Biochemical and molecular alterations and potential clinical applications of biomarkers in keratoconus

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Abstract:

Keratoconus (KC) is a complex multifactorial corneal ectatic disorder, with disease onset commonly in the second-third decades significantly affecting quantity, quality of vision, and quality of life. Several pathways and factors such as eye rubbing, inflammatory, oxidative, metabolic, genetic, and hormonal among others have been studied in the last two decades. However, the management of KC is still based on a few "one-size fits all" approaches and is predominantly guided by topo/tomographic parameters. Consideration of the several novel factors which have the potential to be biomarkers in addressing several unanswered questions in the disease process could help in the better predictive ability of progression or vision loss and customization of treatment options. This article delves into the understanding of these novel factors or biomarkers based on the pathogenesis of KC and features a special focus on their potential clinical applications and their future role in personalized medicine.

Keywords:

Biochemical markers, cyclosporine, inflammation, keratoconus, personalized medicine

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Introduction

Keratoconus (KC) is a corneal ectatic disorder characterized by central and/or inferior corneal thinning and steepening, which causes corneal topographic, tomographic, and aberrometric irregularities and poor quantity and quality of vision.[1] KC can lead to significant emotional and functional impairment and affect the overall quality of life of patients. It usually presents in the second to third decades and is bilateral and asymmetric, but it can be rarely unilateral as well. The prevalence across the world varies between 0.3 and 4790 cases per 100,000 with an average global prevalence of 1.38 cases per 1000 population.[2,3] In the Unites States, prevalence varies between 0.15% and 0.51% across the different states.[4] There is a significant geographic variation, with the highest prevalence in South Asia and the Middle East.[3]

While glasses and contact lenses are the only requirements in most of the early and stable forms of KC, the more common progressive form can lead to rapidly progressive vision loss, corneal hydrops, and scarring. Corneal collagen cross-linking (CXL) is the only proven method to arrest the progression of KC.[5] CXL works by creating chemical bonds between collagen fibrils of the cornea by generating free radicals.[6] While CXL has decreased the requirement of invasive keratoplasty compared to the pre-CXL era,[7,8] it has its limitations. CXL as a stand-alone technique in most cases does not lead to a significant visual gain and requires additional interventions. This could be in the form of glasses, scleral lenses,[9] intracorneal ring segments,[10] topography-guided laser treatments,[11] or implantable Collamer phakic lenses.[12] In pediatric KC eyes, up to 25% failure rates have been described in up to 5 years of follow-up following CXL.[13] Although CXL failure in adults is not as high as in pediatric cases, it is not uncommon.[14]

Although KC was labeled as a degenerative process for a long period of time,[15] there is growing evidence in the last decade pointing toward the involvement of more complex pathways and biological factors, including inflammatory,[16,17] metabolic,[18] oxidative,[19] genetic,[20,21] and hormonal,[22] among others.[23]

Improvement in techniques such as tear sampling, impression cytology, and analysis of the corneal epithelium and stroma using the growing capabilities in the field of proteomics, metabolomics, transcriptomics, and sequencing has made the above discoveries possible.[24]

Personalized medicine is a growing concept where every patient can have access to customized solutions for his/her disease process. In this upcoming era of personalized medicine, there are several unanswered questions pertaining to every single stage of KC. Identifying those who are likely to develop KC, those likely to progress, and those likely to get better/poor visual improvement after CXL or failure post CXL or develop significant haze are some of the several unanswered questions. There is also considerable scope for the development of customized management strategies for ocular surface inflammation, allergy, and dry eye disease in KC patients. Although topo-tomographic and biomechanics research has been trying to answer some of these questions,[14,25,26] inclusion of several novel biochemical and molecular signatures could help in increasing the predictive ability and in unraveling newer diagnostic and customized therapeutic options in the management of KC.

