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Keratoconus: the possible involvement of inflammatory cytokines in its pathogenesis. An experimental study and review of the literature

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Abstract. – OBJECTIVE: Keratoconus (KC) is generally described as a non-inflammatory disease, characterized by thinning in the central region of the cornea with consequent tissue degradation producing impaired visual acuity.

MATERIALS AND METHODS: In our experimental study, we analyzed the presence and implications of several inflammatory cytokines in the corneal tissues of patients suffering from keratoconus by immunohistochemical analysis.

RESULTS: The analysis showed increased levels of inflammatory factors in the pathological tissues compared to controls, confirming that KC cannot be considered an entirely non-inflammatory pathology and that its etiopathogenesis includes several chronic inflammatory events.

CONCLUSIONS: In the light of these results, the classification of KC as an inflammatory pathology or as a pathology related to inflammation might be useful in directing future research aimed at developing effective anti-inflammatory therapies to pharmacologically target the inflammatory mediators which contribute to the development and progression of the disease.

Key Words:
Keratoconus (KC), Inflammation, Cytokines, Interleukin, Wound healing.

Introduction

Keratoconus (KC) is a progressive ectatic disease of the cornea, mainly characterized by thinning of the corneal stroma accompanied by protrusion with consequent scar formation in the central region of the cornea. These changes lead to the development of irregular myopic astigmatism. Described for the first time in 1854 (Nottingham), keratoconus is a term that derives from the Greek words keras (cornea) and konos (cone). Keratoconus is a progressive multifactorial disease on a genetic basis that affects adolescents or young adults[1,2]. However, in the latter, onset may be determined by various factors, such as trauma, infections, environmental factors, lifestyles and habits. Its origin is partly unknown, although there is a significant genetic component as demonstrated by the high incidence of the disease within the same family (6-13% autosomal dominant inheritance with incomplete penetrance). Certainly, it can be counted among the multifactorial pathologies and the underlying pathogenetic mechanism is believed to be the result of a genetic predisposition (less resistant cornea, increase in proteolytic enzymes, etc.) and of environmental and behavioral factors that can favor its onset[1,2]. The genetic component is confirmed by numerous clinical and laboratory evidence such as: a higher incidence in monozygotic twins than in dizygotic twins and a high incidence of the disease among relatives of affected individuals with autosomal dominant transmission with incomplete penetrance. Researchers have

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focused their attention on the study of different genes; in particular, the VSX1 gene located in the 20p11-q11 region is involved, which synthesizes transcription factors and is expressed in the nuclear layer of the retina and in the cornea, of which 4 mutations have been discovered. capable of causing disease. Other mutations potentially involved in the genesis of keratoconus concern the lysyl oxidase gene, responsible for collagen cross-linking, the cell death-inducing DEFA-like effector b gene, implicated in apoptosis, and the jasmine gene, also associated with to reticular corneal dystrophy type II[3-7]. Affecting all ethnic groups and both sexes, its incidence in the population varies between 1/2000 and 1/50,000, Although the need for corneal grafts in these patients appears to have decreased in recent years, it is still one of the main indications for this procedure in other areas of the world[3-5]. In patients affected by early-stage KC with no corneal scarring, therapeutic procedures are represented by a cross- linking procedure or implantation of intrastromal rings. Patients affected by advanced stage KC with corneal scarring often need keratoplastic procedure, either lamellar or full thickness, depending on the extent and depth of the stromal scar.

