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Research Article

Collagen Cross Linking for Keratoconus

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Introduction

Keratoconus is an Eye condition in which cornea gets thinner, conical and gradually bulging outwards into conical shape. This can cause blurred vision and discomfort in light. It is one of the most common corneal ectasia, characterized by progressive, non-inflammatory changes in corneal stromal collagen and manifests as the protrusion and alteration of the central and paracentral cornea **(**1**)**. The progression of the disease usually increasing in the first three decades of life and if not treated on time can result in a decreased visual acuity. The Keratoconus management is decided by many factors such as age, stage of keratoconus, corneal topography and visual acuity. There are many management options available include spectacles, Hard contact lenses, corneal collagen cross-linking with riboflavin, Intacs, penetrating keratoplasty, and deep anterior lamellar keratoplasty. Cross Linking is a method of making the corneal tissue strong by using riboflavin and ultraviolet-A light to stop the progression of keratoconus. In Cross Linking, riboflavin dye act as a photosensitizing agent and UVA light improves the formation of intra and interfibrillar covalent bonds by oxidative photosensitization. This procedure explained the roll of collagen cross-linking in managing keratoconus.

Keratoconus has variable prevalence in the Middle East. Keratoconus can be high as five percent in gulf region who has refractive error. Rubbing, environmental and genetic factors can be responsible for the keratoconus.

The etiology of this condition is unknown. However, there are several ocular and systemic association exist like connective tissue disorders of Ehlers-Danlos and Marfan syndromes, Leber congenital amaurosis, atopy and Down syndrome. It can usually occur in the second or third decade of life with progressive myopia and astigmatism. It can be unilateral or bilateral. It is initially unilateral and latter both eyes are involved **(**2**)**.

On examination, there are several clinical signs. Munson’s sign is a V shape bulging of the lower eyelid on downgaze. A slit-lamp examination can show Vogt striae: fine, vertical, stromal stress lines, and a Fleischer ring: a ring-like configuration of epithelial iron deposits. Distant direct ophthalmoscopy reveals a characteristic “oil-droplet” reflex, and retinoscopy shows an irregular scissor reflex.

Corneal topography and optical coherence tomography shows minor changes in corneal topography and tomography. Amsler-Krumeich system is known classification system which uses the patient’s refractive error, central keratometry readings, central corneal thickness and the presence or absence of scarring. Amsler-Krumeich system does not utilize corneal topographic reading. Various topographic indices have

been proposed for diagnosing forme fruste keratoconus / preclinical and clinical KC. Rabinowitz suggests the following topographical characteristics of KC: increased areas of keratometric readings surrounded by areas of reduced corneal power, inferior-superior symmetry and skewed radial axes (3). Pentacam system (Oculus GmbH, Wetzlar, Germany) utilizes the Belin/Ambrósio Enhanced Ectasia Display (BAD) to screen for KC using maximal keratometry, anterior and posterior elevation, and tomographic thickness data (4).

Treatment of early keratoconus is spectacles to improve vision, but as the disease progresses, rigid gas-permeable contact lenses are required. Advanced keratoconus may need eventual corneal transplantation. Collagen cross linking with ultraviolet A (UV-A) light and riboflavin (vitamin B2) is a relatively new treatment that slows the advancement of the disease in its early stages (5).

Corneal cross linking was invented in the 1990s. Corneal cross linking utilizes riboflavin as a photosensitizer which is exposed to UV-A light to induce chemical reactions in the corneal stroma which finally results in the formation of covalent bonds between the collagen molecules. Collagen cross linking increases the tensile strength and rigidity of the cornea to prevent further thinning of the cornea.

Objectives

- Explain the common clinical and topographic features of keratoconus.
- Explain the mechanism of action of collagen cross linking.
- Indication of the collagen crosslinking procedure.
- Review the results of procedure.

Anatomy

The cornea is densely placed, multi-scale structures of fibers arranged in various layers. Each layer is composed of distinct components having variations in collagen density, elastin, fibronectin, laminin, and proteoglycan (6). These components make mechanical strengths due to different mechanical properties. The cornea is made of six layers and is approximately 550 um thick. Eighty percent light in the visible spectrum can be passed through the healthy cornea (7).

The cornea is made of densely packed proteins called collagen. The collagen of cornea remains for approximately 2-3 years. The corneal transparency is maintained by the regular packing of proteins with a proteoglycan-rich matrix, allowing the light to pass unimpeded **(**8**)**. Collagen crosslinking forms the bonds between the polymeric chains of proteins. In biological tissue like cornea, these bonds strengthen the tissue, making them more resistant to mechanical degradation and deformation **(**9**)**.