The Enigma in the Pathophysiology of Keratoconus

The macro- and ultrastructural changes in all involved layers from the epithelium to the basement membrane, Bowman layer, and the stroma that lead to the typical thinning and steepening of the cornea along with biomechanical weakening have been well-documented.[27] The epithelium is shown to have a typical pattern of thinning over the cone region,[28] and this has been utilized in newer customized laser treatments to improve visual outcomes in patients.[29,30] In the epithelial basement membrane, the expression of extracellular matrix (ECM) materials such as laminin, fibrin, and collagen is altered.[27] Breaks in the Bowman layer along with fibrotic changes have been documented using ultra high-resolution anterior segment optical coherence tomography (OCT) imaging and immunohistochemistry.[31,32] These Bowman layer discontinuities likely play a role in the cross-over of inflammatory mediators from the epithelium to the stroma. In the stroma, the keratocyte numbers, collagen fiber density, diameter of fibrils, alignment, and orientation are shown to be affected.[27,33] This can be studied using *in vitro* histopathological analysis, as well as the novel *in vivo* imaging technique called polarization-sensitive OCT (PSOCT).[34] While the exact order in which the above ultrastructural changes take place in the pathogenetic process of KC is unclear, the changes in the collagen density and orientation are likely to occur before the biomechanical weakening and thinning or steepening. PSOCT is being studied as a potential tool for the clinical detection of the earliest structural changes that can be identified even before biomechanical or topo/tomographic changes in KC.[34,35]

While the above macro- and ultrastructural changes are the end products that manifest the disease, there is an ongoing enigma about the etiology or what triggers these changes. Among the several aetiologies which include inflammatory factors, genetic predisposition, eye rubbing, hormonal changes, oxidative imbalance, and chemical or metabolic alterations, there is no consensus on "what comes first." Certain groups believe that a mechanical compression or eye rubbing alone acts as the trigger.[36,37] *In vitro* study on human corneal fibroblasts has shown that, when subjected to mechanical compression, there is induction of apoptotic and ECM degradation genes including matrix metalloproteinases (MMPs) 1 and 9 and reduction in messenger RNA (mRNA) expression levels of COL1A1, lumican, and vimentin within 24 to 48 h, thereby bringing about a pro-inflammatory collagen degenerative state.[37] While the above could support the role of eye rubbing in KC pathogenesis, there is a significant percentage of KC patients who do not present with a history of eye rubbing or mechanical compression of the eye. Furthermore, there are studies both at population and individual levels, which have shown significant associations of KC with multiple systemic immune-mediated diseases.[38] The studies on tear inflammatory signatures in KC eyes,[16,17] genetic mutations in various collagen genes,[39] and cross-linking enzyme leading to KC[40,41] suggest the possibility of other factors that could trigger the onset of KC pathogenesis. However, regardless of the triggering factor, there are some final common pathways/factors through which the macro/ultrastructural anatomical (ECM) changes are effected [Figure 1].

The purpose of this article is not to rest this longstanding debate on what could be the most dominant initiating factor. However, by delving deep into the individual pathways/factors and their interactions in the pathogenesis of KC, the role of several potential biochemical markers can be understood, which can help in going a step forward toward personalized medicine. It is important to understand that these pathways/factors do overlap and interact with one another. Below, we have discussed the key altered biochemical factors with potential clinical applications wherever possible. Genetic alterations are discussed only in brief as that is not the focus of this article.