This eye disease, associated with atopy and connective tissue alterations[8], induces a significant impairment of visual acuity. The typical alterations associated with KC are represented mainly by the weakening of the corneal collagen. Generally, at its onset, the disease is asymptomatic, but as it progresses the first symptom that the patient notices is a vision loss. The clinical manifestations of KC are marked by astigmatism, myopia and thinning in the paracentral region of the cornea, which exhibits a forward cone-shaped protrusion. In advanced cases breaks in the Bowman's layer occur that will lead to sub-epithelial apical corneal opacities. The disease is almost always bilateral, though asymmetric[9,10]. KC is generally described as a non-inflammatory pathology, and it does not in fact present the typical signs of inflammatory diseases such as heat, redness, swelling and pain. Nevertheless, even in the absence of classic inflammatory symptoms, the presence of high levels of inflammatory cytokines in the tears of patients with KC raises doubts about the non-inflammatory nature of this corneal pathology[11]. Indeed, in light of this finding, it may be more appropriate to classify KC as an inflammation-related disease. In several studies[11-13] describing the expression of inflammatory molecules in the tears of patients suffering from KC, researchers have hypothesized that the development of the disease may be influenced by an inflammatory response[11-13]. Over two decades ago, Pouliquen et al[14] already suggested that the alterations of the extracellular matrix might be caused by the involvement of pro-inflammatory mediators. Hence, in recent years, specific biomarkers of inflammation have become the new target for KC evaluation[15,16]. According to recent research[12,17], it seems clear that inflammation may play a role in the development of several corneal pathologies. Most patients suffering from allergic ocular disorders tend to rub their eyes excessively. It seems that atopy can contribute, by continuous rubbing of the eyes, to the development of KC[18] since macrophages, monocytes and pro-inflammatory cytokines are released at the site of inflammation. Several epidemiological studies[19-21] have demonstrated correlations between KC and diseases characterized by a strong immune response, such as atopic disease[19], allergies[20] and allergic rhinitis[21]. Very often KC is also associated with genetic diseases such as Down syndrome, Ehlers-Danbs syndrome, Marfan syndrome. Recent results[22] argue that KC may be induced by a systemic disorder that also causes alterations in the corneal epithelium. Nemet et al[23] also found strong correlations between KC and several immune diseases. The role played by the immune system in the etiogenesis of KC is uncertain. Although KC is considered to be a non-inflammatory disease, the involvement of some cytokines, the increased MMP9 in KC and the efficacy of drug treatment with cyclosporine and corticosteroids suggests a possible involvement of the immune system and mediators of the inflammatory response in the pathogenesis of KC[19]. After exposure to ultraviolet irradiation, epithelial cells in the cornea release a greater quantity of pro-inflammatory cytokines[24]. Furthermore, compared to healthy subjects, it has been shown that in the corneas of subjects affected by KC there is an increase in reactive oxygen species with a consequent increase in oxidative damage[25]. All this evidence seems to point to a logical explanation, namely, that these corneas are less apt to process the accumulating reactive oxygen species as a consequence of the exposure to ultraviolet radiation[25,26]. Therefore, the possible involvement of an inflammatory response in as-

sociation with oxidative stress would cast doubt on the non-inflammatory nature of the corneal pathology[16,27]. In this regard, the aim of our experimental research was to verify, by means of immunohistochemical analysis, the presence of several inflammatory cytokines in tissue samples belonging to subjects with KC.

Materials and Methods

Ethics Statement and Patients

The experimental protocol was approved by the local Ethics Committee and strictly adherent to the guidelines of the Declaration of Helsinki for research on human participants and in agreement with the ARVO declaration for use of human samples in ophthalmic and vision research. Informed consent was obtained from each subject before any procedure.

In our study, 54 eyes belonging to 30 patients of Italian origin, with advanced KC who could not obtain a correction and sufficient vision with contact lenses or glasses were included and for this reason were treated by surgery. Specifically, they underwent DALK (Deep Anterior Lamellar Keratoplasty - DALK). In addition, 12 eyes belonging to 6 patients of Italian origin, with normal corneal topography undergoing photorefractive keratectomy (PRK) for the correction of refractive errors were used as controls samples. Further immunohistochemical (IHC) studies were subsequently performed on a separate set of archived corneal tissue samples to expand the number of control samples, as described below.

Diagnosis and Classification of Patients

The diagnosis of KC patients was carried out through the use of retinoscopy, slit lamp biomicroscopy and measurements of corneal refraction. The corneal topographic images of the patients were acquired using a Pentacam (OCULUS Optikgeräte GmbH, Germany) and the data obtained were used for the classification of the KC patients included in our study. KC grades were obtained from the biomicroscopic data set and theKC characteristics observed using the slit lamp, included spherical and cylindrical refractive changes, mean central keratometry and corneal thickness measurements, as described by the Amsler-Krumeich classification[28,29]. The classification system for KC patients was based on the methods described in previous studies[30]. Mean keratometry was used as the primary classification variable. The mean keratometric reading was calculated as the average of the steep and flat axis keratometric readings. The eyes of KC patients who had a mean keratometric reading less than 48D were classified as Grade 1. The eyes of KC patients who had a mean keratometric reading greater than 48D and less than 53D were classified as Grade 2. The eyes of KC patients who had a mean keratometric reading greater than 53D and less than 55 were classified as Grade 3, and the eyes of KC patients who had a mean keratometric reading greater 55 D were classified as Grade 4, with Grade 4 being the most severe grade. The KC subjects that were included in our study were all classified as Grade 4. Control samples were collected from PRK subjects who showed no corneal surface distortion and showed no clinical signs of KC.