The tear film is made of lipid, aqueous and the muicin layer which is 3 um thick in healthy subjects. It covers the anterior surface of cornea. The tear film provides the lubrication and protection through soluble factors and maintains a smooth optical surface **(**10**)**. The First layer is the epithelial layer which is drived from surface ectoderm. It is a stratified, squamous and non keratinized epithelium and is made off single layer of basal columnar cells attached by a hemidesmosome to the underlying basement membrane, two to three layers of wing cells and two to three layers of squamous surface cells. The surface area of squamous surface layer is increased by microvilli which help in attachment of the mucin. The surface cells are shed in to the tear film after few days. This is followed by Bowman's layer, an acellular superficial layer of stroma which scars when damaged. It is measuring 17 um and made up of randomly distributed collagen fibers **(**11**)**. Below Bowman's layer is the stroma, which makes ninty percent of corneal tissue. The stroma is 500 um thick which can vary between individuals. The stromal is made up of densely packed collagen fibrils which are organized into lamella and these run approximately parallel to the corneal surface.

The spacing between the lamella and collagen fibril are necessary for corneal transparency and is maintained by the proteoglycans ground substance with interspersed keratocyte. The Dua's or Pre-Descemet's layer is nearly identical to adjacent stromal tissue. There is a difference in proteoglycan distribution due to the higher density of lamella and more spacing between the collagen fibrils **(**12**)**. The next is the layer formed of endothelial cells which is 3 um thick.

Physiology

Epithelium has ability to regenerate so that does not scar. It makes first protective layer of cornea. Epithelial cells have microvilli which help in attachment of mucin. The epithelial stem cells are located at the superior and inferior limbus and are responsible for healthy corneal epithelium. It also acts as a junctional barrier in preventing conjunctival tissue growth on to the cornea. The deficiency of the stem cells may result in epithelial defect and over growth of conjunctival epithelium on the corneal surface and vascularisation.

Stroma makes a major part of corneal thickness and provides the mechanical strength to the cornea.

The endothelial pump has a vital role in maintaining dehydration of the cornea which is mandatory in maintaining the transparency of cornea. It can not be regenerate. As the age is increased the number of endothelial cells is decreased and therefore the neighboring cells enlarge to fill the space.

Indications

Keratoconus, with evidence of progression, is the most common indication for Corneal Cross Linking. Other indications include pellucid marginal degeneration, Terrien marginal degeneration, and post-refractive surgery **(**such as LASIK, PRK, or radial keratotomy**)** ectasia. Many studies have their criteria based on an increase in Kmax value, myopia and/or astigmatism, mean central K-readings, and a decrease in mean central corneal thickness **(**13**)**.

Every keratoconus does not need Corneal cross linking. Stable keratoconus does not need corneal cross linking. Keratoconus usually does not need Corneal cross linking after 3rd decade because cornea becomes hard due to age related changes. Unstable keratoconus only need Corneal cross linking. Corneal cross linking is needed usually before the age of 30 years. Both spectacle corrections and rigid contact lenses form the part of conservative therapy.

Contraindications

Corneal thickness less than 400 microns is considered a contraindication for Corneal Cross Linking, but hypo osmolar Corneal Cross Linking can be performed when corneal thickness varies from 370 to 400 microns. Other contraindications are concurrent ocular infection, presence of corneal opacity / scars, neurotrophic keratitis, severe dry eyes, history of poor epithelial wound healing, autoimmune disorders, pregnancy and prior herpatic ocular infection. There have been reports of herpetic keratitis after Corneal cross linking **(**14**)**. Kymionis et al. reported a case of a young woman who underwent Corneal cross linking and five days later presented with geographic keratitis and anterior uveitis. Similarly Qarni and Harbi reported two cases who underwent Corneal cross linking and later presented with dendritic keratitis in the early postoperative period. Viral keratitis can also develop without prior history of keratitis **(**15**)**.

Equipment

The various equipment and instruments are needed to perform Corneal cross linking:-

1. Diluted absolute alcohol
2. Calliper
3. Ink pen marker
4. Hockey stick blade
5. Crescent blade
6. Riboflavin
7. Ultraviolet light
8. Conjunctival forceps
9. Portable slit lamp
10. Portable Pachymetery
11. Normal saline
12. In the case of accelerated Corneal cross linking, an accelerated Corneal cross linking is needed **(**16**)**.
13. Saline cannula

Team Work

A combined effort of a team of Eye surgeons, an assistant OT nurse, mid-level ophthalmic personnel **(**MLOP**)** and a good counselor is needed. The surgeon performs the surgery, the assistant nurse helps in patient preparation and provides instruments for the surgery, the MLOP helps in patient movement, and the counselor helps in guiding the patient pre-operative and postoperative care **(**17**)**.