Extracellular Matrix Markers

The corneal stroma is composed of the keratocytes along with a rich ECM of different types of collagens, proteoglycans (PGs), and glycosaminoglycans (GAG). The collagen proteins are held cross-linked in their functional form with the help of the endogenous cross-linking enzyme called lysyl oxidase (LOX).[42] The GAGs and PGs interact with the collagens and help in their orderly arrangement, which also ensures corneal optical clarity in addition to biomechanical stability. There is also a fine balance between the proteolytic enzymes MMPs and cathepsins and antiproteolytic enzymes (tissue inhibitors of matrix metalloproteinases [TIMPs]).[43] These components maintain the structural homeostasis of the cornea. Most of these factors can be studied from the tears, epithelium, and stroma and their dysregulation has been reported in KC.[44,45]
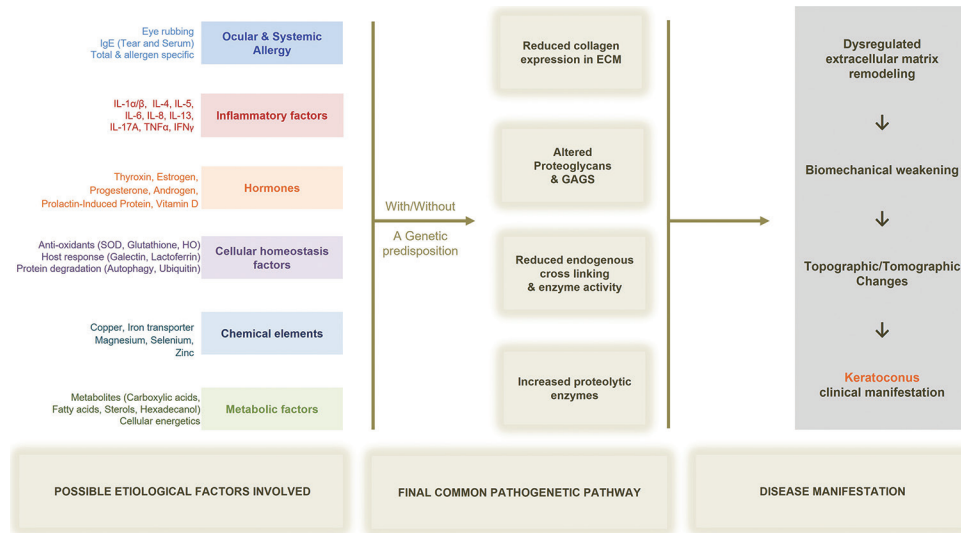
Figure 1: Illustration of how the various factors or pathways in the pathogenesis of keratoconus converge into a final common pathway to effect the ultra/macrostructural and clinical manifestations of the disease

A Wnt ligand, Wnt-10a, is involved in the positive regulation of Type I collagen in epithelium and Bowman layers. Wnt-10a mRNA and protein levels were studied in KC epithelium, and mRNA levels were found to be significantly lower compared to controls.[46] Wnt-10a expression levels also correlated to the disease severity, and hence, Wnt-10a expression is being studied as one of the potential markers of ECM remodeling in KC pathogenesis.[46]

Collagens I, VI, VII, XII, and XIII showed a reduced expression in KC epithelium, and additionally, collagens III, IV, and V were reduced in the KC stromal samples.[47-49] Epithelial collagen I and IV expressions were also found to be different between the cone and the periphery.[44] Prolidase is an enzyme necessary for collagen synthesis or turnover. The activity of this enzyme has been found to be lowered in the tears and serum of KC patients.[50,51] As KC progresses and advances, there is aberrant ECM remodeling with scarring changes, and in the scarred regions of the basement membrane, expressions of collagen IV and VII, fibronectin, and laminins 1 and 5 were increased.[52] This represents a variation in expression of these ECM proteins with different stages of KC severity. Alteration in the PGs in the cornea was studied in KC compared to controls.[53] Specifically, expressions of lumican, osteoglycin, biglycan, perlecan, syndecan 1 and 2, and keratan sulfate were lowered,[47,54,55] and dermatan sulfate, keratocan, tenascin, and decorin were increased in KC corneas.[56-58]

Potential applications

Corneal stromal regeneration is an upcoming field that focuses on increasing the lost stromal bulk in KC. This could either be done by insertion of stromal lenticules or by using mesenchymal stem cells, which start producing ECM components upon transplantation.[59] Several of these collagen markers and GAG could be potentially studied to monitor the response to such novel treatments and help in customizing the type of mesenchymal cells used depending on the specific

deficient markers. A novel *in vitro* study[60] has evaluated the role of arginine supplementation in the production of ECM materials. The study reports that the addition of arginine leads to a significant increase in collagen type 1 production.[60] Future studies are needed to evaluate the possible clinical utility of this amino acid supplementation as a nonsurgical means of strengthening the ECM.