For our study, the exclusion criteria adopted were: patients who habitually wore contact lenses or patients who had used ocular or systemic anti-inflammatory drugs (e.g., anti-allergic, anti-inflammatory drugs) in the previous three months or who had undergone previous surgery (e.g., penetrating keratoplasty/corneal collagen crosslinking, cataract surgery, etc.). Additionally, we excluded patients with a history of allergy or those who had recent infections (less than three months previously) in both eyes. We also excluded subjects with childhood corneal disease. All subjects were evaluated for dry eye using Schirmer's test and corneal staining, and those whose signs confirmed the condition, were excluded. Finally, patients who had any type of systemic inflammatory or autoimmune disease were also excluded.

Surgical procedures were performed under general anesthesia using the same surgical technique (DALK with big bubble described by Anwar and Teichmann) in all patients[31]. If complete denudation of the Descemet membrane (DM) was achieved, the procedure was classified as successful D-DALK, while in the event of failed DM exposure, a predescemetic stromal delamination was performed manually and the procedure was classified as PD-DALK.

A total of 25 unused corneas belonging to donors without clinical symptoms of KC, fixed in formalin and embedded in paraffin (FFPE) were obtained from the histotheca of pathological anatomy of the Policlinico Umberto I, "Sapienza" University of Rome. These samples were used as control samples for IHC studies.

Immunohistochemistry

We conducted the immunohistochemical analysis using the ABC/HRP technique (avidin complexed with biotinylated peroxidase) on 4 μm-thick paraffin sections which had been cut using a rotative microtome. All sections were deparaffinized and later rehydrated using decreasing ethanol series to distilled water, later subjected to microwave irradiation and immersed in citrate buffer (pH=6) twice for 5 minutes each time. Later, endogenous peroxidase activity was quenched using 0.3% hydrogenous peroxide in methanol for 30 minutes. For immunohistochemical analysis, the following antibodies were used: i) rabbit anti-IL-1β polyclonal antibody (1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA); ii) mouse anti-TNF-α monoclonal antibody (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA); iii) rabbit antiIL6 polyclonal antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA); iiii) and rabbit antiTGFβ1 polyclonal antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Immunohistochemical analysis was performed as already described by the authors in a previous work[32,33].

Statistical Analysis

We conducted the statistical analysis using the Student's *t*-test, and the GraphPad Prism (La Jolla, CA, USA). A p-value<0.05 was considered for statistical significance.

Results

Expression of Inflammatory Cytokines in the Corneal Epithelium of KC Patients.

Fifty-four eyes belonging to 30 Italian patients (66.54% males and 33.46% females) diagnosed with advanced KC (24 with bilateral KC, 6 with KC in one eye) who underwent Deep Anterior Lamellar Keratoplasty were included in the present study. The mean age of the KC subjects was 47.7 ± 18.9 years (range 25-77 years).

Twelve eyes of 6 normal subjects of Italian origin, (2 men and 4 women), whose mean age was 22.8 ± 1, 9 years (range 21-25 years) undergoing (PRK) were included as control group.

In addition, a total of 25 unused corneas from 15 donors (9 males and 6 females) with a mean age 61.3 ± 12, 4 years (range 33-77 years) and without clinical symptoms of KC, which had been formalin-fixed and paraffin-embedded (FFPE) were obtained from the Pathological Anatomy Institute of the Policlinico Umberto I, "Sapienza" University of Rome. These samples were used as control samples for IHC studies. For our study the inclusion criteria adopted were: subjects with severe KC (all KC patient samples were classified as grade 4 KC), all subjects with signs. For the samples belonging to the control group, only those with corneal topography were included as normal.

After analyzing the expression of several inflammatory cytokines by IHC in the corneal tissues of KC patients (Figure 1A-1C), the results obtained were compared with the expression of the same cytokines in the control samples. Tumor necrosis factor-α (TNF-α) expression was observed in the corneal epithelial layer, the basement membrane and corneal stroma. In all the KC analyzed corneas, TNF-α showed high levels of expression, while only a small percentage of the control donor corneas showed very low levels of TNF-α expression (Figure 1D). There was considerably greater variability in expression levels in the KC samples than in the controls, as shown in Figure 1A showing TNF-α expression. As expected, IHC study of Interleukin (IL)-6 in KC corneas (Figure 2) showed expression in the epithelial basement membrane and Descemet's basement membrane. Furthermore, IL-6 was also detected in the stroma, and there was a greater expression in the anterior stroma than in the posterior stroma. At higher magnification (Figure 2C), the endothelium and anterior stroma could be seen more clearly. In the anterior stroma, IL-6 was present in both stromal cells and in the surrounding extracellular matrix. In agreement with our findings, Lema et al[12] observed increased levels of IL-6 and TNF-α in keratoconic eyes using the conventional ELISA test[12,34]. Furthermore, in the present investigation, a considerably higher expression of interleukin-1β was observed in the pathological corneal tissue than in the control sample. More specifically, the immunohistochemical study provided evidence of increased Interleukin-1β (IL-1β) expression in the corneal stroma in KC samples (Figure 3A-3C). This over-expression of IL-1β was observed in the epithelial layer. The expression levels of transforming growth factor beta (TGF-β) were then examined in KC corneas. TGF-β expression was observed across the entire corneal stroma in all the pathological specimens examined (Figure 4A-4D), whereas reduced staining intensity was observed in the control samples. It should be not-

