CORNEAL ENDOTHELIAL CHANGES
IN KERATOCONUS PATIENTS

Essay
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Ophthalmology

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Mohamed Gamal Abdel Moez Masoud
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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</tr>
<tr>
<td>BCVA</td>
<td>Best corrected visual acuity</td>
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</tr>
<tr>
<td>CD</td>
<td>Cell density</td>
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</tr>
<tr>
<td>CM</td>
<td>Confocal microscopy</td>
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<td>CV</td>
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<td>D</td>
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</tr>
<tr>
<td>ECD</td>
<td>Endothelial cell density</td>
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<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
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</tr>
<tr>
<td>HLA</td>
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<td>KC</td>
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<td>Km</td>
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<tr>
<td>OD</td>
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INTRODUCTION

Keratoconus (KC) is a degenerative, non-inflammatory corneal disorder characterized by progressive stromal thinning and ectasia. It is commonly bilateral. The disease progresses throughout the second and third decades of life (Fogla, 2013).

Current concepts of etiology include a possible genetic link of an autosomal dominant mode of inheritance (Rabinowitz, 1998). The pathogenesis of KC is not completely known, but it is likely a multifactorial disease, with several genetic and environmental factors contributing to its development (Romero-Jiménez, et al., 2010) (Burdon & Vincent, 2013). Keratoconus has been associated with many systemic conditions, such as trisomy 21, Ehlers–Danlos, and Marfan's syndromes. (Hartstein J, 1968) (Macsai, et al., 1990). Eye rubbing, contact lens wear and atopy have also been reported among mechanical factors to be associated with the disease (Edwards, et al., 2001). A current concept has been proposed that individuals who have a genetic predisposition to keratoconus may develop the condition, if the cornea is subjected to physical forces such as eye rubbing in floppy lid, atopy and contact lens wear (Krachmer, 1997).

The pathology of keratoconus demonstrates breaks in Bowman's membrane with reduplications of the basement membrane of the epithelium and progressive scarring (Teng, 1963). There is thinning of the corneal stroma with marked
alteration of the corneal lamellae centrally. In advanced cone formation, breaks in Descemet's membrane with stromal edema may occur in the form of corneal hydrops.

The corneal endothelium, a monolayer of cells at the posterior surface of the cornea, is crucial for maintaining the transparency of the cornea. It regulates the ion composition of the whole cornea, permitting optimal hydration of the cornea, and thus constant thickness and transparency. Consequently, a disturbance of endothelial functions can provoke corneal edema followed by partial or complete loss of transparency. A decrease in endothelial cell density and an abnormal cell mosaic, which may occur after many conditions, can compromise the integrity of the endothelial monolayer, resulting in corneal decompensation with reduced vision (Vincent, et al., 1996).

Although keratoconus has been well studied, endothelial changes have not been extensively investigated. The corneal endothelium is usually normal in keratoconus patients. However, some abnormalities have been reported, including pleomorphism, and elongation of cells with their long axis toward the cone (Sturbaum & Peiffer, 1993). Gross histopathologic analysis of corneal buttons undergoing penetrating keratoplasty for keratoconus has revealed endothelial cell pleomorphism and polymegathism; endothelial cell degeneration, and evidence of anterior chamber inflammation (Perlman & Zaidman, 1994). Patterns of endothelial damage were variable ranging from isolated cell membranolysis to denudement of Descemet's membrane. Less damage was present at the apex of the
cones than that observed in a circumferential pattern at the bases (Sturbaum & Peiffer, 1993).

Keratoconus management varies depending on the disease severity. Traditionally, incipient cases are managed with spectacles, mild to moderate cases with contact lenses, and severe cases can be treated with keratoplasty. Other surgical treatment options include intra-corneal rings segments, corneal cross-linking, and laser procedures such as: PRK, LASIK, intra-ocular lens implants or a combination of these (Jimenez et al., 2010).

One of the methods of treating keratoconus is DALK. One of the proposed advantages of this procedure is that it preserves the host’s endothelium, which is assumed to be normal. If corneal endothelial abnormalities are present in keratoconus patients, these may theoretically affect the long-term maintenance of corneal graft clarity after DALK. If the extent of endothelial abnormality is correlated with the grade of the disease, this may affect selection criteria for DALK. Based on this, if there is advanced endothelial abnormality, PKP with high quality donor tissue might be a better alternative to DALK in these patients (Krumreich, et al., 2008).
AIM OF WORK

This essay aims to study endothelial integrity, count and morphology in keratoconus patients and to correlate the endothelial changes to the stage of keratoconus.
ANATOMY OF THE CORNEA

The cornea is the major refractive surface of the eye, the anterior surface of the cornea provides approximately 45 diopters of power. It also serves as a strong barrier protecting the inner structures of the eye against trauma and infection (Armitage, 1999).

GROSS ANATOMY

The cornea is a clear transparent tissue that joins the opaque sclera at the limbus and is bounded anteriorly by the tear film and posteriorly by the aqueous humour. When it is viewed from the outside, it has a certain diameter, most commonly now assessed by measures of the horizontal corneal diameter. This is also known as the visible iris diameter or white to white diameter since it is essentially the transition from the visible edge of the iris to the white sclera surrounding the cornea. Human cornea might be considered as round when viewed from the outside. It is slightly oval so that the vertical diameter measures are slightly smaller (Jonuscheit & Doughty, 2009).

Its outer aspect is slightly elliptical with a horizontal axis of 11.7 mm and a vertical axis of 10.6 mm while from the inner aspect it is circular with a diameter of 11.7 mm. The radius of curvature of the anterior surface of the central cornea is 7.8 mm and that of the posterior surface is 6.5 mm, while the peripheral cornea is more flattened (Bron et al., 1997).

Measures of the anterior and posterior curvatures are readily obtained by modern instrumentation using a special scheimpflug photography (pentacam) system (Ho et al., 2008).
The difference in curvature between the anterior and the posterior surfaces results from the central corneal thickness being relatively thinner than the periphery. Maurice reported that the central corneal thickness value is 0.52 mm and the peripheral corneal thickness is 0.65 mm when measured with an optical pachemeter (DelMonte & Kim, 2011).

There is no gender difference in the corneal thickness, but other corneal dimensions are slightly less in females (Armitage, 1999).

**Microscopic anatomy**

Previously, the human cornea, which is approximately 550 microns thick, was thought to be comprised of five layers (figure 1) from front to back, the corneal epithelium, Bowman’s layer, the corneal stroma, Descemet’s membrane, and the corneal endothelium (Bron et al., 1997).

Based on clinical experience with corneal transplants, Dua described a layer that exists between the corneal stroma and Descemet’s membrane (Dua et al., 2013).
**Epithelium:**

It accounts for 10% of the corneal thickness. It is of non-keratinized stratified squamous epithelial layer and becomes continuous with the epithelium of bulbar conjunctiva at the limbus. It consists of 5-7 layers of cells. The deepest (basal) layer is made up of columnar cells of about 18 um in height and 10 um in diameter with a flat basal surface and a rounded apical surface. Moving anteriorly, cells become more flattened to form 2-3 layers of wing or umbrella cells, while those in the outermost layer are highly flattened squamous cells that are only 4 um thick and up to 45 um across. The basement membrane zone “basal lamina” is a complex interface between the basal cells and the underlying bowman’s layer (Armitage, 1999).
The basal cells are connected to one another by desmosomes and to the underlying basal lamina by hemidesmosomes. Both the wing and basal cells possess numerous fibrils called anchoring filaments which pass through the desmosomal structures to be inserted into the underlying basal lamina (Niederkorn & Kaplan, 2007).

The superficial cells are attached to each other by tight junctions zonula occludens in addition to the desmosomal connections (Remington, 2012).

Langerhan’s cells are antigen presenting cells that carry both class 1 and 2 major histocompatibility antigens which are confined to the peripheral 1/3 of the epithelium, but corneal diseases, trauma or chemical stimulators can result in substantial recruitment of it into the central region (Armitage, 1999).

The cornea has a rich supply of sensory nerve fibers, with their main origin being the ophthalmic division of the trigeminal nerve. The corneal sensory nerve extending from the basal to more anterior layers (Muller et al., 1996).

Recent studies on post-mortem human corneas indicate that central nerve fibers form a network that extends uniformly in all directions across the epithelium with estimated densities of some 600/mm² in more anterior layers (Marfurt et al., 2010).

As a result of the differences in the nerve terminals and the organization of the nerve fibers, the touch sensitivity of the central corneal zone is the greatest, with slightly less sensitivity being found for the peripheral cornea. The tactile sensitivity of the cornea is most often determined with a Cochet-Bonnet aesthesiometer (Cocheti & Bonnet, 1960), where the maximal length of a nylon filament that can be detected has been used as a measure of sensitivity. For central corneal zone, the maximal sensitivity is typically observed, as being 60 mm, while that of the peripheral cornea is likely closer to 30 mm (Belmonte et al., 2004).
The corneal sensory system is also able to detect ambient changes in air temperature and changes in tear film osmolarity, (Liu & He, 2009) as well as airborne irritants (Cometto et al., 2007).

**Figure (2) The corneal epithelium and Bowman's layer, showing hemidesmosomes and desmisomes along the basal lamina (Thomas, 2008)**

**Bowman's membrane:**

This layer consists of acellular layer of condensed collagen fibrils of uniform thickness (each is 20-30 nm in diameter). It is better referred to as the Anterior Limiting lamina. It binds the corneal stroma anteriorly with basement membrane of the epithelium. It is about 8-10 μm in thickness. (Merindano et al., 2006)

The anterior surface of the membrane runs parallel with the surface of the cornea, while the posterior surface isn’t well defined and merges with the anterior corneal stroma. Peripherally, the membrane ends abruptly in a rounded border (Nema et al., 1999).
It is not a true elastic lamina and once destroyed does not regenerate. However, it offers a great deal of resistance to infection and injury (Niederkorn & Kaplan, 2007).

**Stroma (substantia propria):**

This layer is about 0.5 mm in thickness and constitutes 90% of total corneal thickness (Remington, 2012). It consists of collagen fibrils (lamellae embedded in hydrated matrix of proteoglycans (PG) which have a major role in controlling the hydration, thickness and transparency of the cornea. This function is necessary for maintaining the rigid curved shape of the cornea. Together with salts and PGs of the extra cellular matrix contribute to the hyperosmotic nature of the corneal stroma, which exerts a swelling pressure of 60 mm Hg (DelMonte & Kim, 2011).

The lamellae are arranged in many layers. In each layer they are not only parallel to each other but also to the corneal plane and become continuous with scleral lamellae at the limbus. The alternating layers of lamellae are at right angle to each other. Among the lamellae are present keratocytes (which can phagocytose particles and undergo shifts in PG and collagen synthesis in response to injury), wandering macrophages, histiocytes and a few leucocytes (Hedbys & Mishima, 1966).
Dua's Layer:

Is a well-defined, acellular, strong layer in the pre-Descemet's cornea. It is hypothetically 15 micrometers thick, despite being thin; the layer is very strong and impervious to air. It is strong enough to withstand up to 2 bars (200 kPa) of pressure. Its recognition will have considerable impact on posterior corneal surgery and the understanding of corneal biomechanics and posterior corneal pathology such as acute hydrops, Descematocele and pre-Descemet's dystrophies. This layer's discovery was reported in May 2013 by Harmande Dua and was confirmed by September 2013 (Dua et al., 2013).