Lysyl Oxidase

The endogenous cross-linking enzyme, LOX, plays a very critical role in the biomechanical stability of the cornea by ensuring endogenous cross-links between collagen and elastin fibrils. LOX expression along with LOX-like (LOXL2), L3, and L4 and the most dominant cross-link type, lysin or leucine, has been found to be decreased in KC corneas compared to controls.[40,49,61] When compared to healthy eyes, the expression of LOX in KC epithelium was found to be reduced significantly. In addition, there was a proportionate reduction in LOX expression levels with increasing severity of KC. In addition to the epithelium, LOX levels and its activity can be successfully measured from the tears, and this was also found to be correlated to the KC disease severity.[44,49] In a study by Shetty *et al*,[62] the gene expression levels of LOX, MMP9, TIMP-1, COL1A1, and COL4A1 from the epithelium of patients undergoing CXL were studied, and it was found that higher expression of LOX, collagens, and TIMP-1 correlated to a better response to CXL in terms of keratometric reduction.[62] The activity of LOX in inducing cross-links and tissue strengthening has also been studied *in vitro* using human corneal fibroblasts.[45]

Potential applications

LOX activity can be studied from the tears, and it is known to correlate with disease activity and CXL outcomes. LOX levels are also negatively impacted by inflammatory markers such as

MMP9.[62] These can be utilized in clinical settings to improve and customize outcomes of CXL. Some possible ways would be by ensuring a lowered ocular surface inflammatory milieu prior to CXL and by using higher fluence levels of UV-A in the cone region. This form of customized CXL has been shown to have better keratometric flattening compared to conventional CXL, but the long-term results beyond 1 year are not known.[63]

"*IVMED-80*" is a novel drug that was been granted orphan drug designation by FDA recently. This drug is used as an eye drop twice daily and acts by increasing the LOX activity in the cornea. IVMED-80 has been used in clinical trials in KC eyes, where it has been shown to decrease progression and induce a corneal flattening effect of up to 1.6Dioptres.[64] Long-term trials are needed to assess the efficacy and potential use as an alternative to CXL.

In one of the first reports on asymmetric bilateral ectasia post Small Incision Lenticule Extraction (SMILE®),[45] it was found that the LOX and COL1A1 levels in the stroma of the ectatic eyes were lower compared to controls even before they underwent SMILE® surgery. This shows the significance of prerefractive surgery molecular testing in detecting cases at risk for ectasia that can otherwise go undetected by existing topo/tomographic and biomechanical assessment alone.

Matrix Metalloproteinases

An imbalance between the proteolytic and antiproteolytic enzymes in the cornea is important in KC pathogenesis. An increase in activity of proteolytic enzymes as collagenases, gelatinase, and peptidase have been reported in KC.[65,66] Cathepsins were also found to be increased in the cornea and tears.[66,67] MMPs are the most important of the proteolytic enzymes and several subtypes of MMPs are reported to be higher. MMP2[68] and 9[49] have been found to be elevated in cornea and serum,[69,70] while MMP9 and 13 are elevated in tears.[43,66,71] TIMP-1, alpha-1 proteinase inhibitor, and alpha-2 macroglobulin are proteinase inhibitors and were significantly lower in KC.[67,72,73] Similar to LOX, epithelial MMP9 is also differentially altered in the cone region compared to the periphery,[44] and MMP9 and 13 levels positively correlated with the disease severity and progression.[49,74]

Potential applications

MMP9 levels have been well studied in tears and they are found to be correlated well with disease activity and progression. Tear MMP9 is also important in predicting response to CXL, wherein higher pre-CXL levels indicate poorer response to CXL and risk of failure.[44] Tear MMP9 can be tested in the clinic using a simple point-of-care diagnostic kit.[75] Treatment with topical cyclosporine 0.05% has been shown to reduce tear MMP9 levels in KC patients.[74] Clinicians can utilize this as a biomarker before CXL and pretreat prior to CXL for a month with cyclosporine 0.05% if MMP9 is elevated. This can potentially help in achieving better CXL outcomes.