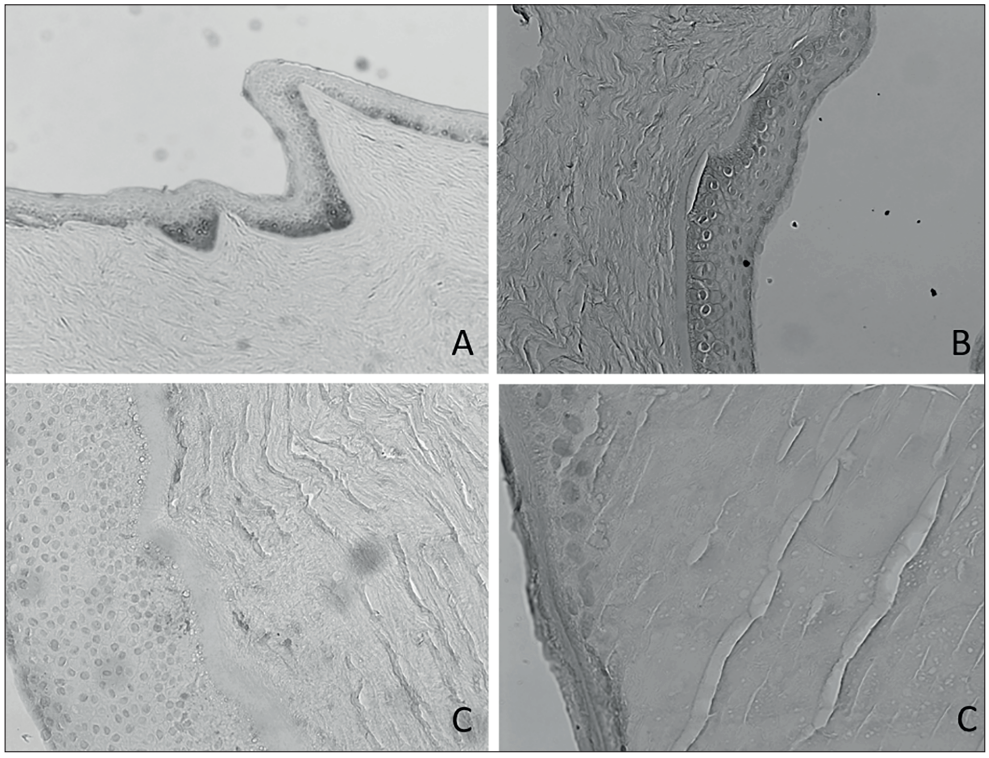

Figure 1. Micrograph of TNF-α immunostaining in human corneal epithelium. Marked expression immunoreactivity for tumor necrosis factor-α (TNF-α) was observed in the corneal epithelial layer, basement membrane and corneal stroma in all pathological specimens (**A-C**). TNF-α was weakly expressed in the control specimens (**D**). (**A-C**) Magnification, ×20; (**D**) Magnification, ×40.

ed that a greater intensity of TGF-β staining was observed near the corneal basement membrane, and none of the control corneas showed the same level of staining intensity (Figure 4D). The results obtained from the statistical analysis are shown in Table I.

