
GAZİ ÜNİVERSİTESİ
Gazi
1926

EVALUATION OF CONJUNCTIVAL SURFACE DAMAGES AND INFAMMATORY CYTOKINES IN GLAUCOMA PATIENTS

Eman MUSTAFA ALHARDODE

Ph. D. THESIS

DEPARTMENT OF IMMUNOLOGY

GAZİ UNIVERSITY

GRADUATE SCHOOL OF HEALTH SCIENCES

MARCH 2023

ETHICAL DECLARATION

In this thesis study prepared in accordance with the Thesis Writing Rules of Gazi University Institute of Health Sciences; I hereby declare that

- I have obtained the data, information and documents that I have presented in the thesis under the academic and ethical rules,
- I offer all information, documents, evaluations and results in accordance with scientific ethics (Human Research Ethics Committee of Gazi University with decision No. 57/25.1.2021),
- I have found the source of the works which I have used for the thesis work,
- I have not made any changes to the data used, in this thesis,
- I have gaven the original work, in this thesis,
- I accept all other rights losses which may arise in the event of a contrary.

Eman MUSTAFA ALHARDODE

01/03/2023

EVALUATION OF CONJUNCTIVAL SURFACE DAMAGES AND INFAMMATORY CYTOKINES IN GLAUCOMA PATIENTS

(Ph. D. Thesis)

Eman MUSTAFA ALHARDODE

GAZİ UNIVERSITY

GRADUATE SCHOOL OF HEALTH SCIENCES

March 2023

ABSTRACT

Glaucoma is one of the most common causes of irreversible blindness in the world. Chronic use of topical medications in glaucoma can lead to ocular surface diseases (OSD). Dry eye is a common type of OSD. Inflammation plays an improtant role in the pathogenesis of DED. Our study aimed to assess the levels of IL-1β, IL-1Ra, VEGF, and PGE2 cytokines in tears and their relation to the clinical severity of dry eye in patients with glaucoma. A total of 45 volunteers participated in the study: 15 glaucoma patients, 15 DED patients, and 15 normal control subjects. All participants were aged between 22 and 65 years old. We use the ELISA technique to assess the levels of IL-1β, IL-1Ra, VEGF, and PGE2 in the tears of the group subjects. The conjunctival impression cytology was used to assess the changes in the GCD and MUC5AC expression at the ocular surface, which are considered significant markers for OSD. In glaucoma and DE groups, the goblet cell density and MUC5AC expression were shown to be significantly reduced. IL-1β, IL-1Ra, VEGF, and PGE2 cytokine levels were increased in the glaucoma group as compared to the normal control group, however, not all the cytokines were statistically significant. Also, significant correlations were found between these cytokines and the clinical parameters of DED. These findings indicate the critical relation between these cytokines and dry eye severity. Our findings clarify the crucial role that cytokines may play in the pathogenesis of DED. Using anti cytokines therapy can be considered as a choice of treatment for DED in the future. Furthermore, we demonstrated that long term use of preservatives containing topical drugs has a negative impact on the ocular surface of glaucoma patients.

Science Code : 1039.2
Key Words : Glaucoma, Dry Eye, Infammation, Cytokines, Goblet Cells, MUC5AC, IL-1β, IL-1RA, VEGF ve PGE2
Page Number : 103
Supervisor : Prof. Dr. Emin Umit BAĞRIAÇIK

GLOKOM HASTALRINDA OKÜLER YÜZEY HASARININ VE INFLAMATUAR SİTOKİNLERİN DEĞERLENDİRMESİ

(Doktora Tezi)

Eman MUSTAFA ALHARDODE

GAZİ ÜNİVERSİTESİ
SAĞLIK BİLİMLERİ ENSTİTÜSÜ

Mart 2023

ÖZET

Glokom, dünyada kalıcı körlük yol açan en sık hastalıklardan biridir. Kronik topikal ilaçların kullanımı glokom hastalrında oküler yüzey hastalığına neden olabilir. Kuru göz, yaygın oküler yüzey hastalıklarından bir tipidir. Enflamasyon, kuru göz patogenezinde çok önemli bir rol oynamaktadır. Çalışmamızda glokomlu hastalarda gözyaşlarında, IL-1β, IL-1RA, VEGF ve PGE2 sitokin konsantrasyolarını ve bunların kuru gözün klinik şiddeti ile ilişkisini değerlendirilmesi amaçlanmıştır. Çalışma 22 ila 65 yaşları arasındaki toplam 45 katılmcı ile gerçekleştirilmiş, bunlar 15 glokom hastası, 15 DED hastası ve 15 normal kontrol. Rutin göz muayeneleri yapıldı. Katılımcıların gözyaşlarındaki IL-1β, IL-1RA, VEGF ve PGE2 düzeylerini ELISA ile hesaplandı. OSD için önemli bir belirteç olarak kabul edilen oküler yüzeydeki goblet hücreleri yoğunluğu ve MUC5AC ekspresyonu değişiklikleri değerlendirmek için konjonktival impresyon sitolojisi kullanıldı. Glokom ve kuru göz gruplarında göblet hücre yoğunluğu ve MUC5AC ekspresyonunun önemli ölçüde azaldığını gösterilmiştir. Glokom grubunda IL-1β, IL-1RA, VEGF ve PGE2 sitokin düzeyleri normal kontrol grubuna göre artmış, ancak tüm sitokinler istatistiksel olarak anlamlı değildi. Ayrıca, bu sitokinler ile kuru gözün klinik parametreleri arasında anlamlı bir korelasyonlar bulundu. Sonuçlar olarak sitokinlerin kuru göz patogenizde kritik rol oynadığı düşünülmektedir. Gelecekte kuru göz tedavisi için anti sitokinler kullanmısı bir iyi seçeneği olarak düşünülebilir. Ayrıca, topikal ilaçlar içeren koruyucuların içeren topikal ilaçların uzun süre kullanımının glokom hastalarının oküler yüzeyi üzerinde olumsuz bir etkisi olduğunu gösterdik.