Inflammatory Markers

A specific signature of inflammatory markers/factors is found to be elevated in the tears and serum of KC patients.[17,76,77] There is also a genetic predisposition to heightened inflammation in KC patients in the form of toll-like receptor 2 (TLR2) and TLR4 overexpression[78] in conjunctival and corneal cells and interleukin (*IL1A*) or *1B* gene polymorphisms.[79] Systemic immune inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio, and monocyte to HDL cholesterol ratio are significantly elevated in the blood of KC patients compared to controls.[80] IL-1 α/β, tumor necrosis factor (TNF-α), and IL-6 are increased in the serum, cornea, and tears and IL-6 is additionally increased in cultured fibroblasts from KC eyes.[66,70,77,81-83] IL-6 levels from epithelium are more elevated in the cone compared to the periphery and tear IL6 levels have been shown to increase with eye rubbing.[44] IL-8, IL-17A, IL-21, IL-23, and transforming growth factor-beta (TGF-β) are all elevated in the tears, and IL-8 is increased in the saliva of KC patients as well.[17,66,81,84] Meibomian gland dysfunction is also found to be present in around 50% of KC patients, which contributes to ocular surface inflammation as well.[85] Different levels of cytokine alterations are also found in the tear film of KC eyes fitted with scleral lenses.[86] Tear levels of IL-4, IL-8, basic fibroblast growth factor, and MMP9 were specifically elevated significantly in KC eyes showing clinical progression, while levels of fractalkine and vascular endothelial growth factor increased significantly in stable KC eyes compared to progressors.[16] There are other specific cytokines that are elevated specifically in response to ocular allergy-related inflammation, which we will discuss in the upcoming section. Recent studies on ocular surface microbiome also show a signature in KC eyes along with correlation to specific inflammatory markers, suggesting a possible role of alteration in microbiome in inflammation and KC pathogenesis.[87]

Potential applications

Inflammatory biomarkers contribute significantly toward clinical utility and personalized medicine in KC. SII index >469 calculated from blood has been shown to have a 79% sensitivity and 72% specificity in predicting KC. High SII values are associated with a heightened systemic inflammation.[80] NLR >2.24 can predict the progression of KC with 79% sensitivity and 81% specificity.[88] These can serve as novel biomarkers in evaluating patients with KC and in predicting progression, and they also offer newer perspectives in the understanding of KC pathogenesis.

An increase in tear levels of IFN-gamma is associated with disease progression and corneal thinning.[89] Epithelial TNF-α levels from the cone region have a positive association with Belin–Ambrosio-enhanced ectasia display scores, corneal deformation, and keratometry, all of which suggest a biomechanical weakening process.[44] TGF-β tear levels indicate a profibrotic state and an increase in the tear levels correlates with corneal remodeling and scarring in advanced

KC.[17,81] The specific molecular signatures in tears in KC could be used as potential targets in the management of inflammation in KC. Newer molecules targeting specific cytokines are being developed for use in other fields[90,91] and these can have potential applications in decreasing the progression of KC.

MARKERS IN OCULAR ALLERGY AND EYE RUBBING

Ocular allergy and eye rubbing are known as key contributors in the pathogenesis of KC. Aggressive and customized management of this condition is key to the management of KC and avoiding progression before and after CXL. Inflammation associated with eye rubbing with or without ocular allergy is also associated with specific tear and serum biomarkers.[92] Immunoglobulin E (IgE) is a vital modifiable biomarker and ocular responses to allergy and atopy are primarily driven by IgE-mediated cellular responses. IgE can be measured from serum and tears.[92] Serum total and allergen-specific IgE are most used, and tear IgE testing needs further studies before being used clinically as a routine. KC patients have been shown to have elevated serum IgE and it is more significant in people with ocular allergy or atopy.[92] Elevated serum IgE is associated with the progression of KC and graft rejection.[93] It is interesting to note that there is also a subset of patients with KC who do not have any ocular allergy but still have high IgE levels in serum.[94] These are the patients who have systemic atopy, asthma, or allergic rhinitis or an unknown systemic inflammation.