Technique:-

Epi-OFF Collagen cross linking

This is known as conventional Corneal cross linking. It is a standard protocol which is follow at majority of eye centers. This technique is named Dresden protocol because it was initially designed at the University of Dresden **(**Germany**)**. This procedure involves the removal of central 8-9 mm epithelium followed by applying 0.1% riboflavin solution followed by 30 minutes of UVA radiation of wavelength 370nm and power 3mW/cm2. Clinical outcomes **(**6-26 months**)** of this technique have been reported very favorable. The Dresden group in 2015 published their 10-year follow-up results of 34 eyes, demonstrating long-term stability and a good safety profile **(**18**) (**19**)**.

Thinner corneal endothelium is more susceptible to radiation damage so that are unsuitable for epi-off Corneal cross linking. The procedure can be done in thin cornea by providing the protection to the endothelium by either retaining the epithelium or using hypo-osmolar riboflavin to increase stromal thickness during the radiation exposure.

Many Modifications were done in dresdon protocol but are not superior than conventional method.

Epi-On Collagen Cross Linking

Corneal epithelium is not removed in this procedure. This technique has given the advantage of no risk for thin cornea, postoperative pain, and persistent epithelial defect. The problem of riboflavin diffusion through the tight epithelium junction has been solved by adding chemicals that cause epithelial disruption, such as surfactant benzalkonium chloride **(**BAC**)** or ethylenediaminetetraacetic acid **(**EDTA**) (**20**)**.

Wollensak and Iomdina conducted a study using iso-osmolar riboflavin **(**20% dextran**)** and BAC which resulted in biochemical and biomechanical changes after cross-linking in comparison to epi-off. Studies have shown that biomechanical stiffening in epi-on corneal cross linking is approximately one-fifth of that induced in epi-off corneal cross linking. Another study of porcine eyes comparing epi-off corneal cross linking versus epi-on corneal cross linking showed that epi-on was 70 % less effective. Although few studies have shown promising results but the efficacy of epi-on is still controversial and may need retreatment in unstable keratoconus.

Accelerated Collagen Cross Linking

Accelerated Corneal cross linking was invented to shorten the procedure length and reduce the exposure time of the cornea to sources of infection. This idea works on the Bunsen-Roscoe law of photochemical reciprocity. The same photochemical effect is achieved with a shorter time by increase in irradiation intensity. The irradiation time is shortened from 30 minutes to 3 minutes with comparable results to the standard epi-off corneal cross linking and may be safe to use in thin corneas. Therefore it is proposed that a higher intensity will be needed with reduced exposure time to achieve the same total energy. In a landmark study by Wernii et al., they studied the result of irradiance between 3 and 90 mW/cm2. They show that radiation intensity above 50 mW/cm2 with a time of fewer than 2 minutes failed to increase the corneal stiffness. It is found that Ultra violet is highly toxic to endothelial cells and has cytotoxic activity. Cytotoxic levels of Ultra violet light have been described as approximately 0.35 mW/cm2, which is twice that of protocol **(**0.18 mW/cm2**)**. Riboflavin helps in reducing the toxic effect of UVA and increases corneal stiffness **(**21**)**.

A cut-off of 400 microns is taken to avoid damaging the endothelium and very rarely endothelial damage can occur even above 400 microns. In a comparative study of four Corneal cross linking protocols by Shetty et al. In steeper corneas, they found that corneas treated with Dresden Corneal cross linking protocol **(**3 mW/cm2 for 30 minutes**)** showed a more significant flattening effect compared to accelerated Corneal cross linking protocol of 9, 18, and 30 mW/cm2. In a study by Kanellopoulos of 21 patients treated with accelerated Corneal cross linking protocol in one eye and Dresden Corneal cross linking in the other, they found equal results in both eyes with no progression of keratoconus, endothelial damage, and there was good improvement in keratometry and visual acuity **(**22**)**.

It is also known that a minimum oxygen concentration is required in the stroma for Corneal cross linking to occur when applying UVA light in the presence of riboflavin. Moreover considering the theoretical model of photochemical kinetics of Corneal cross linking, the UVA radiations produce a reduction in the available oxygen in the riboflavin-applied cornea leading to a secondary reduction in reactive oxygen species. When Ultra violate light is turned off, the oxygen level is returned to the original level from the environment within 3-4 minutes. Another alternative to increase the level of oxygen across the corneal stroma is to pause the Ultra violate light during the cross-linking process to allow reoxygenation during the pause in exposure.