Bilim Kodu : 1039.2
Anahtar Kelimeler : Glokom, Kuru Göz, İnflamasyon, Goblet Hücreler, MUC5AC, IL-1β, IL-1RA, VEGF ve PGE2
Sayfa Adedi : 103
Danışman : Prof. Dr. Emin Umit BAĞRIAÇIK

GENİŞLETİLMİŞ TÜRKÇE ÖZET

1. GİRİŞ

Glokom, retinal ganglion hücrelerinin ve aksonlarının yavaş ilerleyen, optik diskin karakteristik bir görünümüne yol açan ve beraberinde görme kaybı paterni ile ilişkili olan bir optik nöropatidir [1]. Dünyadaki geri dönüşü olmayan körlüğün önde gelen nedenidir ve yaşam kalitesinin düşmesiyle ilişkilidir [2]. Glokom dünya çapında 70 milyondan fazla insanı etkiler ve yaklaşık %10'unda iki taraflı körlüğe neden olmaktadır [3] Glokom, çeşitli tiplerde, sıklıkla yüksek göz içi basıncının (GİB) neden olduğu optik sinir başı (OSB) hasarı ile ayırt edilmektedir [4].

Topikal ilaçlar, glokom için en sık kullanılan birinci basamak tedavidir [5]. Glokom ve oküler yüzey hastalıkları sıklıkla bir arada bulunmaktadır ve her ikisi de genellikle yaşla birlikte ortaya çıkmaktadır [6]. Koruyucu içeren topikal ilaçların uzun süreli kullanımı, glokom hastalarında kuru göz hastalığının gelişmesi için önemli risk faktörlerinden biridir. Glokom hastalarında ortaya çıkan oküler yüzey hastalığı, ilgili semptomlar nedeniyle ilaç uyumunun azalmasına neden olabilmektedir [7]. Ayrıca oküler yüzey hastalıklar, glokom cerrahisinde daha az bir başarı oranı ile ilişkilendirilmiştir [8].

Oküler yüzeyde hasarı gösteren değişikliklerden biri de goblet hücre yoğunluğundaki azalmadır. Goblet hücreler, oküler yüzeyi müsin üreten özel salgı hücreleridir. Ayrıca, konjonktival goblet hücrelerinin, tolerojenik ve koruyucu immünolojik yanıtlar arasındaki homeostazın sürdürülmesinde çok önemli bir rol oynadığı bulunmuştur [9].

Konjonktival goblet hücreleri, MUC5AC'yi oküler yüzeye salgılayan tek hücreler olduğundan, MUC5AC, goblet hücresi tespiti için en iyi göstergelerden biridir [10]. Gözdeki goblet hücresi değişikliklerinin tam mekanizması bilinmemekle birlikte, diğer mukozal dokulardan elde edilen veriler, enflamasyonun önemli bir rol oynayabileceğini göstermektedir [11]. MUC5AC, hücresel kalıntıların, patojenler ve alerjenler gibi yabancı cisimlerin yakalarak uzaklaştırılmasında koruyucu bir rol oynamaktaır [12]. İmpresyon sitolojisi konjunktıvadaki yüzey tabakanın elde edilmesi amacıyla kullanılan bir yöntemdir. Bu yöntemle alınan konjunktival hücrelerin morfolojisi, sitoplazm/nukleus oranı ve

özellikle goblet hücre densitesiyle ilgili detaylı bilgi vermesi dolayısıyla kuru göz hastalığının sebeplerinin incelenmesinin önemli olduğu vurgulanmıştır.

Enflamasyon, kuru göz patogenezisi kısır döngüsünde anahtar rol oynamaktadır. Kuru gözdeki enflamasyonuna katılan elemanları arasında makrofajlar ve T hücreleri gibi hücreleri, proinflamatuar sitokinler (İL-1β, TNF-α), ve matriks metalloproteinazlar (MMPS) vardır [13,14]. Birçok çalışma, kuru göz hastalarında çok sayıda inflamatuar sitokin düzeylerinde ve bu sitokinlerin hastalığın klinik şiddetiyle ilişkisinde anlamlı artış olduğunu göstermiştir [15,16]. Ayrıca, kortikosteroidler ve siklosporin A gibi topikal antiinflamatuar ilaçlar, kuru göz semptomlarını ve bulgularını önemli ölçüde azaltır [17,18].