Potential applications

It is very important clinically to control ocular allergy to slow down the pathogenesis of KC. Apart from the routinely used mast cell inhibitor eye drops such as olopatadine or cromolyn sodium, resistant cases of ocular allergy have been shown to benefit from short-term topical steroids and long-term topical cyclosporine 0.5% or tacrolimus 0.03%, as these are involved in blocking the IgE-mediated cellular (T-cell) activation.[74,95,96] Controlling IgE levels are also considered important in the management of KC. For patients with high IgE, Ahuja *et al*. suggest an algorithmic approach.[92] In short, high serum IgE in the presence of ocular allergy may be brought under control with the treatment of ocular allergy alone. In cases with refractorily high IgE levels despite control of ocular allergy or in the absence of ocular allergy, testing for systemic atopy and allergens along with systemic immunomodulators or SLIT (sublingual immunotherapy) is being recommended.[92] Although long-term results are awaited, this algorithm is showing promise in our practice. Systemic omalizumab (subcutaneous), which is a monoclonal antibody against a specific component of IgE, has also been tried in refractory ocular allergy or systemic atopy cases with varying results and needs further research.[97]

OXIDATIVE STRESS MARKERS

In every tissue in the body, there are pro-oxidant and antioxidant enzymes and cofactors, and the balance between them is important for maintaining cellular homeostasis. An increase in the pro-oxidant pathway by-products leads to oxidative stress in cells and can lead to apoptosis, inflammation, and cell death.[98] A study in a rabbit KC model suggests a possible role of oxidative imbalance in the etiopathogenesis of KC.[99] A recent meta-analysis[19] on oxidative stress markers in human KC eyes which includes data from 1328 KC patients reveals that there is a significant imbalance of the reduction–oxidation homeostasis in tears, cornea, aqueous humor, and blood of KC patients, compared to controls. There is a significant increase in reactive oxygen (ROS), nitrogen species, and malondialdehyde and reduction in aldehyde/NADPH dehydrogenase, lactoferrin, albumin, transferrin, selenium, and zinc.[19] Mean xanthine oxidase levels in KC epithelium are lesser compared to controls.[100] In other studies, increased ROS has been found in tears, 8-oxo-2'deoxyguanosine in the corneal tissue, and higher levels of total oxidant status in serum.[19,101] Antioxidant factors found to be reduced in KC eyes are glutathione, superoxide dismutase, heat shock protein 27, heme oxygenase, and nuclear factor erythroid 2-related factor.[81,102-104] This imbalance leads to cellular stress and accumulation of ubiquitin and decreased autophagy in KC corneas.[81,105] These processes also tip the balance in keratocytes toward apoptosis and ECM degeneration.

Potential applications

Cytological studies of the corneal epithelium in the cone region compared to controls have shown a higher level of pro-apoptotic markers like Bax and lower levels of differentiation marker like cytokeratin 3/12.[106] Patient-specific cellular characterization of epithelial status is possible with this technique, and depending on the level of epithelial maturity or proliferation/differentiation and healing ability (based on apoptotic markers), treatments can be potentially customized for individual KC eyes. With the impression cytology technique, the above can be possibly studied noninvasively in a clinic setting.[107]

Regulation of autophagy plays an important role in preventing oxidative damage in cells. Trehalose is a sugar that has been shown to induce autophagy and reduce oxidative inflammatory damage, thereby potentially slowing down the cell death and progression of KC.[108-110] Trehalose is available in combination with sodium hyaluronate as eye drops and its use has been well-documented in dry eyes and KC.[110,111] It shows potential in improving epithelial healing post CXL[112] and slowing down the progression of KC before CXL but needs larger studies.

Lactoferrin is a key protein involved in the redox pathway, wound healing, and in the modulation of inflammatory signals.[113] Lactoferrin is reduced in KC tears and epithelium,[47] and novel delivery of Lactoferrin using nanoparticles in the form of eye drops is being studied.[114] These can potentially help in reducing the impact of oxidative cell damage and KC progression.

HORMONAL MARKERS

Hormones are coming up as important contributors in KC pathogenesis, especially in women. Receptors for estrogen, progesterone, and androgen are found in the corneal epithelium.[115] These are steroid hormones and act by modifying gene expression within the nucleus. Estrogens act by stimulating MMPs and thereby affecting collagen in the stroma.[116] Raised serum levels of dehydroepiandrosterone sulfate and reduction in serum estrone, estriol, and prolactin have been reported in KC patients.[84,117] Pregnancy and use of oral estrogen/progesterone hormonal replacement therapy can lead to progression of KC and development of postrefractive surgery ectasia.[118,119] This is important to identify early to avoid peripartum vision loss in women. These can also be potentially used as biomarkers in other situations such as hormone-producing ovarian tumors or polycystic ovarian disorder.