In accelerated corneal cross linking, riboflavin induction time 10 minutes, total treatment time 8 minutes pulse mode **(** 1 minute on and one 1 minute off**)** ulta violate irradiation time 4 minute, UV power 30

mW/cm2 and total energy of 7.20 J/cm2 showed safe and effective procedure in halting the progression of keratoconus.

Iontophoresis

Iontophoresis Cross linking which facilitates the penetration of riboflavin through the cornea through a low-intensity electrical current is under trial. This procedure shortens riboflavin penetration time and duration of irradiation and does not require epithelium removal. There are, as yet, no long-term published studies comparing this to conventional Corneal cross linking and the results of short-term follow-up show that it may also be inferior to conventional Corneal cross linking **(**23**)**.

Collagen Cross Linking Plus

Corneal cross linking combining with other procedure was initially invented by Kymionis et al. in 2011, known as Corneal cross linking Plus.' The technique involved doing Photorefractive keratectomy in Keratoconus patients to regularize the astigmatic corneal surface followed by Corneal cross linking to strengthen the corneal biomechanically, thus giving the additional benefit of improvement in visual acuity which Corneal cross linking alone cannot offer. Intracorneal ring segments **(**ICRS**)** have proven helpful in Keratoconus or post-LASIK ectasia patients for regularising corneal astigmatism, but they cannot stop the progression of the disease. According to a few studies combining Corneal cross linking with ICRS does offer the combined benefit of improved visual acuity and strengthened cornea biomechanically.

Complications

Common complications are temporary corneal haze **(**10 to 90%**)**, delayed epithelial closure, sterile infiltrates, and central stromal scars. The literature describes postoperative microbial keratitis from herpetic, bacterial, fungal and protozoal sources. The stromal haze is usually temporary and occurs due to increased edema and keratocyte activation and occurs three to six months post-operatively **(**24**) (**25**)**.

Rarer complications are corneal melts, endothelial failure and Treatment failure. Treatment failure defined as the progression of the condition with an increase in Kmax values of 1.0 D over the pre-operative value or greater than a 10% decrease in pachymetry readings six months post-operatively; this may occur in up to 10% of patients. The corneal melt and endothelial failure are serious complications.

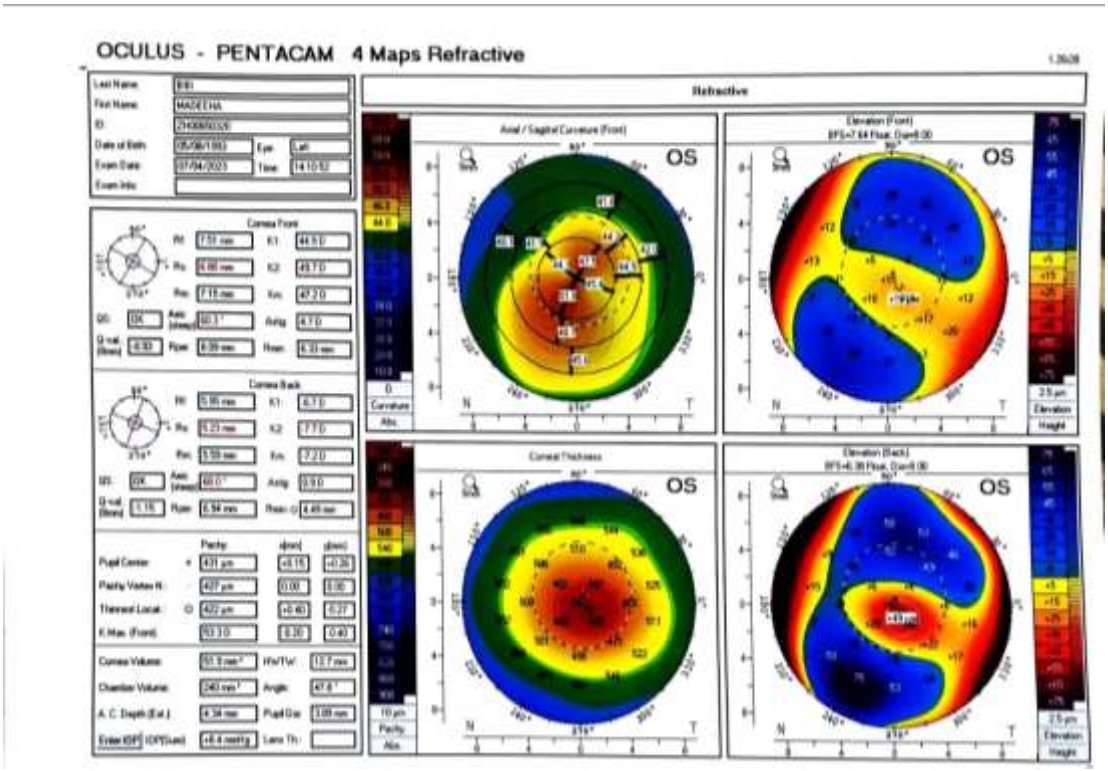
Clinical Significance

Conventional Corneal cross linking has proven a safe and effective means of halting the progression of corneal ectasias and reducing the need for more invasive treatment options. Modified Corneal cross linking techniques had mixed results related to efficacy when compared to conventional Corneal cross linking and long-term follow-up results are still needed. In addition to treating ectasia Corneal cross linking has increasing utilization as a treatment for microbial keratitis and reduction of low myopia and further research into these domains is still underway **(**26**) (**27**)**.