Bu çalışmada glokomlu ve kuru göz olan hastaların gözyaşlarındaki İL-1β, İL-1Ra, VEGF ve PGE2 sitokinleri düzeylerini araştırdık. Sonuçları normal kontrollerle karşılaştırma esasına dayanır. Ayrıca konjonktival impresyon sitoloji tekniği kullanarak, goblet hücre yoğunluğundaki değişiklikleri ve önemli bir belirtisi olan oküler yüzeydeki MUC5AC ekspresyonunu araştırmak için de kullanıldı. Bunlara ek olarak, İL-1β, İL-1Ra, VEGF ve PGE2 inflamatuar sitokinleri ile kuru göz klinikal parametreleri arasında korelasyon olup olmadığını da araştırdık.

2. GEREÇ VE YÖNTEM

Bu çalışma Gazi Üniversitesi İnsan Araştırmaları Etik Kurulu tarafından onaylanmıştır (57/25.1.2021 sayılı Karar). Çalışmaya 15 glokom hastası, 15 DED hastası ve kontrol olarak oküler veya sistemik hastalığı olmayan 15 sağlıklı kişi olmak üzere toplam 45 kişi gönüllü olarak katılmıştır. Tüm katılımcılar 22 ila 65 yaşları arasından seçilmiştir. Gözyaşı örnekleri 7 ay boyunca toplandı (Haziran 2021 – Aralık 2021). Tüm katılımcılar, çalışmanın prosedürlerinin açıklanmasının ardından yazılı bir bilgilendirilmiş onay formu imzaladı. Bilgilendirilmiş onay formu imzaladıktan sonra OSDI anketini doldurdu. Ardından snellen çizelgesi, göz içi basıncı, göz dibi muayenesi, gözyaşı dahil olmak üzere detaylı bir oküler muayeneleri yapıldı.

Tüm katılamcıların gözyaşı örneklerinin toplanması ve impresyon sitolojisini takiben aynı gün içinde film kırılma zamanı, oküler yüzeyin floresan boyaması ve schirmer testi yapıldı.

Örnek toplama Gazi Tıp Fakültesi Oftalmoloji kliniklerinde yapıldı. Numunelerin analizi Gazi Üniversitesi Tıp Fakültesi İmmünoloji laboratuvarında yapıldı.

Çalışmaya dahil edilme kriterleri

1. Glokom tanısı olan hastalar.
2. Kuru göz tanısı olan hastalar.
3. Çalışmaya alınan tüm katılımcıların 18 yaşın üzerinde olmak.
4. Çalışmaya katılmayı kabul etmek.

Çalışmadan dışlama kriterleri

1. Başka bir göz hastalığı olanlar
2. Hasta grubu kriterlerini karşılamaması
3. Diyabet ve romatoid artrit gibi kronik hastalığı olan hastalar
4. Göz cerrahisi geçiren hastalar (son bir yıl içinde)
5. Aktif göz enfeksiyonu olanlar.

2.1. Kuru göz klinikal parametreleri değerlendirmesi

Oküler yüzeyin durumunu değerlendirmek amacıyla testlerin ELISA veya immünofloresan boyama sonuçlarını etkilememesi için gözyaşı örnekleri ve impresyon sitolojisi yapıldıktan sonra aşağıdaki klinkal testler yapıldı.

2.1.1. OSDI anketi

OSDI anketi, kuru göz semptomlerin değerlendirmesi için kullanıldı [191]. Alınan sonuçların hesaplanması Grubbs JR Jr ve arkadaşlarına göre yapılmıştır, OSDI skoru şu şekilde sınıflandırıldı: ≤ 12 normal, 13-32 hafif-orta ve ≥ 33 şiddetli kuru göz olarak [193].

2.1.2. Schirmer I testi

CHO ve arkadaşlarına göre Schirmer I testi yapıldı, topikal anestezi kullanmadan her iki gözün alt kapak kenarının dış üçte birine steril kâğıt şeriti yerleştirildi ve katılımcılardan gözlerini kapatmaları istendi [198]. Islanma uzunluğu, beş dakika sonra milimetre

cinsinden ölçülmüştür. 5 dakikada 10 mm'den fazla skor normal kabul edilimektedir. 5 dakikada 5 mm'den az bir skor gözyaşı yetersizliğini gösterir.

2.1.3. Gözyaşı kırılma zamanı testi (TBUT)

Gözyaşı kırılma zamanı tayini için bir islatılmış floresan kağıt şeriti alt fornikse nazikçe uygulandı. Bundan sonra, boyanın uygun şekilde karışmasını sağlamak için olgudan birkaç saniye boyunca birkaç kez göz kırpmaları istendi. Kornea kobalt mavisi ışıkla gözlenirken, son göz kırpma ile gözyaşı filmindeki ilk kornea koyu lekesinin görünümü arasındaki süre ölçüldü. Bron ve diğerlerine göre 10 saniyenin altındaki bir değer anormal kabul edildi [200].

