & Lee, 2005)***, ***(Mamalis et al., 2006)***.

Histologically the cornea consists of five layers (Fig3):

1-Epithelium:

The corneal epithelium 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 μm thick and consists of five or six layers of nucleated cells ***(Reinstein et al., 1994)***.

It consists of five or six layers. These layers are divided into:

(a) Basal cell layer: cuboidal cells where cell division occurs.

(b) Wing cells: the second layer is wing shaped to fit over the rounded anterior surface of the basal cells.

(c) Superficial cells: the next three layers become increasingly flattened as they progress towards the surface due to mitotic activity in the basal cell layer. The most superficial cells detach from the surface as a normal process of "wear-and-tear". The cells of the epithelium are attached by desmosomes and the basal layer is attached to Bowman's layer by an anchoring complex ***(Weng Sehu & Lee, 2005).***

2- Bowman's layer (anterior limiting lamina):

Bowman's layer is a narrow, a cellular homogeneous zone, 8-14 μm thick, immediately subjacent to the basal lamina of the cornea epithelium ***(Bron et al., 1997)***.

Once destroyed, this layer is never replaced. Its absence indicates previous trauma or ulceration ***(Weng Sehu & Lee, 2005).***

Ultra structurally Bowman's layer consists of a felted meshwork of fine collagen fibrils of uniform size, lying in a ground substance ***(Tripathi and Tripathi, 1984)***.

3- Stroma (substantia propria):

The stroma forms about 90% of the corneal thickness (Fig3), it is about 500μm in thickness, and made up of interlacing layers of collagen fibrils embedded within a matrix of proteoglycans (fig4) ***(Radner et al., 1998)***. Composed

mainly of type 1 collagen fibrils with types III, V, and VI also found ***(Tomkins & Garzozi, 2008)***.

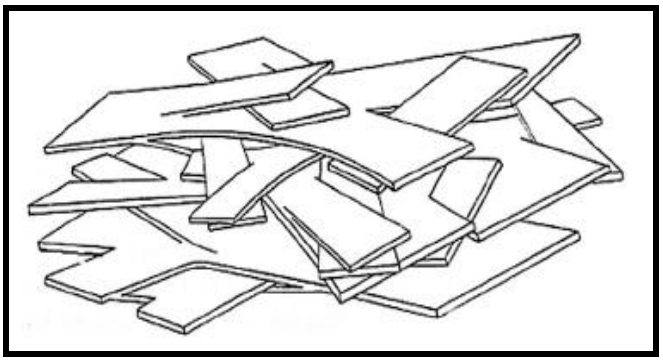

Figure (4): Diagram of the interlacing of collagen lamellae in the corneal stroma ***(Anderson et al., 2004)*.**

Collagen is the major constituent of the corneal stroma. It is arranged in fibers of uniform diameter (typically 25-30nm) spaced in uniform intervals (25-30nm) between adjacent fibrils. Transparency of the cornea depends on the small uniform size of the fibrils and the interfibrillar distances as well as a pump in the endothelium that keeps the stroma properly hydrated. The corneal stroma has the smallest fibrils of any tissue, and increases in the interfibrillar distances in the corneal stroma correlate with opacities in corneal stromal scars ***(Foster et al., 2004)***.

The keratocytes are spindle cells with long branching interconnecting processes. These cells lie between lamellae which contain bundles of uniformly spaced collagen fibrils. The interfibrillar spacing is such that any light scattering is cancelled by interference with light rays from adjacent fibrils

and is the basis for one of the theories to explain corneal transparency (Fig 5). The orientation of the fibrils varies by 60 degrees between lamellae and this provides structural strength ***(Weng Sehu & Lee, 2005)*.**