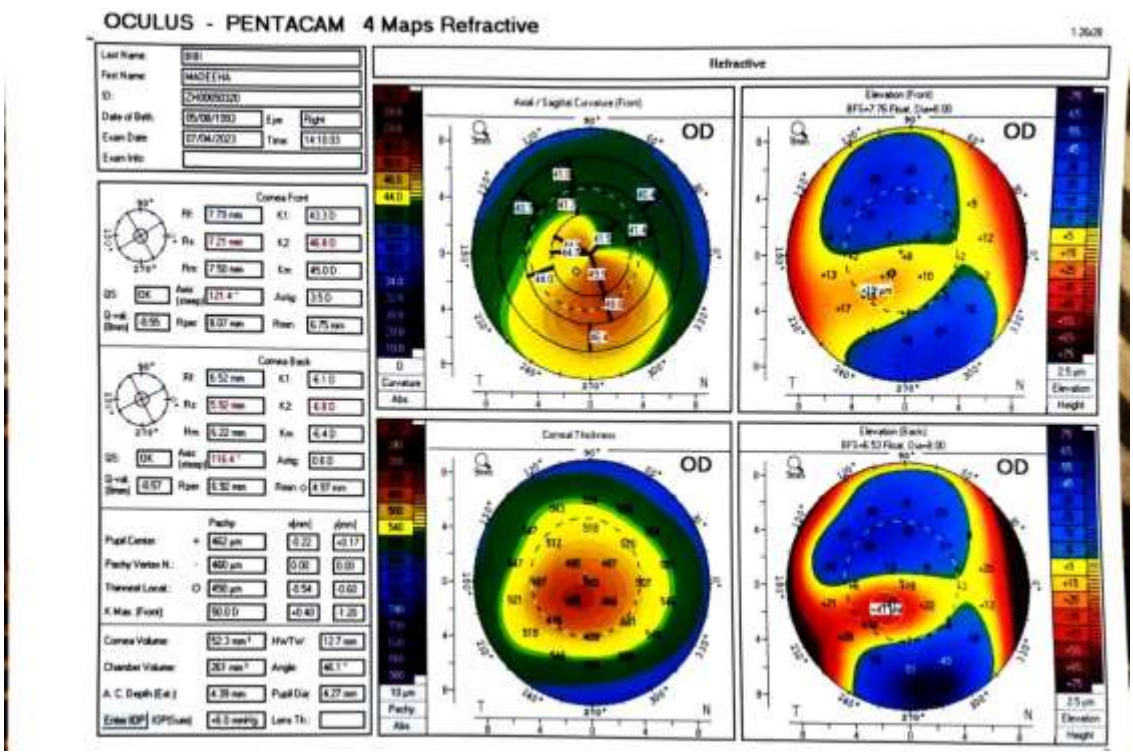
The number of Corneal cross linking procedures performed in the last ten years has increased drastically. The majority of patients are seen in the first two decades of life. Therefore it is very important to select these patients early in the course of the disease, document the progression, and cross-link these eyes to prevent irreversible visual loss. There are treatment options for thin corneas of less than 400 um and vision can be stabilized in most cases.

Good Team Outcomes

Good team approach including subspecialty-trained physicians and ophthalmic-trained nurses providing patient support and follow-up care will lead to the best outcomes. With early detection of keratoconus by healthcare members, corneal crosslinking is an effective way of slowing down and potentially halting progression **(**28**)**.