2.1.4. Korneal floresan boyama

Gözyaşı kırılma zamanı değerlendirmesinin ardından kobalt mavisi ışık kullanılarak kornea boyanması değerlendirildi. Kaido ve arkadaşlarına göre, kornea floresan boyama yoğunluğu 0 (yok) ila 3 (önemli epitel kaybı) arasında skor olarak kaydedildi [199].

2.2. Gözyaşı toplanma yöntemi

Gözyaşı örnekleri bir wash-out yöntemi kullanılarak toplandı. Yaklaşık 50 µl serum fizyolojik gözün alt forniksine pipetlendi ve katılımcılardan gözünü hareket ettirmesi istendi; gözünü kapatmadan yukarı ve aşağı ve soldan sağa ve gözyaşları tekrar mikropipet ile toplandı ve steril PCR tüplerinde konuldu [215]. Toplanan gözyaşı örnekleri hemen kuru buz üzerine yerleştirildi. Laboratuarda, örnekler 4°C'de ve 3000 rpm'de 4 dakika boyunca santrifüj edildi. Ardından her bir örneğe toplam 150µl PBS hacmi eklendi. 200µl hacme ulaştı. Örnekler daha sonra analiz yapılana kadar -80 C derin dondurucuda laboratuvarda saklandı.

2.3. Konjonktival impresyon sitolojisi örnekleri toplanma

MUC5AC immüno floresan boyama için polieter sülfon (PES) filtre kağıtları (1214465, CVS), histokimyasal boyama (PAS-hematoksilen) için selüloz asetat (CA) filtre kağıtları (GSWG047S6, Millipore) kullanılarak impresyon sitoloji örnekleri elde edildi. [208]. Filtre kağıtları kullanılmadan önce 5*5 mm’lik parçalar şeklinde kesilerek sterilize edilmiştir.

Katılımcılardan aşağı ve karşı tarafa bakması istendi ve filtre kağıdı dişsiz bir penset yardımıyla limbustan 3 mm mesafede temporal bulbar konjonktiva üzerinde hafifçe bastırıldı. Soyma hareketi, kağıdı 15 saniye sonra çıkardı. Lokal anestezi kullanılmadı. Bu çalışmada belirtilen impresyon sitolojisi prosedürü ile ilgili herhangi bir komplikasyon olmamıştır. Örneğin PES filtreleri için soğuk metanol, selüloz asetat (CA) filtreleri için %96 etanol ile fiksasyonu yapıldı.

2.3.1. PAS-hemtoksilin boyama tiknik

İmpresyon sitoloji örnekleri %96 etanol ile 10 dakika fiksa edildi, ardından örnektaki hücreler yukarı bakacak şekilde lam üzerine yerleştirildi ve yaklaşık 5 dakika distile su ile yıkandı. Ondan sonra 15 dakika boyunca %0,5 periyodik asitle boyandı. Örnekler distile suda 5 dakika yakandı ve Schiff söylyusyon (1:1) ile yaklaşık 5 dakika boyandı. Ardından, örnek tekrar 5 dakika boyunca distle suyu ile yıkandı ve bir 30 saniye hematoksilin ile boyandı. Örnek daha sonra 5 dakika süreyle distle su içinde üç kez yıkandı. Daha sonra, örnekler, filtre kağıdını şeffaf hale getiren ksilen içine 3 saat süreyle yerleştirilmeden önce artan etanol konsantrasyonunde 5 dakika süreyle dehidre edildi. Örnekler daha sonra kapatma solüsyonu lam üzerine damlatılıp lamel üzerine kapatıldı ve ışık mikroskop altında incelenme yapıldı. Her örnek için resim çekildi.

2.3.2. İndirekt immüno floresan boyama

İndirect immüno floresan boyama şu şekilde yapıldı: Örnekler, 10 dakika süreyle soğuk metanol içinde fiks edildi. Daha sonra hücreler, 1x PBS içinde 5 dakika süreyle iki kez yıkandı ve spesifik olmayan bağlanmayı önlemek için %10 keçi serumu ile 1 saat süreyle bloke edildi. Ardından, örnekler 1:200 tavşan anti-insan MUC5AC antikorlarla +4'te gece boyunca inkübe edildi. Onadan sonra örnekler üç kez PBS ile yıkama yapıldı. Her yıkamada örnekler 5 dk PBS'de bekletildi. Yıkama işlemi bitikten sonra örnekler İkincil antikor olarak FİTC ile işaretlenmiş keçi anti-tavşan antikoru 1/250 oranında kullanıldı. Örnekler ikincil antikor ile karanlıkta 3 saat bekletildi. Sekonder antikorla inkübasyondan sonra örnekler, PBS ile üç kez 5 dakika yıkama yapıldı. Çekirdek boyaması için 1:2000 oranında (DAPI 4',6-diamidino-2-fenilindol) boyası kullanıldı (10 dakika). Floresan boyamalar için kullanılan kapatma solüsyonu lam üzerine damlatılıp lamel üzerine kapatıldı. Sonra floresan mikroskop kullanılarak 20X büyütmede 3 resim alındı.