Descemet's membrane:

Descemet's membrane is a thin, acellular, strong homogenous layer that 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. It is 8-12 μm in thickness. It is continuous peripherally with the cortical zone of the
trabeculae in the trabecular meshwork and is thickened at its peripheral termination to form Schwalbe’s line which is the anterior limit of the trabecular meshwork (Forrester et al., 2003).

It is very resistant to chemical agents, trauma and pathological processes. It consists of collagen and glycoproteins. Unlike Bowman's membrane it can regenerate. Normally it remains in a state of tension and when torn it curls inwards on itself. It is also known as the posterior limiting lamina, posterior elastic lamina, lamina elastic posterior, and membrane of Demours. It was named after French physician Jean Descemet (Johnson et al., 1982).

**Endothelium:**

Endothelium is a single layer of flat polygonal (mainly hexagonal) mitochondria-rich cells which on specular microscope appear as a mosaic. The endothelial cell density (ECD) is rather less than 3000 cells/ mm² in young adults and even closer to 2000 cells/ mm² in elderly. (Edelhauser, 2000).
It is about 5 μm thick. The corneal endothelium governs fluid and solute transport across the posterior surface of the cornea and actively maintains the cornea in the slightly dehydrated state that is required for optical transparency (Nema et al., 1999).

Unlike the corneal epithelium the cells of the endothelium do not regenerate. Instead, they stretch to compensate for dead cells which reduce the overall cell density of the endothelium, which has an impact on fluid regulation. There is a considerable functional reserve for the endothelium. Therefore, corneal decompensation occurs only after more than 75 percent of the cells are lost (Wilson et al., 2012).

### Table 1: Endothelial cell density by age (Edelhauser, 2006) (Phillips, et al., 2005) (Niederer, et al., 2007)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Average endothelial cell density (cells/mm²)</th>
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<tr>
<td>10 - 19</td>
<td>2,900 - 3,500</td>
</tr>
<tr>
<td>20 - 29</td>
<td>2,600 - 3,400</td>
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<tr>
<td>30 - 39</td>
<td>2,400 - 3,200</td>
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<tr>
<td>40 - 49</td>
<td>2,300 - 3,100</td>
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<tr>
<td>50 - 59</td>
<td>2,100 - 2,900</td>
</tr>
<tr>
<td>60 - 69</td>
<td>2,000 - 2,800</td>
</tr>
<tr>
<td>70 - 79</td>
<td>1,800 - 2,600</td>
</tr>
<tr>
<td>80 - 89</td>
<td>1,500 - 2,300</td>
</tr>
</tbody>
</table>
Blood supply:

The cornea is avascular and devoid of lymphatic drainage. The capillary blood vessels derived from the anterior ciliary arteries of the conjunctiva and sclera end at the circumference of the cornea. The cornea is nourished by diffusion from the aqueous humor and from the capillaries at its edge. The central part of the cornea receives oxygen indirectly from the air via oxygen dissolved in the tear film, whereas the peripheral part receives oxygen by diffusion from the anterior ciliary blood vessels (Bron & Tripathi, 1997).

Nerve supply:

Cornea is supplied by anterior ciliary nerves which are branches of ophthalmic division of the 5th cranial nerve. After going about 2 mm in cornea the nerves lose their myelin sheath and divide dichotomously and form three plexuses; the stromal, subepithelial and intraepithelial (Marfurt, 2010).
PHYSIOLOGY OF THE CORNEA

❖ **Epithelium**

The primary function of the corneal epithelium with the tear film is 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. It is probably only minimally involved in active corneal dehydration, but it acts as a barrier that reduces evaporation and minimizes absorption of fluid from tears thus helping to maintain proper corneal hydration (Levin, et al., 2011).

➢ **Epithelial basement membrane**

Composed of Collagen type IV in addition to laminin, perlecan, and possibly fibronectin (Lens, et al., 2008).

❖ **Bowman's layer**

Bowman's layer is the collagen and ground substance lying immediately beneath the corneal epithelium. This area is microscopically distinguishable from the rest of the stroma by the random arrangement of the short collagen fibrils and the lack of cells (Foster, et al., 2004). It is composed of randomly packed type I and type V collagen fibers that are enmeshed in a matrix consisting of proteoglycans and glycoproteins (Panjwani, 1997).
Stroma

The stroma is made up of roughly 200 layers of lamellae, each is 1.5-2.5 μm in thickness and constituted of collagen fibrils enmeshed in a matrix consisting of proteoglycans, proteins and glycoproteins (Panjwani, 1997).

Collagen

Collagen is defined as a structural protein of the extracellular matrix that contains one or more domains having glycine in every third position of the polypeptide chain. This produces a helical structure that can assemble into a triple helix by associating with two other helices to result in a rod-shaped macromolecule. The collagen chains are cross linked to each other and to other triple helices in the matrix. This results in a super molecular structure with incredible tensile strength (Lee and Davison, 1981).

Collagen, which makes up to 71 % of the dry weight of the cornea, is the most abundant protein in the body. It provides the structural backbone of many tissues (e.g., cornea, sclera, cartilage, skin, tendons and cardiac valves). In the eye, collagen is present in the cornea in the subepithelial basement membrane, Bowman's layer, the lamellae of the stroma (mainly collagen type I) and Descemet's membrane. It is present also in the sclera (Linsenmayer, 1981) (Foster et al, 2004).

The collagen type is determined by the type of polypeptide chains (often referred to as alpha chains) present in the triple helix. The alpha chains are distinguished from each other by their amino acid sequence and other physicochemical properties. Different alpha chains are therefore different gene products. Each collagen type consists of three alpha chains that may be the same gene product or three different genes products (Foster et al, 2004).
Based on alpha chain composition, there are 28 types of collagen recognized. Collagen types I, II, III, V, and VI are almost completely collagenous. They appear as classic banded collagen fibrils in the electron micrographs of corneal stroma. Collagen type IV, in contrast, has numerous small non collagenous domains between relatively short collagenous domains. This produces an amorphous matrix in electron micrographs like that of the basement membranes (Foster et al, 2004).

➢ Proteoglycans

Constitute approximately 10% of the dry weight of the cornea. It is the proteoglycans that confer hydrophilic properties to the stroma. They are formed of glycosylated proteins with at least one glycosaminoglycan (GAG) chain covalently bound to the protein core. Four types of corneal stromal proteoglycan core proteins have been identified: decorin, lumican, keratocan, and mimecan (Levin, et al., 2011). GAGs are composed of repeating disaccharides. The GAGs found in corneal stroma include; Keratan sulfate, chondroitin sulfate and dermatan sulfate. Regulation of spacing between the stromal collagen fibrils is thought to result from highly specific interaction between the proteoglycans and the collagen fibrils; when these interactions are disturbed, the ability of the cornea to remain transparent is profoundly affected (Panjwani, 1997).

➢ Matrix metalloproteinases (MMPs)

MMPs are enzymes responsible for degradation of the components of the extracellular matrix during normal development as well as in disease processes. Only MMP2 proenzyme has been found in healthy cornea. However after corneal
injury, additional MMPs (including MMP1, MMP3 and MMP9) are synthesized. The proteinase inhibitors of the cornea play a key role in its protection by restricting damage during corneal inflammation, ulceration, and wound healing. The proteinase inhibitors identified in the cornea include; α1-proteinase inhibitor, α1-antichymotrypsin, α2-macroglobulin, plasminogen activator inhibitors 1 and 2 and tissue inhibitors of metalloproteinase (Panjwani, 1997).

❖ Descemet's membrane

Descemet's layer is a 6μm wide layer of collagen adjacent to the endothelium which is considered as the basement membrane of that cell layer. Although characterized by the high hydroxyproline content and peptide helical features of collagen, the collagen of descemet's membrane is unusual in many respects, including high carbohydrate content and an amorphous electron microscope appearance (Herranz & Herran, 2012).

The basement membrane components, type IV collagen, laminin, fibronectin are also present in descemet's membrane (Herranz & Herran, 2012).

❖ Endothelium

The primary function of the corneal endothelium is to maintain corneal transparency by regulating corneal hydration and nutrition through a “leaky” barrier and metabolic pump function first described by David Maurice (Maurice, 1984). Secondarily; it is also known to secrete the anteriorly located basement membrane, Descemet’s membrane, and a posterior-located glycocalyx layer (Dawson, et al., 2006).
Leaky barrier function

The barrier function of the corneal endothelium is dependent upon a sufficient number of endothelial cells to cover the posterior surface of the cornea and intact macula occludens tight junctions between the endothelial cells resulting in a low-electrical-resistance (25 Ω·cm²) barrier to the aqueous humor flow. Macula occludens tight junctions results in partial obliteration of the intercellular space and partial retention of a 10 nm wide intercellular space (Herranz & Herran, 2012). In healthy human corneas, this barrier prevents the bulk flow of fluid from the aqueous humor to the corneal stroma, but it does still allow moderate diffusion of small nutrients, water, and other metabolites to cross into the stroma through the 10 nm wide intercellular spaces (Lens, et al., 2008).

Despite the loss of endothelial cells that occurs with aging, there appears to be no significant increase in the permeability of normal, healthy aged corneas. Only when the endothelial cell junctions are disrupted does permeability increase (Levin, et al., 2011). Corneal endothelial permeability does, however, gradually increase as central endothelial cell density (ECD) decreases below 2000 cells/mm², but compensatory metabolic pump mechanisms keep the cornea at its normal dehydrated state. These mechanisms can function until a central ECD of 500 cells/mm² is reached (Daniel, et al., 2011) Figure (2).

A number of factors are known to acutely affect the barrier function of the endothelium including reversible disruption of cell junctions during irrigation with calcium-free solutions or glutathione-restricted solutions, mechanical damage during intraocular surgery, or chemical injury due to introduction of non-physiologic or toxic solutions into the anterior chamber. Fortunately, the remaining viable cells are often able to migrate and re-cover the posterior corneal surface by
spreading out over a larger surface area, reestablishing the intercellular cell junctions (Daniel, et al., 2011).

Metabolic pump function

Active metabolic processes in the endothelium are responsible for maintaining corneal dehydration. Subsequent studies demonstrated that transporters, located primarily in the endothelial cell’s basolateral membrane transport ions, principally sodium (Na⁺) and bicarbonate (HCO₃⁻), out of the stroma and into the aqueous humor (Levin, et al., 2011). An osmotic gradient is created and water is thus osmotically drawn from the stroma into the aqueous humor. This osmotic gradient can be maintained only if the endothelial barrier is intact. A major transport protein found to be essential for endothelial metabolic pump function is Na⁺/K⁺-ATPase (Herranz & Herran, 2012). Approximately 3 million Na⁺/K⁺-ATPase pump sites are present in the basolateral membrane of a single corneal endothelial cell (Daniel, et al., 2011) Figure (2).