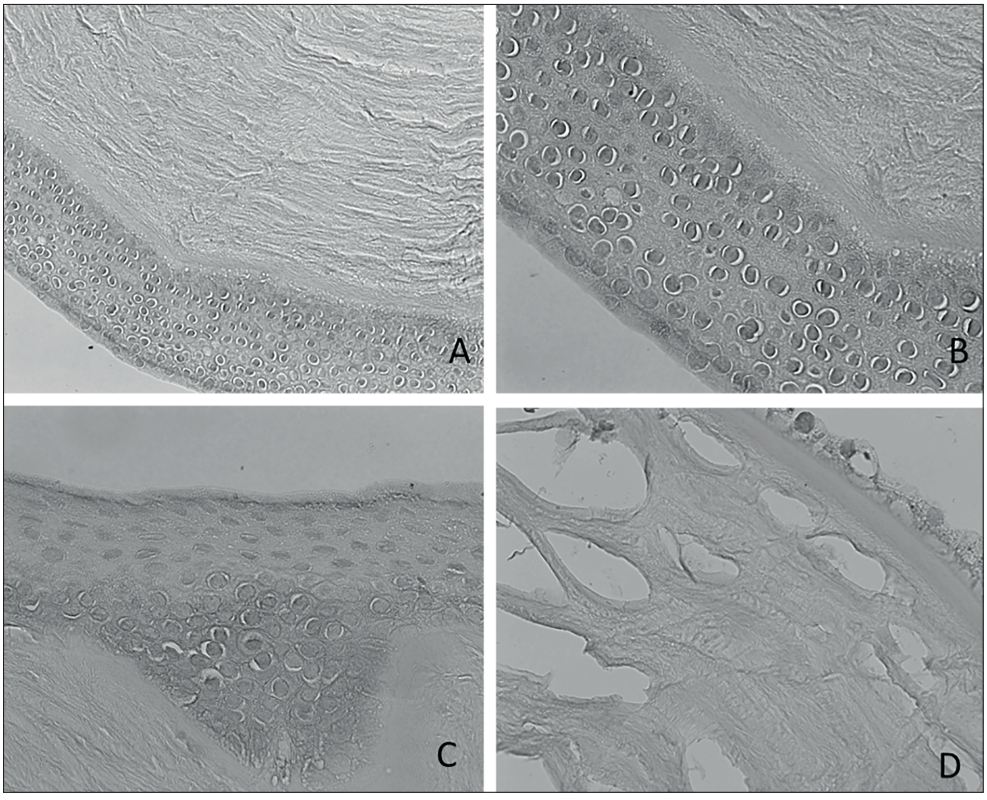

Figure 2. Micrograph of interleukin6 immunostaining in human corneal epithelium. Marked immunoreactivity for IL-6 (**A-C**) was found in the epithelial layer, basement membrane and corneal stroma. Immunoreactivity was weakly expressed in the corneal stroma of the control samples (**D**). (**A**) Magnification, ×10; (**B, C**) magnification, ×20; (**D**) Magnification, ×40.

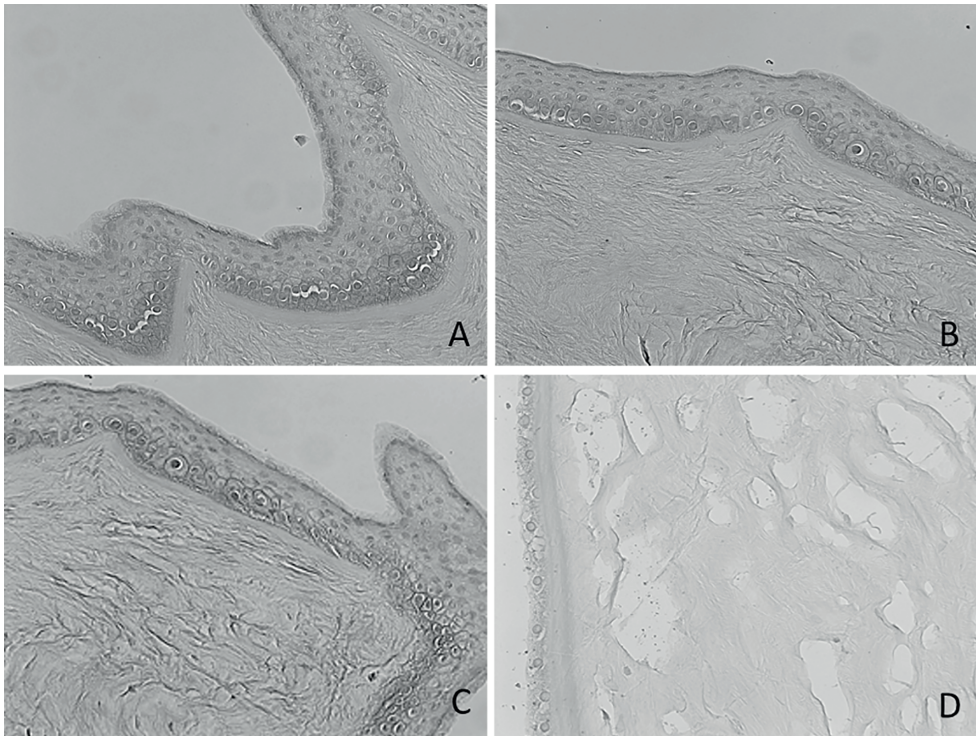

Figure 3. Micrograph of interleukin1β immunostaining in human corneal epithelium. Marked immunoreactivity for IL-1β was observed in the epithelial layer (**A**), evidence of increased Interleukin-1β (IL-1β) expression was observed in the corneal stroma in KC samples (**B, C**). IL-1β was weakly reactive in the corneal stroma in the control samples (**D**). **A-D,** Magnification, ×20.