2.4. ELISA

Gözyaşı sıvısındaki İL-1B, İL-1ra, VEGF ve PGE2 konsantrasyonları tayini için hazır ticari ELISA kitler kullanıldı. ELISA yapmadan önce seçilen sitokinler için referans olarak toplam gözyaşı protein konsantrasyonları quibt protein kiti (Thermo Fisher, katalog no. Q33211) kullanılarak belirlendi. Tüm testler üretici firma prosedürlerine uygun olarak şekilde çalışılmıştır.

2.4.1. İL-1β ELISA kiti protokolü

ELİSA kiti üretici firma prosedürlerine uygun olarak çalışılmıştır (Abcam). Kisaca, üretici firma talimatına göre ilk olarak antikor kokteyli hazırlandı. Daha sonra 50 µl örnekler ve standartlar uygun kuyucuklara eklendi. Ardından 50 µl bir antikor kokteyl uygun kuyucukları ilave edildi. Plak 2 saat boyunca 400 rpm'ye ayarlanmış bir plaka çalkalayıcı oda sıcaklığında inkübe edildi. İnkübasyondan sonra plak üç kez yıkama solüsyonu ile yıkandı. Otomatik bir yıkayıcı (HydroFlex, Tecan, İsviçre) kullanıldı. Yıkama işlemi bitikten sonra 100 µl tetrametilbenzidin (TMB) substratı her kuyucuğa eklenip karanlıkta 15 dakika inkübe edildi. Daha sonra her bir kuyucuğa 50µl stop solüsyonu ilave edildikten sonra bir mikroplaka okuyucusunda (Synergy, ABD) 450 nm'de okutuldu. Optik dansite değerleri ve sitokin konsantrasyonları arasındaki ilişki dağılım grafiği ile gösterildi.

2.4.2. İL-1ra ELISA kiti protokolü

ELİSA kiti üretici firma prosedürlerine uygun olarak çalışılmıştır (Abcam). Kısaca, önceki kite yapıldığı gibi, antikor kokteyli hazırlandı. 50 µl örnekler ve standartlar uygun kuyucuklara eklendi. Ardından 50 µl antikor kokteyli uygun kuyucukları ilave edildi. Plak oda sıcaklığında 400 rpm'de bir plaka çalkalayıcıda 1 saat inkübe edildi. İnkübasyondan sonra, plaka otomatik bir yıkayıcı (HydroFlex, Tecan, İsviçre) kullanarak üç kez yıkama solüsyonu ile yıkandı. Ardından 100 µl TMB substratı her kuyucuğa eklenip karanlıkta 10 dakika boyunca bir plaka çalkalayıcı üzerinde inkübe edildi. Daha sonra 100 µl stop solüsyon her kuyucuğa ekleyerek 450 nm'de OD mikroplak okuyucu (Synergy, Biotek, ABD) okutuldu. Optik dansite değerleri ve sitokin konsantrasyonları arasındaki ilişki dağılım grafiği ile gösterildi.

2.4.3. VEGF ELISA kiti protokolü

ELİSA kiti üretici firma prosedürlerine uygun olarak çalışılmıştır (Abcam). Kısaca antikor kokteyli hazırlandı. 50 µl örnekler ve standartlar uygun kuyucuklara eklendi. Ardından 50 µl antikor kokteyli uygun kuyucukları ilave edildi. Plak oda sıcaklığında 400 rpm'de plak çalkalayıcıda 1 saat inkübe edildi. İnkübasyondan sonra, plaka yıkama için otomatik bir yıkayıcı (HydroFlex, Tecan, İsviçre) kullanıldı. Yıkama işlemi bitikten sonra 100 µl TMB substratı eklenip karanlıkta 10 dakika boyunca bir plaka çalkalayıcı üzerinde inkübe edildi. Ardından 100 µl stop solüsyon her kuyucuğa eklenip 450 nm'de OD bir mikroplaka okuyucu (Synergy, Biotek, ABD) kullanarak okundu. Optik dansite değerleri ve sitokin konsantrasyonları arasındaki ilişki dağılım grafiği ile gösterildi.

2.4.4. PGE2 ELISA kiti protokolü

ELİSA kiti üretici firma prosedürlerine uygun olarak çalışılmıştır (Abcam). Kısaca 100 µl gözyaşı örnekleri ve standartlar, keçi anti-fare IgG ile kaplanmış 96 kuyucuklu plaka ilave edildi. Ardından 50 µl PGE2-AP konjugatı her kuyucuğa aktarıldı. Plak 2 saat oda sıcaklığında 500 rpm'de bir plaka çalkalayıcıda inkübe edildi. İnkübasyondan sonra, plaka bir yıkama solüsyon ile otomatik yıkayıcı (HydroFlex, Tecan, İsviçre) kullanılarak üç kez yıkandı. Yıkama işlemi bitikten sonra 200 µl pNpp substratı her kuyucuğa eklenip karanlıkta 1 saat 37°C'de çalkalanmadan inkübe edildi. Ardından 50 µl her kuyucuğa stop solüsyonu eklendi ve 405 nm'de bir mikroplaka okuyucu (Synergy, Biotek, ABD) kullanılarak OD hemen ölçüldü. Optik dansite değerleri ve sitokin konsantrasyonları arasındaki ilişki dağılım grafiği ile gösterildi.