A number of factors are known to alter endothelial pump function including pharmacologic inhibition of Na⁺/K⁺-ATPase, decreased temperature, lack of bicarbonate, and a chronic reduction in ECD from mechanical injury, chemical injury, or disease states. Fortunately, with regard to the latter, compensatory metabolic pump mechanisms prevent corneal edema from occurring to a certain degree when central ECDs are between 2000 and 550 cells/mm². This occurs by either increasing the metabolic activity of pump sites already present, which requires more ATP production by the cell, and/or by increasing the total number and density of pump sites on the lateral membranes of endothelial cells (Levin, et al., 2011).
- 21 -

Figure (5) Location of corneal endothelial metabolic pumps and barriers.

(Waring, 1982)

➢ **Basement membrane and glycocalyx**

A secondary function of the corneal endothelium is its ability to secrete a basement membrane along its basal surface the posterior amorphous nonbanded zone of Descemet’s membrane, which is continuously deposited throughout life (Dawson, et al., 2006). Finally, the endothelium is also known to secrete a 0.7 μm thick glycocalyx layer on its apical or posterior surface (Edelhauser, 2000). Functionally, it is thought that the glycocalyx layer may protect the internal (or posterior) surface membrane of the endothelium (Daniel, et al., 2011).
KERATOCONUS

Keratoconus (KC) is an entity characterized by ectasia of the cornea. In the absence of inflammation, a cone-like anterior protrusion of the cornea occurs, generally involving the central and inferior paracentral areas. The involved stroma is thin, has decreased tensile strength, and may scar. A high degree of irregular, myopic astigmatism results, causing a variable amount of visual impairment. In most cases, keratoconus occurs bilaterally; although the condition may be considerably more pronounced or appear considerably earlier in one eye than the other (Smadja, et al., 2013).

❖ Prevalence

Reported estimates of the frequency of keratoconus vary widely. Most estimates fall between 50 and 230 per 100,000 (Wang, et al., 2000), and is the major cause of cornea transplantation in the Western world (Alessandro, et al., 2005).

❖ Sex distribution

Keratoconus affects both genders, although it is unclear whether significant differences between males and females exist. Some studies have not found differences in the prevalence between genders, others have found a greater prevalence in females while other investigators have found a greater prevalence in males (Owens and Gamble, 2003).
Age distribution and course

Keratoconus commonly first presents during the second decade of life. Many of the cases slowly and gradually progress in severity, but the rate of progression and the length of time that the entity remains actively progressive varies considerably. Progression is highly asymmetric. The ectasia may progress slowly but continuously for years and then stabilize permanently, or periods of progression may alternate with periods during which the ectatic process appears to have arrested. In some cases, the ectasia remains stationary after its initial appearance. It is uncommon for the condition to progress after the affected individual has attained the age of 40 years, but exceptions can occur. The factors governing the progression and stabilization of keratoconus are not known (Ambrosio, et al., 2014).

Hereditary and familial factors

Most cases of keratoconus are sporadic. Hereditary transmission of keratoconus has also been reported. Dominant, recessive, and irregular transmissions all appear to be implicated. A family history can be demonstrated in 6-10% of cases of keratoconus (Ambrosio, et al., 2014).

Immunological factors

Some studies of the major histocompatibility complex (MHC) suggest an increase of human leukocyte antigen HLA-B5 and a decrease of HLA-DR7 in patients with keratoconus. These data implicate immunogenetic factors in the etiology of keratoconus (Vincent, et al., 2014).
**Pathogenesis of keratoconus**

Despite extensive research, the etiopathogenesis of keratoconus is still unknown, but it is generally accepted that keratoconus is a multifactorial condition or that it represents the final stage of a variety of different pathological processes. Both genetic and non-genetic or environmental factors have been implicated. There is strong evidence for genetic factors in the literature. Although usually sporadic, a family history of keratoconus is not unusual with 14% of 1209 patients in the Collaborative Longitudinal Evaluation of Keratoconus study having a family history of keratoconus (Wagner et al, 2007).

**Biochemical abnormalities and enzymatic role**

Increased collagenolytic activities in keratoconic corneas compared with the activity in normal control corneas have been reported. It was suggested that alterations between one of the enzymes, Matrix metalloproteinases, and its inhibitor may play a role in the stromal thinning that characterizes keratoconus. Also, levels of lysosomal enzymes, including acid esterase seem to be elevated and the levels of proteinase inhibitor (antitrypsin) are reduced in corneas from patients with keratoconus but not in those from patients with other corneal disease. It was found that fibroblasts from keratoconic corneas have four-fold more binding sites for interleukin-1 than do fibroblasts from normal corneas, resulting in a modification of fibroblast proliferation and collagen synthesis (Sugar, et al., 2012).

It was also found that keratan sulfate, a major proteoglycan molecule in normal corneas, is decreased; its structure is modified and its ratio to the proteoglycan dermatan sulfate is reduced in patients of keratoconus. Also the level of alpha 2-macroglobulin, a protein that regulates the degradation of the
extracellular matrix components and other macromolecules, was found to be reduced in keratoconic corneas (Rabinowitz, 1998).

Nerve growth factor (NGF) levels are markedly decreased in corneas of patients affected with keratoconus compared with healthy donors. NGF and its signaling molecules have a crucial role in trophism and wound healing of the cornea. NGF also promotes corneal nerve function, and impairment of corneal innervation has been suggested to play a role in the pathogenesis of keratoconus (Brookes, et al., 2003) (Ruddle, et al., 2003).

➢ **Alteration in Protein/Non protein components**

Corneas obtained from keratoconus patients contain significantly less total protein per milligram of dry weight than corneas of normal controls (Rabinowitz, 2000).

➢ **Changes in collagen synthesis, orientation, and distribution in keratoconus cornea**

Sugar and his colleagues in 2012 found that the amounts of collagen synthesized by corneal cells in keratoconus are slightly lower than those in normal cornea (Sugar, et al., 2012).

Many of the clinical features of keratoconus can be explained by a biochemical hypothesis, which proposes that corneal thinning and ectasia is the result of an intralamellar and interfibrillar slippage of collagen within the stroma, due to loss of cohesion between collagen fibrils and the non-collagenous matrix (Wolffsohn, et al., 2012).
The central and inferior regions of the cornea are likely to be affected preferentially (the main region of cone formation), since intralamellar cohesive strength is at a minimum in that area in normal cornea (Wolffsohn, et al., 2012).

Meek and his co-worker (2005) studied collagen orientation and relative distribution of collagen fibrillar mass in keratoconus corneal buttons using x-ray scattering. They have shown that in keratoconus, formation of the cone is associated with displacement of the axes of the collagen fibrils and distortion of the orthogonal matrix. This implies a degree of lamellar fluidity and slippage.

They hypothesized that in keratoconus, an unidentified primary event, which may be under genetic control, triggers a breakdown of the "glue" that stabilizes collagen fibrils and facilitates the process of lamellar or fibrillar slippage and this could explain the slow evolution of keratoconus from childhood and the early teens.

This hypothesis has therapeutic implications, and it is relevant that attempts are being made to slow down the progression of keratoconus by enhancing cross-linking (Joo, et al., 2004).

Figure (6) Thin keratoconic cornea: note hypercellularity (increased number of fibroblasts) and disorganization of collagen lamellae (Leibowitz and Morales, 1998).
Associated diseases and conditions

A) Ocular diseases and conditions:

In various documented reports and studies, many ocular disorders have been found to be associated with keratoconus including: retinitis pigmentosa, Leber congenital amaurosis, floppy eye lid syndrome and Fuch’s heterochromic iridocyclitis (Yagci, et al., 2001).

Many other isolated ocular pathological conditions have been associated with keratoconus; posterior polymorphous dystrophy, lattice-granular (Avellino) dystrophies and essential iris atrophy. Also, blue sclera, microcornea, congenital cataract, ectropia lentis, lenticous, macular coloboma and retinal dysplasia have been found to be associated with keratoconus (Leibowitz & Morales, 1998).

B) Systemic diseases and conditions:

Keratoconus has been associated with numerous genetic systemic disorders. A number of these keratoconus related disorders involve abnormalities of collagen elasticity and connective tissue, including joint hypermobility, osteogenesis imperfecta, Ehlers Danlos syndrome, Marfan syndrome, mitral valve prolapse, and pseudoxanthoma elasticum. In addition, many keratoconus associated syndromes have a high incidence of eczema and atopy, such as Down syndrome, hyper-IgE syndrome, ichthyosis, and oculodentodigital syndrome, Turner syndrome (Edwards et al, 2001).
Pathology

Histopathologic examinations of cornea removed from conical corneas at the time of penetrating keratoplasty confirms the clinical observation that the tissue is thinner centrally than peripherally and that it gradually and progressively becomes thinner as one approaches the apex of the cone. Although all layers of the cornea ultimately may show microscopic alterations, the earliest changes occur in the superficial layers of the cornea (Wolffsohn, et al., 2012).

1- Epithelial changes:

The basal layer of the epithelium is involved at a relatively early stage. Some of the basal cells become pale, edematous and acquire pyknotic nuclei. In advanced stages of keratoconus, the cell membrane may break up; the basal cells eventually disappear, leaving only one or two layers of flattened superficial epithelial cells lying on an altered basement membrane, on Bowman's layer, or directly on the anterior stroma (Ambrosio, et al., 2014).

An important sign of keratoconus is the Fleischer ring, which is a ring of iron pigments revealed by electron microscopy as ferritin particles. These particles accumulate within and between the epithelial cells, particularly in the basal epithelium (Feder, 1997)

2- Bowman's membrane changes

Initially there is swelling and degeneration of the fibrils in Bowman's layer. In the more advanced stages of keratoconus, Bowman's layer is destroyed, shows
multiple narrow gaps and takes a wavy appearance. These gaps lie at the base of the cone as well as at its apex, and are filled either with newly formed connective tissue or epithelium and may correspond to the linear superficial scars (Wolffsohn et al., 2012).

3- Stromal changes:

At the apex of the ectasia, the stroma may be one third or less of its normal thickness. Thinning is attributable to a decrease in the number of collagen lamellae rather than their size (Smolek & Beekhuis, 1997). Feder, in 1997, found that collagen lamellae were released from their attachment to each other and become able to slide. This results in thinning without collagenolysis (Feder, 1997).