KC patients have a higher prevalence of thyroid gland dysfunction, thyrotoxicosis, and Hashimoto thyroiditis.[115,120] Higher levels of thyroxine have been shown in the aqueous and tears in KC patients along with an increase in expression of thyroid receptors in the stroma and epithelium.[115,121] Although a case report shows that a thyroid dysfunction leads to a new-onset development of progressive KC in a 53 year old,[122] no other studies have shown a direct causal relationship or a strong association of KC with thyroid dysfunction.[123]

Vitamin D plays an important role in systemic and ocular surface immunomodulation and its role has been previously described in relation to evaporative dry eyes and photorefractive keratectomy outcomes.[124,125] In patients with KC, serum Vitamin D levels are found to be significantly lower than in controls. However, no difference has been found in levels between stable and progressive KC patients.[126,127] Vitamin D has been shown to reduce oxidative stress by enhancing autophagic lysosomal clearance in KC eyes.[128] It may be worthwhile to monitor Vitamin D levels in KC patients, and further studies are needed to determine its role as a biomarker.

CHEMICAL AND METABOLIC MARKERS

Among the chemical elements, copper (Cu) is the most studied. Cu is a co-factor in the LOX enzyme-mediated endogenous cross-linking, and altered levels of Cu have been reported in KC. While KC patients have been found to have lower serum Cu levels,[129] very high levels are found in the tears.[130] The hypothesis is that the center of the cornea remains in a Cu deficient state, while the periphery has abundant deposits of Cu. Lower Cu in the central cornea could lead to poor LOX activity and thus play a role as a pathogenetic trigger for KC.[131] The formation of hydrolysine which is a precursor in collagen formation is affected by iron deficiency, and thus, altered iron metabolism is also implicated in KC pathogenesis.[129] Zinc, selenium, and magnesium are elements required in the antioxidant pathways for maintaining oxidative balance and these elements were found to be lower in the serum of KC patients.[129,132] However, none of the elemental markers have been studied extensively enough to be able to clinically utilize them in the management of KC.

Metabolomic studies in KC patients have identified certain specific pathway alterations. Alteration in the metabolism of cytokeratins, urea and citrate cycle, and oxidative stress metabolites is found in KC patients.[133] Specifically downregulated metabolites in KC corneas are fatty acids, sterols, hexadecanol, and carboxylic acids.[18] *In vitro* studies using cultured KC fibroblasts have also shown alteration in cellular metabolism, and tear studies post CXL have also been able to demonstrate changes in certain metabolites post CXL.[134] The role of these metabolites for clinical utility is not clear yet.

CONCLUSION AND FUTURE

The pertinent questions still prevail on "what is the factor/pathway that is the most dominant in the pathogenesis of KC," and likely, it is dependent on individual patients and their predisposing factors. Like several other diseases, KC may follow a "Nature-Nurture" model as well, where there is a component of genetic predisposition and subsequent environmental or biological propagation that leads to disease manifestation. Many carriers of several genetic mutations may not develop KC unless that genetic factor has a strong penetrance. But when acted upon by environmental or biological stimuli, the cascade of pathogenesis begins. Our aim was not to solve the confusion but understand the various pathways and factors in the pathogenesis of KC. This process also helps in understanding the role of several potential biomarkers in Keratoconus. It is important to note that multivariate indices which combine several biomarkers will have a higher diagnostic or predictive ability compared to single ones. A strong clinical acumen is necessary to interpret these and integrate them into clinical practice. Novel biomarker kits, which can provide levels of various cytokines from a drop of tear fluid as a point-of-care diagnostic, are in development, and this will revolutionize the ability to test several biomarkers at a simple clinic setting without the need for complex laboratory setups. The future of the application of such biomarkers toward personalized medicine would rely on "BIG DATA" analysis, where information from topo/tomographic, biomechanical, genetic, demographic, and molecular data is fed and is processed to provide customized care to every patient with KC.

Conflicts of interest

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