2.5. İstatistiksel analiz

Çalışma verilerinin istatistiksel analizi için GraphPad Software Inc.'in Prism 9 ve IBM SPSS versiyon 29 yazılımı kullanıldı. Her değer, ortalama± standart sapma olarak belirlenmiştir. Gruplar arası karşılaştırmalar t- testi ve tek yönlü ANOVA testi ile yapıldı. Grupların klinik parametreleri, goblet hücre yoğunluğu, MUC5AC ekspresyonu ve sitokin konsantrasyonu arasındaki korelasyonun analizi Pearson korelasyon analizi ile yapıldı. $P< 0.05$ bir istatistiksel anlamlı olarak kabul edildi.

3. BULGULAR

Çalışma Haziran 2021-Aralık 2021 tarihleri arasında Gazi Üniversitesi Tıp Fakültesi Göz Hastalıkları Anabilim Dalı'nın katkısıyla İmmünoloji Anabilim Dalı tarafından gerçekleştirildi. Çalışmaya toplam 30 hasta (15 kuru göz ve 15 glokom) ve kontrol grubunu (KG) 15 sağlıklı katılımcı dahil edildi.

3.1. Çalışmaya alınan olguların demografik ve klinik özellikleri

Olguların ortalama yaşı (ortalama ± SS) Glokom hastalarında 52,87±11,84, kuru göz hastalarında 47,60±10,58 ve kontrol grubunda 46,27±7,066 idi. Yaş açısından üç grup arasında istatistiksel olarak anlamlı fark bulunmadı (P=0.1754). Cinsiyete göre kadın ve erkek oranı glokom grubunda %60/40, kuru göz grubunda %66,6/33,3 ve kontrol grubunda %66,6/33,3 idi.

OSDI anketi sonuçlarına göre yedi (%46,66) glokom hastasında hafif ile orta derecede, sekizinde (%53,33) şiddetli kuru göz vardı; kuru göz grubu ile ilgili olarak, iki (%13,33) kişinin oküler yüzeyi normal, sekiz (%53,33) kişinin hafif-orta ve beşinin (%33,33) şiddetli göz kuruluğu vardı. Kontrol grubu için OSDI puanı normal olan 13 kişi (%86.66), OSDI puanı hafif olan 2 kişi (%13.33). OSDI, schirmer testi, TBUT ve kornea boyama için glokom, kuru göz ve normal olguların arasında istatistiksel olarak anlamlı bir fark bulunmuştur ($p<0,0001$).

3.2. Konjonktival impresyon sitolojisi

3.2.1. PAS boyama sonuçları

Nelson skorlama sistemi

Örnekler, Nelson tarafından açıklanan kriterlere göre değerlendirilerek skorlama yapıldı. İmpresyon sitolojisinin sonuçları üç grup arasında farklılık gösterdi. Glokomda hastalarda bası sitolojisi 4 olguda (%20.66) derece 1, 9 olguda (%60) derece 2 idi ve 2 olguda derece 3 (%13.33). Kuru göz grubunda impresyon sitolojisi 9'da derece 1 idi. Olgularda (%60) ve 6 olguda (%40) derece II. Kontrol grubunda bası sitolojisi 13 olguda (%86.66) derece 0, 2 olguda (%13.33) derece I idi.

PAS boyamasında goblet hücre yoğunluğunun (GCD) belirlenmesi

Ortalama goblet hücreler yoğunluğunun, 1 mm konjonktival epitelyal başına goblet hücreleri sayılarak belirlendi. Glokom hastalarında GCD 4.120 ± 4.437 idi. Kuru göz hastaları ve kontrol grubunda GCD sırasıyla 5.653 ± 2.951, 16.97 ± 5.380 idi. Glokom ve kontrol grubunda goblet hücre yoğunluğunun arasında anlamlı bir fark saptanmıştır (P<0.0001). Ayrıca, kuru göz ve kontrol grupları arasında goblet hücre yoğunluğununde anlamlı bir fark görülmüştür (P<0.0001). Glokom ve DE grubu arasında goblet hücre yoğunluğununde anlamlı fark görülmemiştir (P= 0.2745).

3.2.2. İndirekt immüno floresan sonuçlarıyla MUC5AC'nın ekspresyonu değerlendirilmesi

Anti-MUC5AC antikoru kullanılarak impresyon sitolojisi örneklerinin indirekt immüno floresan boyaması ile glikoprtien ekspresyonu değerlendirilmiştir. İndirekt immüno floresan sonuçlarının değerlendirilmesi, pozitif MUC5AC hücreleri sayılarak yapıldı. Kontrol grubunda MUC5AC ekspresyonunun (ortalama ± standart sapması) 18.42 ± 8.179 ve glokom olgularında 8.450 ± 7.619 ve bu iki grup arasında anlamlı bir fark bulunmuştur (P = 0.0018). Ayrıca kontrol ve kuru göz (10.89 ± 9.803) grupları arasında anlamlı fark saptanmıştır (P=0.0301). Glokom ve kuru göz grupları arasında anlamlı bir fark görülmemiştir (P = 0.4532).