4- Dua's layer

According to a 2013 paper by Harminder Singh Dua's group at the University of Nottingham, Dua`s layer is a layer of the cornea that had not been detected previously. It is hypothetically 10.15 ± 3.6 microns thick at the posterior surface of the corneal stromal. The authors emphasize that this layer is not "residual stroma." It is the fourth layer from the front, and located between the corneal stroma and Descemet's membrane. Despite its thinness, the layer is very strong and impervious to air. Corneal hydrops, a buildup of fluid in the cornea in patients with keratoconus might be caused by a tear in Dua's layer. Dua hypothesizes that such a tear would allow water from inside the eye to pass through and cause fluid buildup (Dua et al, 2013).
5- Descemet's membrane changes:

There are no microscopic alterations observed in Descemet’s membrane until later stages of the disorder when Descemet’s membrane may rupture in the region of greatest ectasia allowing aqueous access to the corneal stroma, and resulting in corneal edema (corneal hydrops) (Wolffsohn et al., 2012).

6- Endothelial changes

Sturbaum and Peiffer (1993) found that in the early stages of keratoconus, the endothelium appears to be normal. As the condition progresses, endothelial cells flatten and their nuclei lie further apart. Presumably, this reflects stretching of individual endothelial cells as they attempt to maintain their continuity over the progressively ectatic posterior surface.

Later in the course of the disease, endothelial changes include pleomorphism, polymegathism, endothelial cell degeneration, and deposition of fibrin, inflammatory cells, or both on the endothelial surface (Sturbaum & Peiffer, 1993).

Breaks or tears in the endothelium and Descemet's membrane occur in 11% to 35% of keratoconic corneas, and the healing of these defects also may result in pleomorphism and polymegathism (Sturbaum & Peiffer, 1993).
Figure (7) An anteroposterior section of the central 1 mm of a keratoconic cone from penetrating keratoplasty surgery. The tissue has been labeled with Cell Tracker Green (Molecular Probes) to mark viable cells and then counter-stained with antibodies to integrin (red) and fibronectin (blue). The cross-section shows some of the classical features of keratoconic pathology. Areas of the cornea are highlighted to show position and type of pathological features in keratoconus (Sherwin et al 2002).

Clinical picture
Keratoconus is characterized by progressive thinning and steepening of the central cornea. As the cornea steepens and thins, the patient experiences a decrease in vision which can be mild to severe depending on the amount of corneal tissue affected (Feder, 1999).
Clinical classification of keratoconus

- Based on the shape of the cone:

  Early keratoconus usually manifests as a small island of irregular astigmatism in the inferior paracentral cornea. Moderate to advanced keratoconus falls into these three categories (Goebels, et al., 2015)

  **Nipple form (5 mm):** This manifests as small, near-central ectasia of 5mm in diameter or less. The mid-peripheral area surrounding the cone base often retains normal thickness and curvature.

  **Oval (from 5-6 mm):** This is the most common shape in advanced keratoconus. Displacement of the corneal apex below the midline results in an island of inferior mid-peripheral steepening. This steepening often creates an area of normal or flatter-than-normal superior cornea 180° away from the cone.

  **Globus (> 6 mm):** This form of the disease encompasses nearly three-quarters of the corneal surface. Unlike the nipple or oval forms of advanced keratoconus, the globus cone has no surrounding island of normal mid-peripheral cornea.
- Based on the severity of curvature:

Keratoconus was classified into mild, moderate, advanced and severe (Goebels, et al., 2015).

<table>
<thead>
<tr>
<th>Keratoconus stage</th>
<th>Keratometer reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>&lt;45 D</td>
</tr>
<tr>
<td>Moderate</td>
<td>45-52 D</td>
</tr>
<tr>
<td>Advanced</td>
<td>52-62 D</td>
</tr>
<tr>
<td>Severe</td>
<td>&gt;62 D</td>
</tr>
</tbody>
</table>

➢ *Forms of Keratoconus*

In clinical practice, three distinct forms of keratoconus have been identified, each with a unique clinical presentation. Differentiating between the three forms can be helpful in counseling patients about what to expect regarding eventual progression of the disease.
• **Puberty-Onset Keratoconus**

Puberty-onset keratoconus is by far the most common form of the condition, and, as its name indicates, it begins in early adolescence at about age 14 to 16 years. The condition is usually bilateral and asymmetrical. Following its onset, there is often a dramatic progression of the condition for the next 8 to 10 years. This is typically followed by stabilization of the condition.

Clinical experience has shown that the earlier in life the keratoconus occurs, the more severe the condition will be.

• **Late-Onset Keratoconus**

In late-onset keratoconus, the earliest signs and symptoms begin in the late twenties or early thirties. Both eyes are frequently affected to a similar degree. This is often a more benign form of the condition, and, unlike puberty-onset keratoconus, its progression is significantly less severe, rarely requiring surgical intervention in the form of a corneal transplant.

• **Forme Fruste keratoconus**

The third form of keratoconus is called “forme fruste” and was first described by Amsler in 1938. It is essentially an extremely mild form of keratoconus that can occur at any time throughout life. The condition manifests as a central or para-central zone of irregular astigmatism of unknown etiology. The most striking hallmark of form fruste keratoconus is its lack of progression with the condition staying stable throughout the patient's life.
**Symptoms**

Keratoconus is characterized by progressive thinning and steepening of the central cornea. As the cornea steepens and thins, the patient experiences a decrease in vision which can be mild to severe depending on the amount of corneal tissue affected (Feder, 1999).

**Signs**

- **Mild keratoconus:**

  Identifying moderate or advanced keratoconus is fairly easy. However, diagnosing keratoconus in its early stages is more difficult, as corneal changes are too subtle for detection in the early stages of the disease, requiring a thorough case history, a search for visual and refractive clues and the use of instrumentation. The first or earliest clinical sign probably is the development of astigmatism. Often, keratoconic patients have had several spectacle prescriptions in a short period, and none has provided satisfactory vision correction. Refractions are often variable and inconsistent (Smadja, et al., 2013).

  From a practical standpoint, mild forms of keratoconus are detected by looking for distortion of the rings of the Placido disc or the keratometer mires in the image on the anterior corneal surface and by observation of abnormalities of the red reflex (spinning reflex) with the streak retinoscope. However, many studies have demonstrated that keratometry and photokeratoscopy are not sensitive enough to detect changes in early stages of keratoconus (Smadja, et al., 2013).
- Moderate keratoconus:

With continued progression of keratoconus, several typical signs can be seen with the slit-lamp biomicroscopy. Corneal nerves may become increasingly visible, and corneal sensation may be reduced, especially in the inferior cornea. Deposition of iron in the basal epithelial cells in a ring shape pattern at the base of the conical protrusion resulting in Fleischer ring (50 % of cases) . Thin, vertical stress lines (Vogt's striae) that tend to parallel the steep axis of the cone become visible in the deeper stromal layers. They may disappear transiently if gentle pressure is applied to the apex of the cone. Corneal transparency is retained, but the apex of the cone protrudes anteriorly and is often accompanied by thinning of the corneal stroma (Smadja, et al., 2013).
Advanced keratoconus:

In advanced cases of keratoconus, the diagnosis can be made by gross inspection. When viewed from the side, the cornea is seen to assume the shape of a cone. Two external findings are associated with keratoconus diagnosis; Munson's sign and Rizzuti's sign (Smadja, et al., 2013).

**Munson’s sign:** When the patient is asked to look downward toward the floor, a V-shaped profile of the lower lid margin can be seen. Moderate-to severe KC tends to produce Munson’s sign, while mild cases of KC will not produce this sign since corneal bulging is more subtle (Li et al, 2004).
**Rizzuti’s sign**: This sign is observed by seeing a light on the nasal anterior sclera when the light is directed into the cornea from the temporal direction. This is because of the total internal reflection of light due to the optical properties of the cone. As with Munson’s sign, this Rizzuti’s sign is more reliable for screening moderate to severe cones and is less sensitive for mild KC (Li et al, 2004).

In the more advanced cases, characteristic ruptures in Descemet's membrane occasionally occur and may result in corneal hydrops. Corneal hydrops generally occurs in advanced cases. When Descemet's membrane ruptures, aqueous flows into the cornea then the rupture reseals. Corneal hydrops causes edema and
opacification. As Descemet's membrane regenerates, edema and opacification diminish. Occasionally, hydrops can benefit keratoconus patients who have extremely steep corneas. If the cornea scars, a flatter cornea often results, making it easier to fit with a contact lens. Ruptures in Bowman's layer may also occur producing superficial linear scars in later stages; the apex of the cone becomes hyposthetic and may show considerable scarring over time (Li, et al., 2004).

Figure (16) Corneal scarring
(Rabinowitz, 2005)

Figure (17) Corneal hydrops
(Tuft, 1994)
Methods of investigations

Histological background

In 1629, Christoph Scheiner calculated the refractive properties of the various transparent media of the eye. In 1728, Pourfour de Petit invented the ophthalmometer to measure the curvature of the cornea. In 1840, Kohiraush measured the reflected image of an object on the cornea using a Kepler telescope (Bores, et al., 1993).

Corneal topography systems

Corneal topography devices currently used clinically can be classified as placido-disc systems and slit-imaging systems (Wang, et al., 2005).

Placido-Disk Topography Systems

The placido-disk topography systems are based on the reflection principle. A placido disc is projected into the cornea and the images of the placido disc reflected off the cornea are captured. Information regarding the position of the placido-disk rings is used to reconstruct the corneal shape (Wang, et al., 2005).

These devices have excellent accuracy and reproducibility in anterior corneal curvature measurement. Limitations of these systems include the following: data at the central zone have to be interpolated (although this unmeasured central zone is very small in some devices), the quality of tear film is critical since the images are obtained from light rays reflected off the tear film, and data are less accurate when mapping aspherical or irregular surfaces due to the
assumption of sufficient smoothness in the radial direction used by some devices (Wang, et al., 2005).

- **Slit-Imaging Systems**
  
  The Orbscan system uses the principle of projection. Forty scanning slit beams (20 from the left and 20 from the right with up to 240 data points per slit) are used to scan the cornea from limbus to limbus and to measure independently the x, y, and z locations of several thousand points on each surface. The Pentacam images the anterior segments of the eye using a rotating Scheimpflug camera and pictures in three dimensions of the anterior segment are shown by this rotating process. The images captured are then used to construct the anterior corneal surface, posterior corneal surface, and anterior iris and anterior lens surfaces. Data regarding the corneal pachymetry and anterior chamber depth are also displayed. In the newer version of the Orbscan system, a placido disk has been mounted to this device in order to improve the accuracy of the curvature measurements (Wang, et al., 2005).

- **Analysis Methods**
  
  - **Keratometry**
    
    Javal, (1883) and Helmholtz, (1924) measured the anterior corneal curvature by using an instrument called keratometer.

    The keratometer measures the central anterior corneal curvature. Its principle is contingent upon accurately determining the size of a reflected image from the front surface of the cornea (the first Purkinje-Sanson image) (Ucakhan, et al., 2011).
Keratometry has the limitation of measuring the corneal shape from only 2-3 points approximately 3 mm apart. The remainder of the cornea is ignored. With keratoconic corneas the area of main concern may not be included in the area (Ucakhan, et al., 2011).

Based on Keratometer readings, keratoconus can be classified into mild, moderate, advanced and severe (Ucakhan, et al., 2011) (Table 1).