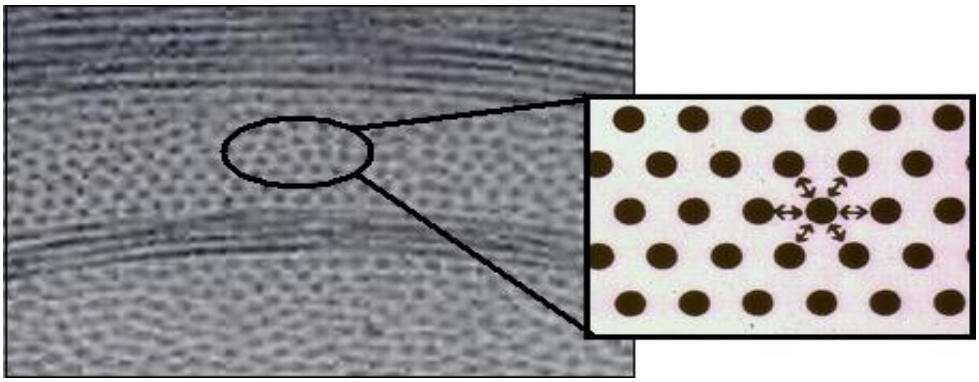

Figure (5): Transmission electron microscopy of the human corneal stroma show lamellar structure of collagen fibers**. The magnified View** shows collagen fibrils lattice pattern arrangement ***(Modified from (Nishida, 2005) & (Stein et al., 2001)*.**

4 -<u>Descemet's membrane (posterior limiting layer):</u>

A thin acellular layer possessing high tensile strength and containing proteoglycans and glycoproteins in addition to collagen. It serves as the modified basement membrane of the corneal endothelium, from which the cells are derived. This layer is composed mainly of collagen type IV fibrils, less rigid than collagen type I fibrils, and is around 5-20 μm thick, depending on the subject's age ***(Linsenmayer, 1981)***.

Just anterior to Descemet's membrane, a very thin and strong layer, the **Dua's Layer**, 15 μm thick and able to withstand 1.5 to 2 bars of pressure, may exist according to one

study, but to date (November, 2014) this has not been replicated in another laboratory ***(Dua et al, 2013).***

The membrane stains intensely pink with the Periodic acid-Schiff **(**PAS) stain. At the ultrastructural level, two zones can be identified, an anterior banded zone which is formed in fetal life and a posterior non-banded zone which increases in thickness throughout adult life (Fig 6) ***(Weng Sehu & Lee, 2005)***.

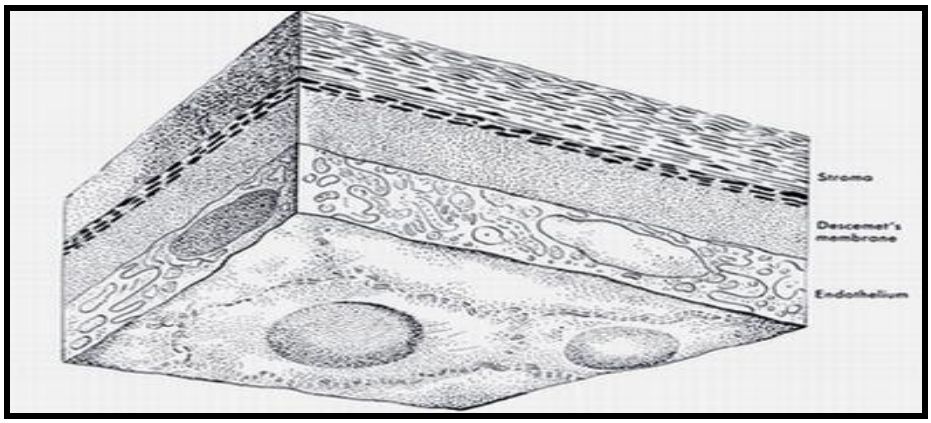

Figure (6): Diagram of Details of the inner portion of the cornea, including stroma, Descemet's membrane, and endothelium ***(Stein et al., 2001).***

5- Endothelium:

The endothelium is a single layer of hexagonal, cuboidal cells applied to the posterior aspect of Descemet's membrane in a well arranged mosaic pattern. The endothelial cells are 5 μm thick and 20 μm wide in normal corneas, the dimensions of the endothelial cells are quite uniform ***(Bron et al., 1997)***.

Corneal endothelium is a neural crest-derived cellular monolayer, that utilizes an ATP dependent pump to maintain physiologic stromal hydration necessary for corneal clarity. Corneal endothelial cells in humans do not normally proliferate in vivo. Corneal endothelial cells are normally lost throughout life at an estimated rate of 0.6% per year, although higher rates of cell loss occur in the settings of trauma (both surgical and nonsurgical). Corneal endothelial cell loss is compensated for through flattening and enlargement of remaining cells without cell division in order to maintain a continuous monolayer ***(Suh et al., 2008)***.