3.3. Gözyaşı örneklerindeki sitokinlerin değerlendirmesi

Glokom, kuru göz ve kontrol gruplarından alınan gözyaşı örneklerinde IL-1β, IL-1Ra, VEGF ve PGE2 sitokinlerinin konsantrasyonu belirlenmiş ve anlamlı farklılıklar olup olamadığını araştırılmıştır.

3.3.1. İL-1β

İL-1β konsantrasyonu hem glokom hem de kuru göz gruplarının gözyaşındaki kontrol grubuna göre önemli ölçüde daha yüksekti. İL-1β'nin gözyaşındaki ortalama konsantrasyonu ve standart sapması kontrol grubunda 242,4 ± 111,1 pg/ml, kuru göz grubunda 418,5 ± 318,0 pg/ml ve glokom grubunda 710.1 ± 621.2pg/ml. Glokom ve kontrol grupları arasındaki fark istatistiksel olarak anlamlı bir fark bulumuştur (P= 0.0137). Kuru göz grubu ile kontrol grubu karşılaştırıldığında, iki grup arasında anlamlı bir fark

saptanmamıştır (P = 0.0515). Gözyaşındaki İL-1β düzeyinin glokom hastalarında kuru göz hastalarından daha yüksek olduğu ancak bu farkın istatiksel olarak anlamlı olmadığı görülmüştür (P=0.4509).

3.3.2. İL-1Ra

İL-1Ra konsantrasyonu hem glokom hem de kuru göz gruplarının gözyaşı sıvısında, kontrol grubuna göre önemli ölçüde daha yüksekti. Gözyaşındaki İL-1Ra düzeyi, kontrol grubunda 3661 ± 3975 pg/ml, kuru göz grubunda 6056 ± 6323 pg/ml ve glokom grubunda 7617 ± 9022 pg/ml. İL-1Ra konsantrasyonu glokom grubunda diğer gruplara (kuru göz grubu, kontrol grubu) göre daha yüksek olmasına rağmen, glokom ile kuru göz grubu (P=0.7006) veya glokom ile kontrol grubu (P= 0.2012) arasında istatiksel olarak bir farkın anlamlı olmadığı görülmüştür. Ayrıca kuru göz grubu ile kontrol grubu arasında istatistiksel olarak fark bulunmadı (P = 0.5907).

3.3.3. VEGF

Gözyaşındaki VEGF düzeylerinin ortalama değerleri kontrol grubunda 454 ± 262 pg/ml, kuru göz grubunda 818,8 ± 642,7 pg/ml ve glokom grubunda 1276 ± 1060 pg/ml. Gözyaşındaki VEGF seviyesi glokom grubunda kontrol grubuna göre istatistiksel olarak fark saptanmıştır (P= 0.0069). Glokom ve kuru göz grubu arasında VEGF konsantrasyonlarında istatistiksel olarak anlamlı fark gözlenmemiştir (P=0,1645). Kuru göz ve kontrol grubu arasında da istatistiksel olarak anlamlı bir fark bulunmamıştır (P=0.0513).

3.3.4. PGE2

Gözyaşlarındaki PGE2 sitokin düzeylerinin ortalama değerleri kontrol grubunda 325.2 ± 164,2 pg/ml, kuru göz grubunda 503,3 ± 331,4 pg/ml ve glokom grubunda 930,9 ± 880,7 pg/ml. Glokom grubunda gözyaşındaki PGE2 sitokin düzeyi kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlı bir fark saptanmıştır (P=0.0141). Glokom ve kuru göz grupları arasında PGE2 konsantrasyonunda anlamlı fark bulunmamıştır (P =0.0894). Kuru göz ve kontrol grupları arasında istatistiksel olarak anlamlı bir fark olmadığı belirlenmiştir (P = 0.0726).

3.4. Korelasyon analizi

3.4.1. Gözyaşı sitokinleri ile oküler yüzey parametreleri arasındaki korelasyonlar

Gözyaşındaki sitokinleri ile OSDI anketi, göz yaşı kırılma zamanı (TBUT), Schirmer testi ve kornea boyama oküler yüzey parametreleri arasındaki korelasyonları araştırdık. OSDI anketi ile şu sitokinler arasında zayıf pozitif ancak anlamlı olmayan korelasyon bulundu: İL-1β (r = 148), VEGF (r = 262) ve PGE2 (r = 163). OSDI ile İL-1Ra arasında korelasyon bulunmamıştır (r = 0.053).

Göz yaşı kırılma zamanı ile şu iki sitokin arasında güçlü negatif ve anlamlı korelasyonlar saptanmıştır: VEGF (r = -0,306, P =0,041) ve PGE2 (r = -0,302, P =0,044). Ancak göz yaşı kırılma zamanı ile İL-1β (r =-0.244, P = 0.106) ve İL-1Ra (r = -0.139, P = 0.362) arasında zayıf bir negatif ve anlamlı olmayan korelasyon saptanmıştır.