- **Keratoscopy**

  The keratoscope is an instrument that reflects a series of concentric circular rings of the anterior corneal surface. Visual inspection of the shape and spacing of the concentric rings provides a qualitative assessment of topography; small diameter, closely spaced indicates high power (steeping region) while large diameter, widely spaced and broadened mires indicates low power (flat region). A photokeratoscope is a keratoscope equipped with a camera, which can provide a permanent record of the corneal topography. Computer-assisted photokeratoscopy has sophisticated image analysis programs to provide quantitative corneal topographic data (Ucakhan, et al., 2011).

  Elliptical distortion of the mires is the hallmark of regular astigmatism. The tortuous shapes of mires with irregular spacing between them are characteristics of irregular astigmatism and can occur in ectatic cornea. In advanced keratoconus, there is narrowing of the spaces between mires especially in the inferior region of paracentral zone and the central mires become smaller than the normal diameter and no longer concentric, and take a tear-drop shape termed “keratokyphosis” (Ucakhan, et al., 2011).
Ultrasonic pachymetry

The ultrasound pachymeter is the most widely used clinical method to measure corneal thickness using the Doppler Effect. It has the advantages of ease of use, portability, and low cost (Sanctis, et al., 2007).

In keratoconus the cornea tends to be thinner than normal, both centrally and peripherally so that reproducible, repeatable, and accurate measurements of corneal thickness are required for the diagnosis, staging, and follow-up of this disease, as well as for planning surgical procedures (Melles, et al., 2000).
Optical pachymetry produces thickness maps which may be more useful in identifying early thickness changes, such as in forme fruste keratoconus (Tracy, et al., 2007).

However, limitations of this system include measurements requiring corneal contact and therefore topical anesthesia, possible incorrect and unrepeatable probe placement, lack of a fixation light for gaze control, ill-defined points of reflection of ultrasound within the cornea, and the variability of sound speed in wet and dry tissues (Bechmann, et al., 2001) (Suzuki, et al., 2003) (Javaloy, et al., 2004).

The pachymetry fail to exclude keratoconus because of its high false negative and false positive rates and presence of large variation in corneal thickness of normal population (Rabinowitz, 1998).

Zadnik and his colleagues, (1996) estimated the normal corneal thickness and classified keratoconus according to the corneal thickness in microns into early, moderate and advanced (Zadnik, et al., 1996) (Table 2).

Table (3) Corneal thickness in normal cornea and in stages of keratoconus. (Zadnik, et al., 1996)

<table>
<thead>
<tr>
<th>Cornea</th>
<th>Thickness in Microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cornea</td>
<td>540 – 515</td>
</tr>
<tr>
<td>Early keratoconus</td>
<td>515 – 480</td>
</tr>
<tr>
<td>Moderate keratoconus</td>
<td>480 – 450</td>
</tr>
<tr>
<td>Advanced keratoconus</td>
<td>Less than 450</td>
</tr>
</tbody>
</table>
• **Computer-assisted videokeratography**

It provides automated analysis of corneal topography using computers to map the cornea and digitize the position of mires in three dimensions of the corneal shape (Schwiegerling & Greivenkamp, 1996).

It provides a color-coded topographical map of the corneal surface. The dioptric power of the steepest and flattest meridian and their axes also calculated and displayed (Wilson, et al., 1993).

**Normal corneal topography**

Normal cornea tends to have a uniform color coded topographic appearance with a dioptric power close to 43 diopters in the center of the cornea. The dioptric power appears to decline toward the periphery particularly in the nasal portion which helps in distinguishing the right (OD) and left (OS) eyes (Barbero, et al., 2002).

![Figure (19) Keratography of normal cornea (Smolek and Klyce, 2000)](image-url)
Topographic analysis of keratoconus stages

- Forme Fruste keratoconus topography

There are no clinical diagnostic criteria for Frome Fruste keratoconus or keratoconus suspect (e.g., munson’s sign corneal thinning in slit lamp, etc) but it usually starts as inferior localized steeping (Barbero, et al., 2002).

The quantitative screening criteria that should raise suspicion of keratoconus are well recognized, such as: apical power greater than 47.0D, differences of greater than 1.0 D in apical power between eyes, surface asymmetry index (SAI) of greater than 1.0 (usually <0.5), elevated values of proprietary automated indices of keratoconus such as Rabinowitz I-S (inferior-superior) ratio, and the increasing value of anterior and posterior elevation topography (Goebels, et al., 2015).

Figure (20) Forme Fruste keratoconus (Smolek and Klyce, 2000)
• **Mild keratoconus topography**

It is characterized by:

1. Superior/inferior corneal curvature discrepancy greater than 1.5 diopters should raise suspicion.

2. Superior/corneal curvature discrepancy greater than 2.5 D

3. Against-the-rule astigmatism (Smadja, et al., 2013).

It is topographically characterized by contour power that tends to be less than 55 diopters at any point on the map. The cone may be located on any position of the surface but it is usually in inferior portion (Barbero, et al., 2002).

![Figure (21) Mild keratoconus (Smolek and Klyce, 2000)](image)

• **Moderate keratoconus topography**

It is characterized by maximum contour at or above 55 diopters. There are no discontinuation contour colors or disruption of the mires images pattern in the cone apex (Barbero, et al., 2002).
It should be noted that the upper half of the cornea appears normal. This is expected since keratoconic changes usually affect the inferior portion of the cornea first (Wang, et al., 2005).

Figure (22) Moderate Keratoconus (Smolek and Klyce 2000)

- **Advanced keratoconus topography**
  In advanced keratoconus, the contour power is at or above 55 diopters, but there are mire pattern interruptions at or near the cone apex (Barbero, et al., 2002).
Artemis ultrasound digital topography

This technique provides a topographic evaluation of the thickness of anterior corneal layers in normal and pathologic corneas with high precision. The resolution of the Artemis is sufficient to distinguish individual corneal layers such as the epithelium, stromal component of the flap, and residual stromal bed. In addition, the technique is not limited to optically transparent tissue (Reinstein, et al., 1994).
Optical coherence tomography (OCT)

Anterior segment OCT pachymetry map-based analysis could detect abnormal corneal thinning in keratoconus eyes. It has micron-level high resolution and can accurately map the corneal thickness of normal, postoperative, and opacified corneas. (Li, et al., 2006) (Khurana, et al., 2007).

One limitation of the OCT technology is that interpolation is used in the central 0.5- to 1.0-mm diameter and among the 8 radial scans. Thus, small areas of corneal thickness variation might be missed (Li, et al., 2006). Another limitation of the OCT system used is that it is not fast enough to measure the corneal topography (Yan, et al., 2008). Spectral domain technology compensates the current time domain systems, by faster capturing and acquisition processing; it reduces the examination time and therefore lessens the effect of the patient’s eye movements.
Microscopic systems

- Ultrasound biomicroscopy (UBM)

In the late nineties, the ultrasound biomicroscopy (UBM) was revealed as a new and useful tool in the study of keratoconus (Castiglione, 2000). UBM is used in estimating corneal thickness, both in patients affected with early-stage keratoconus and in normal subjects. The method proved to be very sensitive, detecting values significantly different in healthy subjects and keratoconic patients. It provides a reliable measure of corneal thinning related to the severity of the disease in keratoconic eyes and allows us to follow the development of the disease. In addition, UBM allows dynamic visualization of the thinnest site of the conus, differently from ultrasound pachymetry (Tello, et al., 1994).

Unfortunately, like any biometric estimation procedure with UBM, it is liable to subjective interpretation. The intraobserver variability is reduced by averaging the measurements on different images of each eye.

- Confocal microscopy (CM)

It is a noninvasive technique allowing visualization of the living human cornea at magnifications of up to 700 times. The CM allows the cornea to be observed in vivo at a cellular level without the need for mechanically section the cornea (Patel & McGhee, 2007). Specifically, it is possible to observe individual cells, cell nuclei in the various layers of the epithelium and endothelium, cell borders, nuclei of stromal keratocytes, Langerhans cells, and the fine epithelial sub-basal nerve plexus. The extent of tissue compromise can be assessed at a cellular level to aid diagnosis and can be used to evaluate the recovery process and
the efficacy of different treatment interventions (Efron & Hollingsworth, 2008). The limitation is the necessity to obtain multiple sections to evaluate larger areas of cornea and the time of image acquisition and processing. Time to acquire a single image is typically less than 1 second, and the observation time in clinical settings is around 10 minutes. The maximal depth of imaging is around 2.7 mm (Patel & McGhee, 2007).

- **Specular microscopy (SM)**
  Specular microscopy is a non-invasive photographic technique that allows the visualization and analysis of the corneal endothelium. The instrument projects light onto the cornea and captures the image that is reflected from the optical interface between the corneal endothelium and the aqueous humor. The reflected image is analyzed by the instrument and displayed as a specular photomicrograph (Figure 3). Viewing the cells by specular microscopy allows the evaluation for pachymetry, cell density, variation in size (polymegathism), and variation in shape (pleomorphism). In clinical practice, specular microscopy is the most accurate way to examine the corneal endothelium.
Corneal endothelial cell density determination:

- **Comparison method**: compare to known “honey comb” pattern.

- **Frame method**: count the number of cell within a frame.

- **Corner method**: determine cell area from a polygon digitization by locating cell border intersections.

- **Center method**: determine cell area from adjacent polygon centers, “center to center” (Dot center of contiguous cells).

The advantages of this method include operator independency and non-invasiveness (Langenbucher & Seitz, 2001). However, because of its operating principle, SM requires clear reflections of the epithelial and endothelial surfaces for this, its clinical use is limited to corneas that are free of edema, scarring, deposits, or opacities that may distort light transmission (Tam & Rootman, 2003).
- **Normal corneal endothelium**

   With specular microscopy, the corneal endothelium appears as a somewhat regular array of cells, known as the endothelial mosaic. In this mosaic configuration, all of the endothelial cells appear to be approximately the same size and shape (Phillips, et al., 2005) (Figure 4).

- **Stability of the endothelial mosaic:** In a normal endothelium, more than 60% of the endothelial cells are six-sided. The size and shape of the endothelial cells is important because adjacent cells with similar dimensions best maintain the fluid barrier function of the endothelium (Liesegang, 2002).

- **Rate of polymegathism:** Complete coverage of the posterior corneal surface is required to maintain the barrier function and the active transport mechanism of the corneal endothelium. Because of normal attrition, the central cornea loses 100 to 500 endothelial cells per year. When these cells die, they slough off the posterior surface of the cornea into the anterior chamber, creating a gap in the endothelial mosaic that compromises both the barrier and pump functions of the endothelium.

   To repair the gap, the endothelium relies on cellular migration and cellular fusion. In this wound repair mechanism, the endothelial cells adjacent to the defect move to fill in the space vacated by the sloughed cell. The endothelial cells either stretch and slide into a different position, or they fuse together to re-establish complete coverage of the posterior surface of the cornea. This movement of the endothelial cells creates a variation in cell size known as polymegathism (Edelhauser, 2006).
Because polymegathism is a reflection of the normal endothelial cell movement that characterizes the wound repair mechanism, there is always some degree of polymegathism in the corneal endothelium. The rate of polymegathism is represented by the coefficient of variation (CV). CV values measured between 0.22 and 0.31 are considered normal (McCarey, et al., 2008).