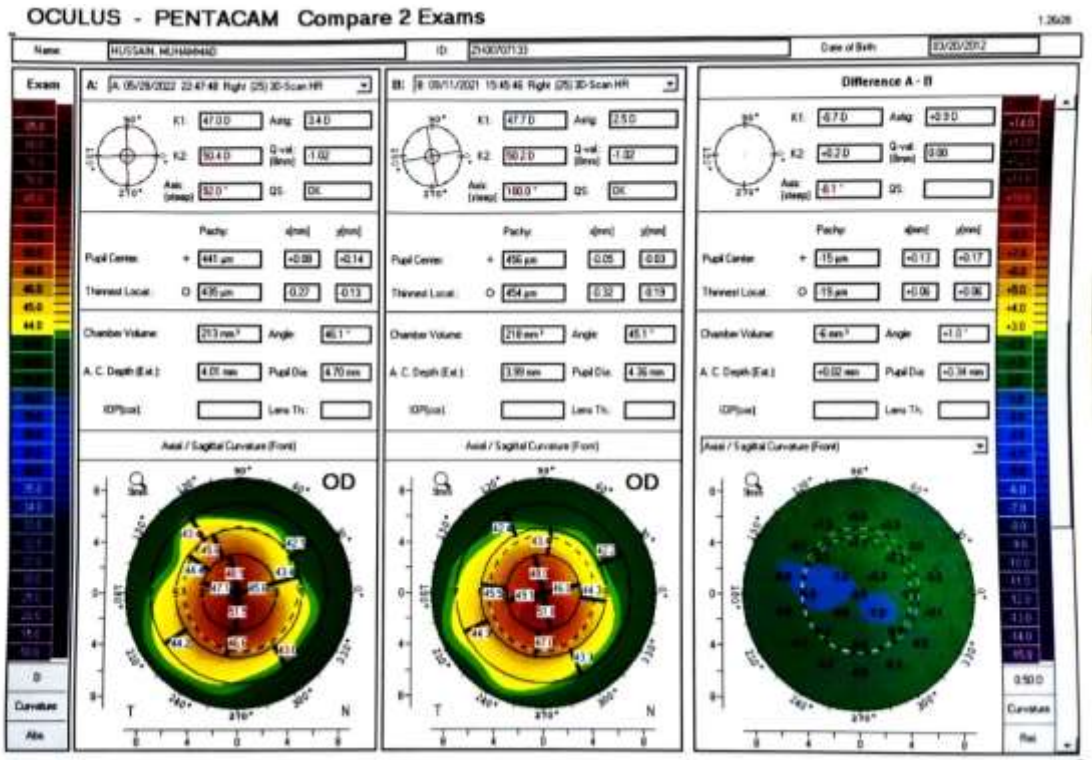


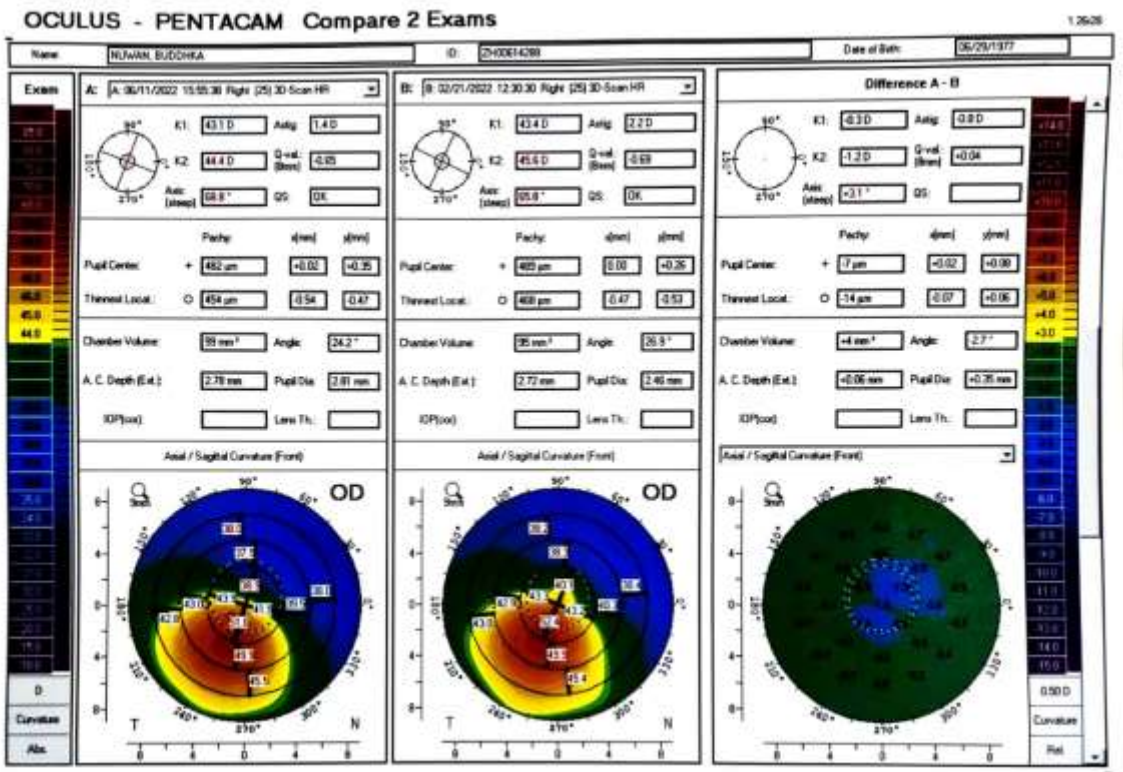
Corneal Topography Report Of Keratoconus