Examination of the posterior surface by scanning electron microscopy reveals that the endothelial cells are arranged in a uniform hexagonal pattern (fig 7) ***(Weng Sehu & Lee, 2005).***

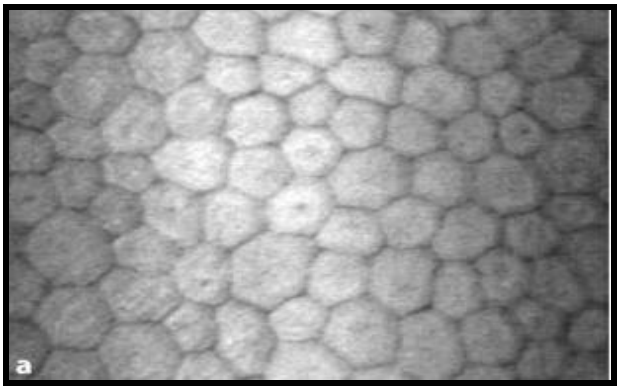

Figure (7): In vivo confocal microscopy image of normal corneal endothelial cells. Note ordered, hexagonal array of cells ***(Suh et al., 2008).***

Nerves of the cornea:

The cornea is supplied by the ophthalmic division of the trigeminal nerve via the anterior ciliary nerves and those of the surrounding conjunctiva. There is also a supply from the cervical sympathetic providing adrenergic fibers to the limbus ***(Bron et al. 1997).***

Physiology and biochemistry of the cornea

Epithelium:

The primary function of the corneal epithelium is, with the tear film, to provide a very smooth refracting surface at the front of the eye. Interference with this surface by drying, edema, or epithelial defects can have severe visual consequences. The epithelium is a relatively impermeable barrier to water-soluble agents from the tear film and to bacterial and fungal infections, though perhaps not to virus. It is probably only minimally involved in active corneal dehydration, but its barrier reduces evaporation and minimizes absorption of fluid from the tears, thus helping to maintain proper corneal hydration. The suprabasal epithelial cell layer appears to represent the main barrier site to the passage of small molecules and cells in the cornea ***(Dohlman, 1971)***.

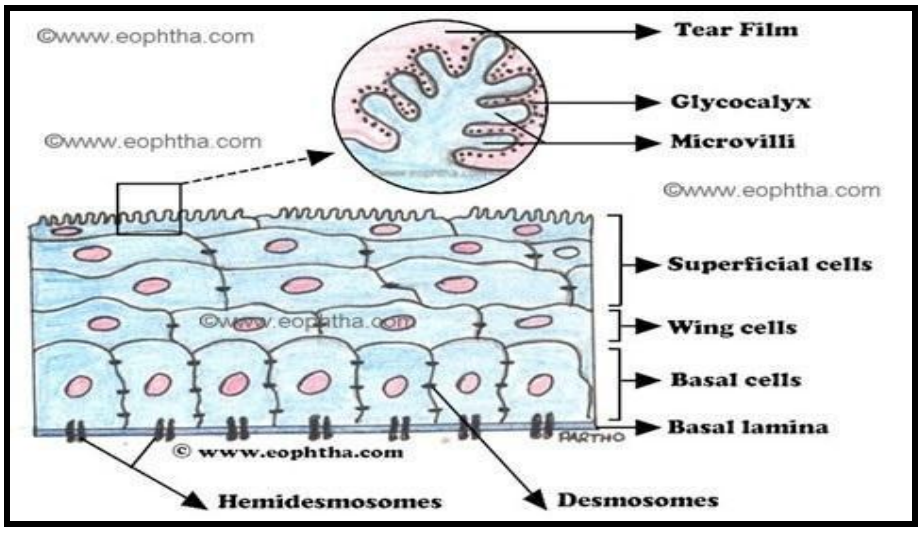

Figure (8): Corneal epithelium ***(Dohlman, 1971)***

Metabolism:

Glucose and Glycogen

The principal substrates for energy production in the corneal epithelium and endothelium are glucose and glycogen. Most of the glucose for the cornea, including the epithelium, comes from the aqueous humor; there may also be glycogen stores in the endothelium. The mechanism of glucose transfer from aqueous to endothelium is probably facilitated transfer ***(Foster et al., 2004)***.Only about 10 percent of the glucose required to support the epithelium diffuses from the limbus or comes from the tears. In addition to the free glucose available, epithelium has large glycogen stores ***(Friend, 1979)***.