- **Abnormal Corneal Endothelium**

  Using specular microscopy, endothelial disease may be characterized by one or more abnormalities of cell morphology.

  - **Presence of pleomorphism:** Pleomorphism is a significant disruption in the regular hexagonal pattern of the endothelium that causes a decrease in endothelial mosaic stability (Taravella & Walker, 2009) (Liesegang, 2002) (McCarey, et al., 2008).

  If a patient’s corneal endothelium demonstrates less than 50% hexagonally-shaped cells, it is considered to be clinically significant pleomorphism. Because of its effect on the fluid barrier function of the endothelium, the presence of pleomorphism increases the patient’s risk of developing iatrogenic corneal endotheliopathy and postoperative corneal edema (Phillips, et al., 2005).

  - **Elevated or abnormal rate of polymegathism:** An elevated or abnormal rate of polymegathism is usually the first sign of endothelial disease. This finding indicates physiological stress to the corneal endothelium and an overactive wound repair mechanism. CV values from 0.32 to 0.40 are elevated, and CV values above
0.40 are abnormal. Although endothelial function may still be adequate in these corneas, the endothelium may be more susceptible to additional trauma from insults, such as intraocular surgery, glaucoma, diabetes, uveitis or contact lens wear (Phillips, et al. 2005).

![Cornea with polymegathism and pleomorphism]

**Figure (27) Cornea with polymegathism and pleomorphism**

**Abnormal reduction in endothelial cell density:** Advanced age, disease and injury may produce significant reductions in endothelial cell density. When present, endothelial cell loss should be bilaterally symmetrical; a difference of more than 280 cells/mm² is clinically significant (Phillips, et al., 2005).
### Amsler's classification of keratoconus

**Table (4) Amsler classification of keratoconus** (Amsler, 1961)

<table>
<thead>
<tr>
<th>Stage of Keratoconus</th>
<th>Clinical findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage 1</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Eccentric corneal bulging</td>
</tr>
<tr>
<td></td>
<td>• Myopia and/or astigmatism &lt; 5 Dioptres</td>
</tr>
<tr>
<td></td>
<td>• Corneal power &lt; or = 48 Dioptres</td>
</tr>
<tr>
<td></td>
<td>• Vogt’s striae, no corneal opacities</td>
</tr>
<tr>
<td><strong>Stage 2</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Myopia and/or astigmatism &gt; 5 and &lt; 8 Dioptres</td>
</tr>
<tr>
<td></td>
<td>• Corneal power &lt; or = 53 Dioptres</td>
</tr>
<tr>
<td></td>
<td>• No central opacities</td>
</tr>
<tr>
<td></td>
<td>• Pachymetry &gt; or = 400 µm</td>
</tr>
<tr>
<td><strong>Stage 3</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Myopia and/or astigmatism &gt; 8 and &lt; 10 Dioptres</td>
</tr>
<tr>
<td></td>
<td>• Corneal power &gt; 53 Dioptres</td>
</tr>
<tr>
<td></td>
<td>• No central opacities</td>
</tr>
<tr>
<td></td>
<td>• Pachymetry: 200-400µm</td>
</tr>
<tr>
<td><strong>Stage 4</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Refraction impossible to determine</td>
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<tr>
<td></td>
<td>• Corneal power &gt; 55 Dioptres</td>
</tr>
<tr>
<td></td>
<td>• Central scars</td>
</tr>
<tr>
<td></td>
<td>• Pachymetry &lt; 200 µm</td>
</tr>
</tbody>
</table>
Lu and Azar Keratoconus Scoring System (Combining History, Examination, and Topographic Indices)

Table (5) Lu and Azar Keratoconus Scoring System. (Lu & Azar, 2003)

<table>
<thead>
<tr>
<th>Values</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Corneal hydrops by examination or history</td>
<td></td>
</tr>
<tr>
<td>Yes Corneal hydrops by examination or history</td>
<td></td>
</tr>
<tr>
<td>No Two clinical keratoconus findings by examination or history</td>
<td></td>
</tr>
<tr>
<td>Yes Two clinical keratoconus findings by examination or history</td>
<td></td>
</tr>
<tr>
<td>Central corneal power ≥ 47.2 D</td>
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</tr>
<tr>
<td>47.2 - 48.7 D</td>
<td>1</td>
</tr>
<tr>
<td>&gt;48.7 D</td>
<td>2</td>
</tr>
<tr>
<td>Difference of central corneal power between right and left eyes&lt; 1.9 D</td>
<td>0</td>
</tr>
<tr>
<td>&gt;1.9 D</td>
<td>1</td>
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<tr>
<td>&lt;1.4 D</td>
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<tr>
<td>1.4-1.9 D</td>
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<tr>
<td>&gt;1.9 D</td>
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<td>Diagnosis based on total scores</td>
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</tr>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Suspect</td>
<td>1-3</td>
</tr>
<tr>
<td>Early keratoconus</td>
<td>4-5</td>
</tr>
<tr>
<td>Advanced keratoconus</td>
<td>6-9</td>
</tr>
</tbody>
</table>
DISCUSSION

The corneal endothelium is a single layer of hexagonal cells on the inner surface of the cornea. The endothelial integrity and metabolic activity are essential for continuous maintenance of corneal dehydration and transparency. Endothelial alterations studies are essential in estimating the safety of surgical techniques and their outcomes (Tuft & Coster, 1990).

Endothelial injury produces corneal edema owing to loss of specialized junctions between the cells as well as reduction in the deturgescing/pumping action at the site of the injury. With reestablishment of the endothelial sheet continuity by cell spreading over an injury site, the specialized junctions and normal pumping and permeability characteristics return, with disappearance of the corneal edema. Otherwise, apart from reduced cell density, an increase in polymegethism and pleomorphism correlates with reduced ability of the endothelial cells to deturgesc the stroma (Leung, et al., 2011).

A localized endothelial cell injury initially stimulates repair by cells in the immediate vicinity of the wound. As these cells flatten and slide to cover the defect, damaged endothelial cells are desquamated into the anterior chamber. As cells more distant from the injury become involved, a density gradient is established with larger cells at the site of the injury and increasing polymegethism. If the posterior limiting lamina or the Descemet membrane is damaged at the time of surgery, the cut edges retract elastically and curl toward the stroma. A fibrin clot forms on the exposed stroma, which is then covered by migrating endothelial cells. The cells covering the stromal bed secrete a new basement membrane to replace the damaged posterior limiting lamina (Tuft and Coster, 1990).
Acute corneal hydrops was found to have occurred in 2.8% of eyes (4.5% of patients) in a series of 2723 cases of keratoconus. In postacute hydrops eyes, there was a localized area in which the endothelial cells were 7 to 10 times larger than normal, apparently being a response to rupture of the posterior limiting lamina at that site. The high incidence of acute hydrops in penetrating keratoplasty buttons may be a consequence of more advanced keratoconus cases proceeding to keratoplasty. However, this observation may also be a consequence of relative ease of ex vivo examination of buttons that enables a more thorough and systematic examination to find evidence of abnormalities (Tuft, et al., 1994).

However, by the simple expediency of producing new posterior limiting lamina in early keratoconus, there is ample opportunity for repair of microscopic posterior limiting lamina ruptures, without the actual production of clinical hydrops, giving the microscopic appearance of solid invaginations or folds in the posterior limiting lamina. That keratoconus specimens obtained at penetrating keratoplasty indicate a higher incidence of apparently previously unrecognized acute hydrops might be due to the presence of microscopic ruptures and changes that are similar to acute hydrops. These findings suggest that a significant number of keratoconus patients could have been found to have evidence of endothelial wound repair because of both acute and subacute posterior limiting lamina rupture. Microscopic ruptures of the posterior limiting lamina may only be associated with small areas of edema (hydrops) that are not detected subjectively. Under these circumstances, both patient and practitioner may remain unaware of their occurrence (McMonnies, 2014).

Until today, researches have been concentrated on the anterior part of the keratoconic cornea, investigating the epithelium, the Bowman’s layer, and the stroma. Limited attention has been paid to the endothelium in keratoconus.
Currently, little is known about endothelial cell density in keratoconus, but this information may enhance our understanding of the disease process. Furthermore, this information may lead to significant modifications in surgical treatment protocols to be implemented in keratoconus cases (e.g. penetrating keratoplasty versus anterior deep lamellar keratoplasty) (Hollingsworth, et al., 2004).

However, an assessment of endothelial cell density in keratoconus is confounded by using contact lenses. Patients with keratoconus are typically fitted with contact lenses to neutralize the corneal distortion and afford satisfactory vision. The more severe the condition, the more likely the use of contact lenses. With the exception of a few research groups, the general consensus in the literature is that in normal subjects, contact lens wear causes an apparent decrease in endothelial cell density, with no clinical significance (Sanchis-Gimeno, et al., 2003).

All cells are susceptible to mechanical trauma, which, especially in keratoconus, might be rubbing related. Compared with normal control subjects, who also routinely wore rigid contact lens, rubbing was reported to occur significantly more frequently in keratoconus patients during teenage years and as adults, after they were counseled to avoid it. Wiping, massaging, or rubbing the eye involves compressive forces being applied to the anterior corneal surface through the lids with associated intraocular pressure elevation, which can rise to many times greater than normal. Elevated intraocular pressure increases the distending forces applied to the posterior corneal surface (McMonnies, 2009).

These activities cause the cornea to be sandwiched between opposing anterior compressive and increased posterior distending forces. The associated elevated corneal hydrostatic pressure increases the risk of mechanical trauma to
cells. Elevated intraocular pressure will initially produce corneal thinning and edema is only produced if the endothelial pump is overwhelmed, possibly because of endothelial cell dysfunction resulting from the mechanical trauma (McMonnies, 2009).

The endothelium can adapt to a gradual increase in intraocular pressure, but rapid rises in intraocular pressure result in endothelial cell damage. For example, the endothelial cell loss after an acute angle-closure glaucoma attack is roughly proportional to the duration of stromal swelling during the attack. A mean cell loss after an acute glaucoma attack of 23% has been reported. In contrast, cell loss has not been consistently demonstrated in primary open-angle glaucoma. A higher and more rapid rate of elevation in intraocular pressure during an acute glaucoma attack may be a key determinant of endothelial trauma. These findings also suggest that the very high and rapid onset intraocular pressure elevation during eye wiping, massage, and rubbing, especially in the case of habitual severe knuckle rubbing in keratoconus patients, may be more likely to be associated with endothelial damage (McMonnies, 2013).