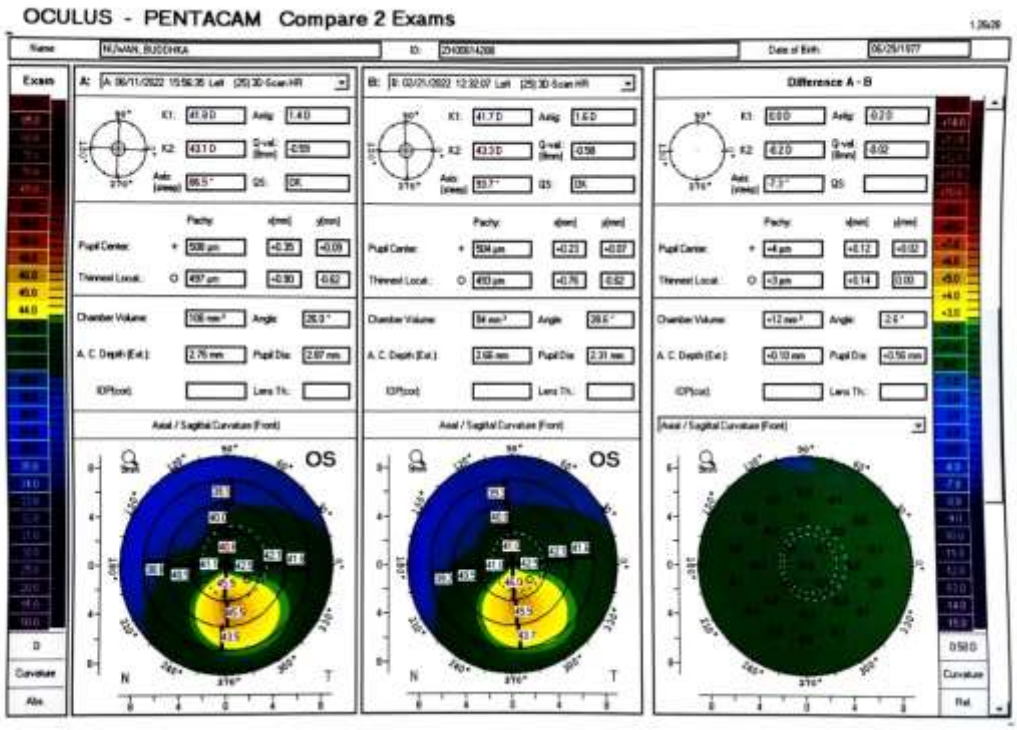


Corneal Topography Report of Keratoconus

Stablized Corneal Topography Report Post Corneal Cross Linking



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