Discussion

KC is an ocular disease that negatively influences the quality of life in affected patients. Our study analyzes the involvement and role played by the inflammatory response in the development and an etiology of this pathology, focusing on the important question of whether it is appropriate, in

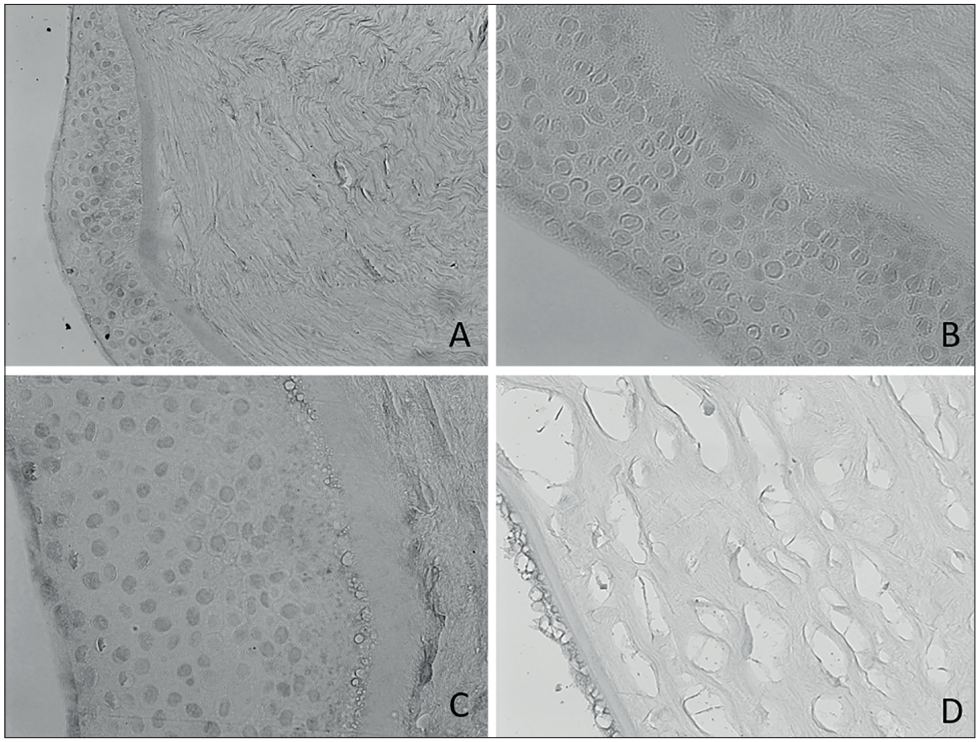

Figure 4. Micrograph of TGF-β immunostaining in human corneal epithelium. In the Immunoreactivity analysis for TGF-β, a staining near the corneal basement membrane and in the epithelial layer was observed (**A-C**); however, none of the control corneas showed staining for TGF-β (**D**). (**A-D**) Magnification, ×20; (**B, C**) Magnification, ×40.

Table I. Expression levels of inflammatory cytokines (IL -1β, IL- 6, TNF- α) in corneal tissues of KC patients (n=54 eyes) and in the control specimens (n=37 eyes), and respective levels of statistical significance (*t*-test).

	Corneal tissue of KC (n = 54 eyes)	Corneal tissue of control specimens (n = 37 eyes)	*p*-value
IL-1α	*72.00 ± 7.27	*8.11 ± 2.15	$p < .00001$
IL-6	*68.30 ± 2.45	*8.62 ± 2.29	$p < .00001$
TNF-α	*61.90 ± 6.56	*6.38 ± 2.20	$p < .00001$
TGF-β	*57.50 ± 5.36	*9.76 ± 2.68	$p < .00001$

Data are the mean ± SD and are considered statistically significant at $p<0.05$. *Values in percentage. Percentage of inflammatory cytokines-positive cells in a 100-μm^2 area.

the light of recent findings, to continue to consider and consequently to treat KC as a non-inflammatory disease.

Several researchers[16,35-37] have highlighted the relationship between the apical and stromal thinning of the cornea and the alteration of the physiological balance caused by an increase in proteolytic enzymes and a decrease in their own inhibitors.

Galvis et al[16] have widely explained, in one of their works concerning the involvement and role played by inflammation in KC, the existence of a sharing of different mediators such as inflammatory molecules, proteases, protease inhibitors associated with free radicals and oxidants which together determine an increase in the apoptotic process[16].