The cornea absorbs approximately 80% of incident ultraviolet-B radiation, and there is the associated potential for generating significant amounts of free radicals and reactive oxygen species. Consequently, high exposure to solar radiation challenges the cornea with an intense burden of reactive oxygen species. Prolonged exposure to broadband ultraviolet-B radiation has been shown to induce endothelial swelling, desquamation, and the development of microvilli. The amount of radiant energy incident at the posterior limiting lamina/endothelium interface is governed by absorption in the anterior and midcorneal layers. Thinning of keratoconic corneas appears likely to expose the
endothelium in the thinned cone area to greater risk of damage from exposure to solar ultraviolet (Marchitti, et al., 2011).

Thinning of keratoconic corneas may help to explain why they are found to have increased oxidative damage compared with normal corneas. Accumulation of reactive oxygen species results in the deposition of cytotoxic by-products that can damage corneal tissue. A review of the pathobiology of keratoconus postulated that underlying abnormalities in stromal repair and reactive species–linked activities, as well as the interaction between these phenomena, are implicated in the development of the ectasia. Consequently, geographic regions that provide increased exposure to solar ultraviolet radiation (increasing with temperature, hours of sunlight, lack of rain, and elevation above sea level) can be associated with increased prevalence of keratoconus independently of or in conjunction with genetic predisposition for keratoconus (Cheung, et al., 2013).

In addition to geographic location, increased exposure to ultraviolet during vocational or leisure activities may be important considerations. For example, work- or leisure-related ultraviolet exposure associated with fishing, sailing, water and snow skiing, or time spent at the beach can be very high for some people. Tanning lamps are another possible source of excess ultraviolet exposure, even with closed lids, and a rare complication of corneal perforation in an eye with keratoconus suggests that keratoconic eyes may be more susceptible to tanning lamp trauma. Evidence of apoptosis in the endothelium was found in 13 of 16 corneal buttons examined, suggesting apoptosis as a mode of cell death in keratoconus (Funnell, et al., 2006).

Apoptotic endothelial cell loss may be accelerated in some keratoconic corneas and/or accompanied by necrotic cell loss associated with ultraviolet
exposure. Contact lens materials that include ultraviolet blocking properties are protective, but corneal rigid lenses do not protect the peripheral cornea and limbus. Similarly, ultraviolet blocking spectacle lenses do not provide complete protection because radiation that does not pass through the lens may reach the cornea. Ultraviolet blocking scleral lenses provide protection for the whole cornea as well as potentially equally important protection for the limbus and, depending on diameter, the paralimbal area as well (McMonnies, 2014).

Specular microscopy is a practical tool not only for the cornea subspecialist in evaluating donor corneas and corneal dystrophies but also for the ophthalmic surgeon in identifying subtle as well as macroscopic changes in the cornea prior to surgery. A clear cornea with a normal pachymetry reading is not an assurance of a normal endothelial morphology or cell density (Tuft & Coster, 1990).

One of the methods of treating keratoconus is deep anterior lamellar keratoplasty. One of the proposed advantages of this procedure is that it preserves the host’s endothelium, which is assumed to be normal. Deep anterior lamellar keratoplasty is a successful form of transplantation with healthy endothelium (Sarnicola, et al., 2012). If corneal endothelial abnormalities are present in keratoconus patients; these may theoretically affect the long-term maintenance of corneal graft clarity after deep anterior lamellar keratoplasty. If the extent of endothelial abnormality is correlated with the grade of the disease, this may affect selection criteria for deep anterior lamellar keratoplasty. Based on this, if there is advanced endothelial abnormality, penetrating keratoplasty with high quality donor tissue might be a better alternative to deep anterior lamellar keratoplasty in these patients (Krumeich, et al., 2008).
Laing and in his co-workers, in 1979, studied the corneal endothelium in keratoconus by specular microscopy. Their study included 12 eyes. There appeared to be an increase in cellular pleomorphism of the endothelium among the corneas in this series, a finding that was particularly unusual in view of the relative youth of the study population. After clinically scanning the photomicrographs, the examiners were left with the distinct impression that there were two populations of cells, one larger than normal, the other considerably smaller than normal. The most striking was a directional enlargement of many endothelial cells. The long axis of these cells seemed oriented toward the apex of the cone and the cells themselves appeared to have been stretched by the ectatic process (laing, et al., 1979).

This observation is consistent with the current concept of acute corneal hydrops, wherein the stretching of the endothelium and Descemet's membrane ultimately results in the rupture of both structures, allowing considerable aqueous humor to enter the corneal stroma. In most cases, dehydration of the cornea ultimately occurs. It is presumed that, with time, the endothelial cells adjacent to the area of rupture enlarge, fill in the defect, and ultimately effect regeneration of Descemet's membrane. The single cornea in the series with a history of acute hydrops contained a localized area of endothelium in which the cells were seven to ten times larger than normal. In other areas of this cornea, the endothelial cells were normal both in size and in morphological appearance. This suggests that the endothelium and Descemet's membrane ruptured in the area of enlarged cells and that the dramatic increase in the size of these cells reflects the changes necessary to repair the affected site (laing, et al., 1979).

The alterations we observed appear consistent with our understanding of acute corneal hydrops. Gross observation of the specular photomicrographs disclosed that many of the endothelial cells contained a dark structure. In all
instances this was completely contained within the cell. There was a normal-appearing area between the dark structure and the cell boundary, and the latter was normal. The intracellular dark structures seemed to occur less frequently in larger endothelial cells. They are consistent in appearance with previously described blebs or vacuoles, but their role in the pathogenesis of keratoconus is completely open to speculation (laing, et al., 1979).

Similarly, Matsuda et al., (1984) compared the corneal endothelium of 21 eyes with keratoconus and 15 eyes of age-matched controls using specular microscopy. Of the 21 eyes, 15 had definite keratoconus while six apparently normal fellow eyes were designated as latent. The endothelium in keratoconus showed a significant increase in the extent of polymegathism as compared with controls. The relative frequency of hexagonal cells in keratoconus was significantly lower than that of controls. Additionally, an increase of various cell shapes was noted, indicating that there was also a significant increase in cellular pleomorphism (Matsuda, et al., 1984).

Sturbaum and Peiffer, (1993) studied the endothelium of 14 keratoconus corneal buttons obtained by penetrating keratoplasty using light microscopy, scanning electron microscopy and transmission electron microscopy. Observations were correlated with patient history. Corneas demonstrated: endothelial cell pleomorphism and polymegathism (6 corneas); endothelial cell degeneration (13), and evidence of anterior chamber inflammation (4). Patterns of endothelial damage were variable ranging from isolated cell membranolysis to denudement of Descemet’s membrane. Less damage was present at the apex of the cones than that observed in a circumferential pattern at the bases. In general the damage observed correlated with the severity and duration of the keratoconus with
9 years being the dividing time between mild and severe endothelial cell damage. These observations support other studies that implicate contact lens wear as a cause of pleomorphism and polymegathism in these patients. Endothelial cell alterations are likely a secondary event occurring due to mechanical stresses (Stubaum and Peiffer, 1993).

The earliest study in the literature on evaluation of in vivo confocal microscopy and endothelial cell density of the cornea was conducted by Holligsworth et al., who reported that endothelial cell density of central keratoconic corneas were significantly higher than that of controls (Holligsworth, et al., 2005).

In a study by Uçakhan, et al., (2006) 48 eyes of 24 consecutive patients with keratoconus (Group A) were examined by in vivo confocal microscopy. Forty-four eyes of 22 healthy subjects served as the control (Group B). According to the mean keratometric readings obtained from computerized topography, eyes with keratoconus were classified as mild (< 47 D), moderate (47–55 D), or severe (>55 D). In their study, central corneal structures could be successfully visualized in all patients. Pleomorphism and enlarged endothelial cells were seen in six (12.5%) eyes with severe keratoconus. Although the mean endothelial cell density was lower in group A than in group B, this difference did not reach clinical significance. However, when group A was broken down as mild, moderate, or severe keratoconus, in eyes with severe keratoconus, the mean endothelial cell density was statistically significantly lower than in eyes with moderate (P<0.05) or mild (P<0.05) keratoconus.
The mean endothelial cell hexagonality percentage in Uçakhan et al’s study was statistically significantly lower in group A than in group B (P<0.05). When broken down, although the mean hexagonality percentage in eyes with severe keratoconus seemed to be low, when compared to those with mild or moderate keratoconus, this difference did not reach clinical significance.

Probably because of the limited number of eyes with mild keratoconus. Considering these findings, one may propose that in keratoconus, the endothelium becomes particularly unstable and susceptible to damage in advanced stages of the disease (Uçakhan, et. al., 2006).

In a report by Weed et al. in 2007, nineteen keratoconus subjects were categorized using Orbscan-derived corneal apex power and pachymetry as exhibiting moderate (n=7) and advanced (n=12) keratoconus. Control subjects included 23 noncontact lens wearers (Group A) and 15 contact lens wearers (Group B). All subjects underwent Confoscan slit scanning in vivo confocal microscopy. The severity of keratoconus was classified on the basis of corneal thickness (< 400µm >) and apical power (< 55 D >). Group B was used only to investigate the effect of contact lens wear on normal corneal stromal keratocytes. They showed that the presence of keratoconus did not statistically affect the endothelial cell density (P=0.54) (Weed, et al., 2007)

Niederer et al., in 2008, examined 52 eyes with keratoconus and 52 age-matched control eyes with laser scanning in vivo confocal microscopy. The severity of keratoconus was classified according to the steepest simulated keratometry reading on the keratometric map (mild < 45 D; moderate, 45–52
D; Severe >52 D). They observed a small but statistically significant lower endothelial cell density in keratoconic eyes 15.2 % (P < 0.001) than in control eyes. They theorized that the distortion of corneal shape in keratoconus, with a resulting increased posterior surface area, could also explain the relative decrease in density with the original number of endothelial cells covering a greater area (Niederer, et al., 2008).

Similarly, Mocan et al., (2008) evaluated the corneas of 68 eyes with keratoconus and 22 controls using in vivo confocal microscope (Confoscan). Compared with corneas of control subjects, patients with keratoconus had a significantly lower endothelial cell density (2924 ± 300 cells/mm² vs. 2719 ± 279 cells/mm²) (p = 0.004) (Mocan, et al., 2008).

One exception is a recent study conducted on subjects with mild to moderate keratoconus, which reported no change in ECD (Yeniad, et al., 2010).

A study by Kubaloglu and co-workers showed that endothelial cell loss was higher following penetrating keratoplasty than deep anterior lamellar keratoplasty within the first four postoperative years (Kubaloglu, et al., 2012). And since authors like Krumeich and co-workers showed that penetrating keratoplasty and deep anterior lamellar keratoplasty yield similar results in terms of best corrected visual acuity, it would be more reasonable to consider deep anterior lamellar keratoplasty rather than penetrating keratoplasty in patients with a normal preoperative endothelial cell counts, to preserve the endothelium and hence the long-term stability of the graft (Krumeich, et al., 2008).