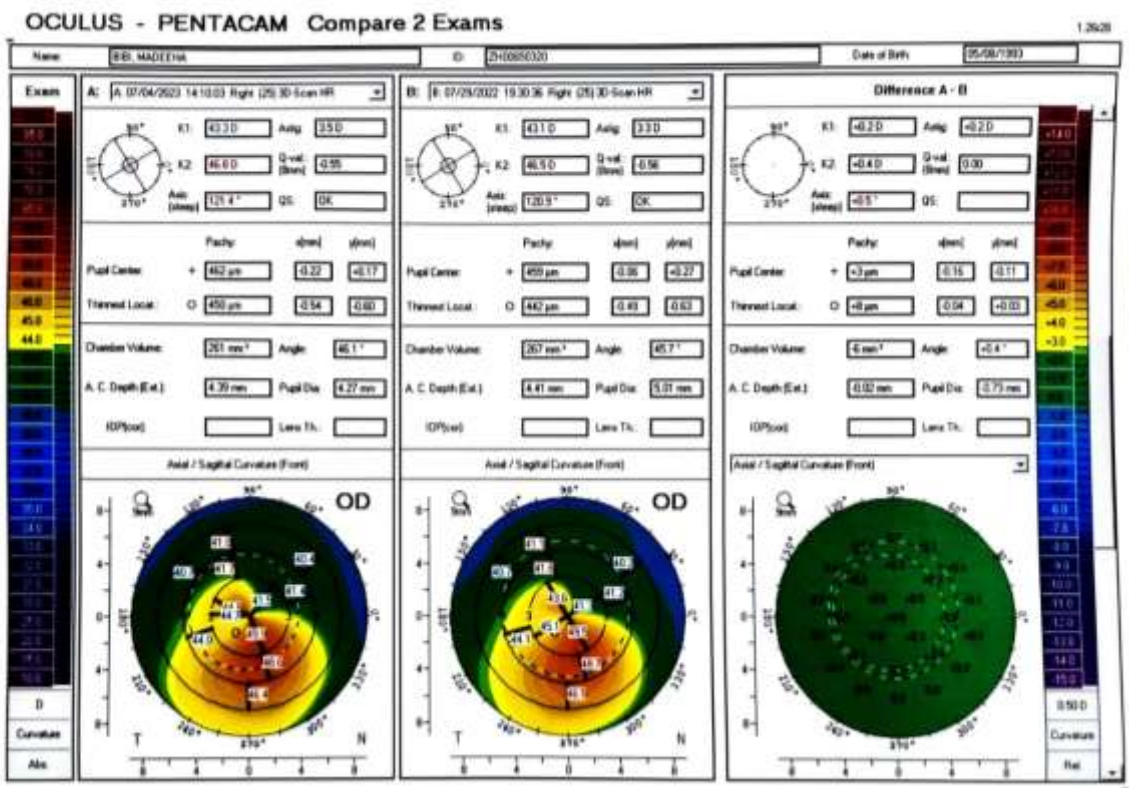
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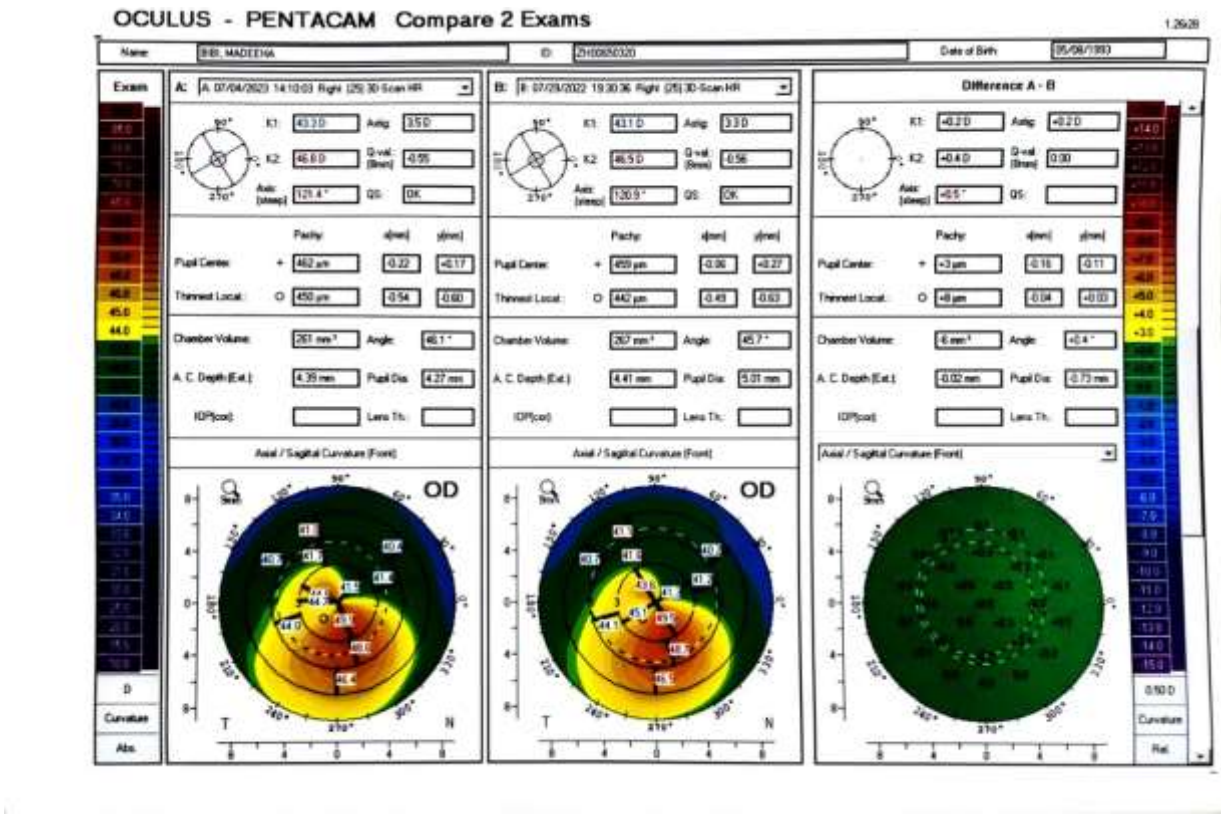

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