High levels of ROS and oxidative stress also play an important role in the development of various eye diseases, such as keratoconus, Fuchs dystrophy and type 2 granular corneal dystrophy. In addition, ROS also led to a reduction of the derived neurotrophic factor from the brain (BDNF). This growth factor regulates axonal growth and synaptic activity. BDNF depletion caused by increased ROS induces cell apoptosis[38-40]. The progression of the pathology determines biochemical and molecular changes that involve the entire corneal structure[41]. Consequently, there are modifications that alter the tissue composition, such as variations concerning the different types of collagens that characterize the corneal stroma, hence the pathological corneal thinning characteristic of KC. In previous studies[11,12,41-43], a high expression of some inflammatory mediators such as IL-6, TNF-α, MMP9 was observed in the tears of KC subjects. MMPs are involved in the cleavage of type IV collagen and their main function is represented by degradation with removal of the damaged extracellular matrix (ECM) during the inflammatory phase. The activity of MMPs is closely linked to Interleukin-6 (IL-6) and is involved in the alterations of type I and type IV collagen fibers that occur in some eye diseases[44]. Since collagen is a fundamental protein of the corneal tissue, it is necessary to identify the pathological mechanisms that induce modifications of the corneal collagen fibers[12]. Matrix metalloproteinases are enzymes involved in the degradation of extracellular matrix proteins. These proteins are released following the high expression of cytokines and growth factors, such as Interleukin (IL)-6 and tumor necrosis factor (TNF). KC is widely described as a non-inflammatory degenerative disease. Typical clinical signs of KC such ascorneal thinning, ectasia, alteration of Bowman's membrane, fibrotic process and apoptosis of corneal keratocytes suggest involvement of the inflammatory response in the progression of KC. Following damage to the corneal stroma, the keratocytes are transformed into cells similar to fibroblasts participating in the apoptotic process. The fibroblasts begin remodelling of the stromal tissue and subsequently there is an increase in the expression of cytokines, growth factors and MMPs. Growth factor TGF-β1 differentiates keratocytes into myofibroblasts. TGF-β induces the overexpression of inflammatory molecules and subsequent cellular apoptosis. TNF-α also induces apoptosis of keratinocyte cells. The onset of this process determines the formation of corneal fibrosis[44,45]. Our investigation confirmed a high expression of IL-6 and TNF-α in all the pathological tissues that were compared with controls, pointing to a cytokine imbalance in the corneas affected by KC interfering with normal corneal homeostasis. Our findings are consistent with those of Lema et al[46,47], who reported elevated levels of the same cytokines. KC is generally associated with allergies. In fact, in most cases, high levels of inflammatory molecules were found in the tears of patients with KC. Several authors

supported the thesis that atopy is associated with KC and that eye rubbing could induce an increase in the tear levels of MMPs and various cytokines. In addition, the elevated levels of immunoglobulin E detected in serum samples of patients with KC supports the hypothesis that keratoconus may be linked to atopy[48]. In allergic subjects, rubbing of the eyes is a negative factor that could favor the development of the disease, because rubbing and the allergic situation favor the production of pro-inflammatory factors. Therefore, the atopy that induces mechanical rubbing of the eye represents a risk factor for KC[49-51].

This finding reinforces the assumption that atopic diseases interact with the pathological processes of KC leading to a chronic evolution of an inflammatory response. In these conditions, the expression of TNF-α is particularly high. In addition, in advanced KC, the formation of a corneal scar frequently occurs.

IL-1β plays a fundamental role in the process of the inflammatory cascade. In a recent study on mice[52,53], it was observed that, after damage to the corneal epithelium, IL-1β was responsible for activating the apoptotic process of corneal keratocytes. In fact, this cytokine is believed to be responsible for triggering the inflammatory process. The loss of keratocytes is the first sign that occurs during the course of damage to the corneal stroma. Indeed, in patients suffering from KC, a decrease in keratocytes is found in comparison to control subjects[54]. An excessive proliferation of collagen fibers and the consequent activation of myo-fibroblasts by the growth factor TGF-β were also confirmed by our immunohistochemical analysis. The involvement of different inflammatory mediators has also been observed in other studies performed in ocular fluids and in the conjunctiva[55]. All these modifications of the ocular microenvironment may increase the release of pro-inflammatory factors, thus contributing to the development of further disorders, such as dry eye, which enhances the progression of KC[24]. Hence, the eye appears to be involved in an inflammatory process that affects the entire ocular surface, making it impossible to distinguish which was the first triggering factor or stimulus.