Amiri et al., in 2015 examined 26 mild and moderate keratoconic eyes (case group) with no history of contact lenses wear or eye surgeries and compared
them with 25 normal eyes (control group) that corneal power based topographic images is lower than 47.2 diopter. This comparison were done based specular microscopy images in 5 corneal regions (central, superior, inferior, nasal, temporal). Then the information related to the cell density, Coefficient of Variation of polymegathism and pleomorphism of cells were analyzed and concluded that Keratoconus does not have any considerable effect on cell density, polymegathism and pleomorphism, in mild and moderate stages and corneal opacity risk caused by intraocular surgeries (such as: Cataract or Glaucoma surgeries) and some diseases (such as diabetes and uveitis) is similar in keratoconic and normal eyes (Amiri, et al., 2015).

In a recent study done by Bitirgen, et al., on Seventy-eight eyes, they found that there is a statistically significant reduction in endothelial cell density was observed in keratoconic corneas compared with the controls. Additionally, variation in the size and the shape of the endothelial cells in eyes with keratoconus was apparent. There are conflicting results among the studies analyzing the endothelial layer of keratoconic corneas (Bitirgen, et al., 2015).

Evaluation of ECD in keratoconic corneas with in vivo confocal microscopy (IVCM) has revealed contradicting results in different trials. A significant issue at this point is the comparability of keratoconic corneal ECD values determined through different measurement protocols. Mocan, et al. recommended standardization of the methods used in the cellular assessment of the cornea. Uniform (standardized) methods are essential in the assessment of parameters for comparable trial outcomes. Discrepancies in inclusion/exclusion criteria, measurement devices or techniques in different trials will preclude a comparison of the trial outcomes (Mocan, et al., 2008).
The ideal solution is to apply strict inclusion and exclusion criteria during patient selection, and to implement a uniform experimental methodology in image acquisition and cell density calculation to assess the possible presence of cell loss associated only with keratoconus. The following inclusion criteria in the cellular assessment of keratoconic corneas with IVCM have to be proposed: strict selection of corneas with no current or previous history of contact lens use, selection of one eye per patient, utilization of a single confocal microscopic technique (scanning slit or laser scanning), selection of the area to be imaged (central or apical cornea), selection of a uniform counting technique (automated or manual) for quantitative cell analysis, and enrollment of similar age groups (Timucin, et al., 2013).

Stromal hypoxia, hypercapnia, and thinning are well known to be associated with contact lenses with low O2 permeability. There is a cause-and-effect relationship between contact lenses and changes in the corneal endothelium reflected by an increased variation of endothelial cell size, shape and density. Long-term wear of rigid or soft contact lenses with limited oxygen permeability seems to contribute to corneal endothelial changes. The mechanisms proposed to explain contact lens-induced endothelial changes include lactate accumulation, changes in pH, increase in CO2 content, decrease in endothelial adenosine triphosphate levels, and disturbed calcium homeostasis (Liesegang, 2002).

In addition, when compared with healthy corneas, keratoconic corneas are more susceptible to contact lens-related mechanical trauma due to apical protrusion. Chronic mechanical injury of the corneal epithelium is known to increase the release of cytokines, such as IL-1, and to accelerate apoptotic changes in the cornea. The IL-1 protein has been found to increase also in the endothelial layer of keratoconic corneas. It has been suggested that wearing of CLs in patients with keratoconus contributes to mechanical trauma, which stimulates the release of
apoptotic cytokines. Perhaps the combination of mechanical trauma with CLs with low oxygen permeability has contributed to the decrease in ECD. These data may explain the apoptotic process in the endothelial cell layer in corneas with keratoconus and thus, the decrease in the ECD (Wilson, et al., 2001).

In several studies, endothelial cell density was investigated in patients with keratoconus. In most of these studies, current or previous use of contact lenses (CL) was not indicated as an exclusion criterion. Hence, no details with regard to the use of CLs are available in the majority of these studies, whereas the presence of CL is a significant factor that may affect the assessment of ECD. Patients with keratoconus use rigid CLs in order to neutralize the corneal distortion and to provide satisfactory vision. As the severity of the disease increases, the need and probability of using rigid CLs increases. The alteration in the endothelial cell count among patients with keratoconus may be due to previous or current use of CLs, or due to progressive disease. Thus, determination of whether the change in ECD associated with keratoconus is due to the impact of lens wearing, to lens-wearing history, to the direct pathological effects of keratoconus, or possibly due to both factors, is essential (Timucin, et al., 2013).

A decrease in endothelial cell density may also be anticipated in keratoconic corneas with no CL use. Increased oxidative stress and increased lysosomal enzyme activity have been shown in corneas with keratoconus. Zhou, et al., in 1996 showed that IL-1 protein is increased in the epithelium and endothelium of corneas with keratoconus. Wilson et al. in 2001 found that apoptotic cytokines involved in keratocyte apoptosis following epithelial injury were also expressed in the corneal endothelium. Therefore, it has been suggested that the tissue degradation processes may be abnormal in keratoconus. Endothelial cell loss may also develop as a result of tears in the Descemet’s membrane. These
data may explain the apoptotic or degenerative cell death in the endothelial cell layer in corneas with keratoconus and thus, the decrease in the ECD. Considering these findings, one may propose that in keratoconus, the endothelium is particularly unstable and susceptible to damage, regardless of contact lens use (Ucakhan, et al., 2006) (Mocan, et al., 2008).

A decrease in ECD has also been reported in severe keratoconus cases, while there is no history of acute hydrops or use of CL. At this point, one may postulate that the endothelium becomes particularly unstable and susceptible to damage in advanced stages of the disease, regardless of a history of acute hydrops, Descemet’s tears or contact lens wear (Timucin, et al., 2013).
SUMMARY AND CONCLUSION

Keratoconus is a progressive, ectatic corneal disorder, characterized by protrusion of the cornea and thinning of the corneal stroma, causing astigmatism.

The etiology of keratoconus is still largely unknown, although many biochemical and pathological changes at the structural and cellular level of the corneal abnormalities have been suggested.

Patients with keratoconus often complain of decrease in visual acuity which can be mild or severe depending on the degree of corneal tissue affection. Keratoconus can be classified according to the severity of the clinical and topographic signs into mild, moderate and advanced.

The clinical manifestations of keratoconus include steepening of the cornea, especially inferiorly, thinning of the corneal apex, corneal scarring, Vogt’s striae and Fleischer's ring. In advanced keratoconus, two findings are associated with keratoconus diagnosis; Munson's sign and corneal hydrops.

Early in the disorder the astigmatism can be corrected by glasses. With the progression of the protrusion, the astigmatism needs hard contact lenses or even keratoplasty in advanced cases.

The keratometer aids in the diagnosis of keratoconus. The initial keratometric sign of keratoconus is absence of parallelism and inclination of the mires. The photokeratoscope or placido disc can provide an overview of the cornea and can show the relative steepness of any corneal area. The even separation of the
rings in the spherical and the astigmatic cornea and the uneven spacing of the rings especially inferiorly in the keratoconic cornea should be noted.

Ultra-sonic Pachymetry is a technique for measuring corneal thickness that aids in diagnosis and evaluation of the stages of keratoconus.

Keratoconus is more accurately distinguished from the normal population by videokeratography. Videokeratoscopy is used clinically to demonstrate the topography changes in keratoconus distinguishing the different stages of keratoconus. Confocal microscopy and specular microscopy allow the visualization of more details in the corneal layers.

The aim of this work was to study the corneal endothelial integrity, count and morphology in keratoconus patients, and to correlate the endothelial changes to the stages of keratoconus.

The critically important functions of the endothelium appear to require its careful assessment in KC corneas. Apparently, as a consequence of problems with different methods of examination used for endothelial assessment, current evidence of changes that have been detected in KC corneas is contradictory. Observations cited in this review raise the possibility that guttae, polymegethism, and pleomorphism could be significant findings in KC, perhaps especially for the cone area and particularly in any location of episodes of acute or subacute hydrops. Any heterogeneity of the endothelium in a KC cornea may not be fully characterized unless observations are made from multiple areas including some in the cone region. Contact lens intolerance in KC may be associated with endothelial changes such as reduced cell density, guttae, polymegethism, and pleomorphism.
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المملوء
المقدمة

القرنية المخروطية هي اعتلال للقرنية يتميز ببروز القرنية، مع ترقق في سدي القرنية، يؤدي إلى حدوث لا نظفية (استيجماتيزم)، وتكون النقطة في بداية المرض منتظمة وقابلة للتصحيح بنظافة طبية، ومع ازدياد البروز، تحول إلى لا نقطة غير منتظمة غير قابلة للاصلاح بنظارة طبية، وتحتاج إلى عدسة لاصقة صلبة، أو حتى زراعة قرنية جديدة في الحالات الشديدة.

أسباب القرنية المخروطية غير معروفة إلى الآن، وترجح النظريات أن السبب هو خلل في الخصائص الفيزيائية والكيميائية لبروتين الكولاجين الذي يكون النسيج القرني.

يشتكي مريض القرنية المخروطية عادة من ضعف الإبصار والذي يمكن أن يكون بسيطا أو شديد، وذلك يعتمد على مدى تأثر القرنية وتنقسم القرنية المخروطية حسب شدة إلى بسيطة وعالية شديدة.

الأعراض الإكلينيكية للقرنية المخروطية عديدة، وتتضمن زيادة تكورية خصوصا من أسفل وتوقف قمة القرنية وحدود ندبة في القرنية وخطوط توتر عميق، والتي تتنبأ عندما يتم الضغط على الأذن أثناء الفحص الإكلينيكي بالمصاب الشقى، وقد تترسب حقبة من الحديد تدعى حقبة فلا يشير من النسيج الطلائي بقاعدة القمع.

يستخدم جهاز مقياس منحنينات القرنية (الكيراتومتر) لقياس درجة تكور القرنية وتحديد مستوى تحدب القرنية المخروطية، كما يستخدم المكشاف القرني (الكيراتاسكوب) لقياس درجة تقؤر القرنية بحالة ضيق الحلقات الساقطة من الجهاز على المنطقة المركزية للسطح الامامي للقرنية. كما يستخدم جهاز (الباكاميتر) لقياس سمك وتضاريس القرنية.

يُعتبر جهاز تحليل تضاريس القرنية بالكمبيوتر اهم الأجهزة لرسم خرائط ملونة ومرونة للقرنية ثلاثية الأبعاد يتم تحليلا من خلال فيديو مكشاف القرنية (فيديوكيراتاسكوب)، ومن خلال هذا الجهاز يمكن تصنيف التغييرات التضاريسية للقرنية المخروطية على حسب مراحلها ابتداءا من الشكل الطيفي مرورا بالنوع المتوسط وانتهاءا بالشكل المتقدم للقرنية المخروطية.

ويستخدم الفحص المجهرى البراق والمجهرى البؤري لرؤية التفاصيل في طبقات القرنية المختلفة.
الهدف من هذا البحث هو دراسة صلاة وعدد وشكل خلايا بطاقة القرنية في مرضى القرنية المخروطية والربط بين التغييرات في بطاقة القرنية ومراحل القرنية المخروطية.
التغيرات في بطاقة القرية في مرضى القرية المخروطية

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كلية الطب جامعة بىها

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