A tissue is usually defined as inflamed when there is an increase in the permeability of the capillaries associated with consistent dilation of the vessel walls, neo-vascularization and excessive presence of exudates. In the case of KC, since the pathology affects the cornea, a tissue free of vessels, the typical clinical manifestations of inflammation cannot be identified. It is therefore more complicated to establish whether the pathology may be truly inflammatory or not. According to the results reported in our experimental study, it seems that IL-6, IL-1β, TNF-α and TGF-β could be responsible for the activation of the inflammatory cascade, and thus, contribute to the changes in the extracellular matrix with subsequent differentiation of fibroblasts into myofibroblasts. The action of TGF-βmay contribute to worsening of the corneal scar. Oxidative stress also seems to play a fundamental role in the development of the pathology. Some studies[56,57] have reported an increase in free radicals and reactive substances in pathological samples when compared to controls. These substances induce modifications of the extracellular matrix and oxidative stress with variations in the cell population, leading to a greater susceptibility to damage[58]. Therefore, it is not clear whether oxidative stress may be responsible for activating the inflammatory response or whether inflammation induces oxidative stress with consequent tissue damage. A recent study[59] has shown a significant involvement of enzymes, cytokines and free radicals; even if KC does not possess the typical clinical symptoms of inflammatory diseases, the role of the inflammatory response and oxidative stress, which appear to be closely related, cannot be ignored. Undoubtedly, KC is a complex disorder, and its development is marked by a series of events/alterations, including the modification of the corneal stroma, an imbalance between the concentrations of pro-inflammatory and anti-inflammatory cytokines, an involvement of enzymes that induces apoptosis and oxidative stress due to free radicals. All these changes take place almost simultaneously and, to date, it remains unclear if one of them precedes the others, and above all, which one is the decisive factor in the development of KC.

Collagen appears to be fundamental in the composition of the corneal stroma and a decrease in collagen fibers in the corneas of KC patients compared to healthy corneas has been reported[60]. This decrease in collagen fibers could result in the thinning of the cornea in the central region, thus making the tissue more sensitive to damage. In our study we found an increase in IL-1β and TNF-α, both of which are known to induce apoptosis in keratocytes and to produce collagen modifications.

The correlation between KC and inflammatory processes could open the door to a new therapeutic approach in the treatment of this disease

by means of the use of anti-inflammatory drugs. To date, no ocular or systemic anti-inflammatory therapy has been shown to help in reducing the damage to corneal collagen of patients with KC, but anti-inflammatory therapy could be used in the first phase, useful for inhibiting and preventing the high expression of inflammatory cytokines that induce the release of MMPs that are involved in degradation of the collagen fibers of the ECM. Tetracyclines have anti-inflammatory and antibacterial properties and could also be used to treat diseases such as KC since they are able to reduce the levels of MMP, IL-1 and TNF-α. Considering KC as an inflammatory disease, Shetty et al[50] suggested the use of cyclosporine A, an immunosuppressive drug with an anti-inflammatory effect, as a possible drug alternative in KC. In the study performed by Shetty et al[50] the eyes of some KC subjects were treated with topical cyclosporine A (CyA) for about six months; treatment with CyA led to the reduction of MMP-9, IL-6 and TNF-α levels in the tears of KC patients and it also reduced corneal curvatures[50,61]. Other anti-inflammatory drugs might be considered as potentially therapeutic agents. Therefore, if imprecise, the current definition of KC as a non-inflammatory pathology could actually be harmful because it automatically excludes drugs useful in defusing inflammatory events.

Conclusions

Although in KC it is difficult to distinguish primary disease mechanisms from secondary inflammatory or degenerative effects, the presence of higher levels of proinflammatory mediators in pathological samples than in controls suggests that inflammation plays an important role in the pathogenesis of the disease. According to Jun et al[13] inflammation signs in KC can be found even during the subclinical stages and persist until evidence of KC becomes apparent. Scientific research has confirmed that KC is a multifactorial disease involving various genetic, environmental and even behavioral factors. Moreover, continuous rubbing of the eyes induces and facilitates the onset of the disease by stimulating inflammatory processes[49,51,62,63]. It is now clear that corneal ectasia is closely linked to the tissue degradation that occurs through modifications of the extracellular matrix due to the involvement of inflammatory mediators, such as IL-1β, IL-6 and TNF-α[56-58,64-66], However, the contribution of each inflammatory mediator to the onset and progression of KC remains unclear. To gain a better understanding of the underlying pathophysiological mechanisms that determine the development of KC represents a crucial step in identifying and formulating new therapeutic approaches which may be helpful for preventing irreversible damage to delicate eye tissues. Our study has limitations, such as the small number of subjects analyzed and the failure to measure serum levels of cytokines. Despite these limitations, this study should be considered as a basis from which to deepen and to intensify research in order to better define the mechanisms underlying the enzymatic imbalances that favor the development and progression of KC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Availability of Data and Materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' Contribution

ST, MR, PG and AM designed the study. MN, GS, VP, AG, MA, VS, PF and AMP consulted literature and collected data, ST and MR wrote the paper. PG and AM reviewed and edited the manuscript. All authors read and approved the manuscript.

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