3.4.2. Enflamatuar sitokinler ile impresyon sitolojisi sonuçları arasındaki korelasyon (GCD, MUC5AC ekspresyonu)

Enflamatuar sitokinler ve impresyon sitoloji sonuçları (goblet hücre yoğunluğnu, MUC5AC ekspresyonu) arasında negatif bir korelasyon bulunmuştur, ancak bu korelasyonun anlamlı olmadı saptanmıştır.

4. TARTIŞMA

Glokom olan hastalarında oküler yüzey hastalıklarını gelişme riski daha yüksektir, çünkü bu iki hastalıklar yaşlı insanlarda daha sıklıktır. Ayrıca, koruyucu madde içeren anti-glokomatöz göz damlalarının kronik kullanımı oküler yüzey hastalıklarını neden olabilir. Oftalmolojik ilaçlarda en yaygın kullanılan koruyuculardan biri Benzalkonyum klorürdür. Benzalkonyum klorürünün toksisitesi, konjonktival hücrelerde daha önce saptanmıştır [299], Benzalkonyum klorür düşük bir konsantrasyonda bile bir hücresel ölüm indükleyicisi olarak tanımlandı. Oküler ve konjonktival epitel hücrelerinde, Benzalkonyum klorür oksidatif stres ve inflamatuar reaksiyonları indükleyebilen hidrojen perokside dönüşür [300]. BAK, uygulamadan sonra 1 haftaya kadar birçok oküler dokuda kalabilir ve oküler yüzeydeki epitelini toksik etkilere karşı savunmasız bırakır [301].

Kuru göz hastalığı (KGH), yaş, kornea iltihabı ve hormonal değişikliklerle ilişkili ve dijital ekranların uzun süre kullanılmasıyla şiddetlenen kronik bir göz bozukluğudur. COVID 19 pandemisinden sonra insanlar tarafından uzun süre yüz maskesi kullanılması nedeniyle kuru göz prevalansının arttığı söylenebilir.

Bu çalışmada, topikal ilaçlar alan glokom hastalarının yanı sıra kuru göz hastalarının gözyaşlarındaki bazı enflamatuar sitokinlerin ekspresyonunu sağlıklı kontrollerle karşılaştırmayı amaçladık. Ayrıca bu hastalarda oküler yüzey değişikliklerini değerlendirdik.

Glokomlu hasta grubumuzun ilaç öykülerini alarak, hastaların büyük çoğunluğunun birden fazla ve bir yıldan uzun süredir ilaç kullandığını öğrendik. Lavin ve arkadaşları, en az bir yıl boyunca medikal tedavi görmüş olanlarla ameliyat öncesi ortalama sadece iki hafta medikal tedavi almış hastaları karşılaştırdığında filtrasyon cerrahisinin başarılı olma oranının yüksek olduğunu bildirmişlerdir [302].

Bu çalışmada OSDI anketi, oküler yüzey hastalıkları prevalansını belirlemek amacıyla çeşitli çalışmalarda sık kullanılmıştır. Çalışmamızda OSDI değerleri, üç grup arasında istatistiksel olarak anlamlı farklılıklara saptanmıştır. Kontrol grubundaki katılımcıların çoğu, normal ile hafif OSDI ile başvurdu. Bunun dışında glokom ve kuru göz hastalarının OSDI skoru kontrol grubuna göre daha yüksekti (P=0.0001).

Kuru göz hastalığı için yapılan klinik testlerle ilgili olarak hem glokom hem de kuru göz olan hastalarında ciddi anormallikler saptanmıştır. Schirmer testinin glokom ve kuru göz hastalarında sağlıklı kontrollere göre oldukça düşüktü (P=0.0001), bu da her iki hastalık grubunda da gözyaşı üretmini azaldığını gösteriyor. Ayrıca göz yaşı kırılma zamanı, sağlıklı kontrollere kıyasla glokom hastalarında ve kuru göz hastalığında (P = 0.0001) istatistikal anlamlı olarak daha kısaydı. Bu veriler sağlıklı kontrole kıyasladığımızda hem glokom hem de kuru göz hastalığı gruplarında gözyaşı filminin instabilitesini yansıtıyor. Sonuçlarımız, topikal ilaçlar alan glokom hastalarında oküler yüzey hastalıkları prevalansında artış gösteren önceki çalışmaların sonuçlarıyla da uyumludur [303,304].

Göz yaşı fonksiyon birimi (LFU), konjonktival goblet hücreleri, yüzey epiteli, göz kapakları, lakrimal bezler, meibom bezleri, lakrimal drenaj sistemi, mukozal bağışıklık

sistemi ve bunları birbirine bağlayan sinir ağından oluşmaktadır. Bu dokular, gözyaşı filminde immün ekspresyon seviyelerini düzenleyen, oküler yüzeyin bütünlüğünü koruyan ve korneanın şeffaflığını koruyan işlevsel bir etkileşime sahiptir [305].

İnflamasyonun oküler yüzey hastalıkları etiyolojisinde çok önemli bir rolü oynadığı daha önce belirlenmiştir. Kuru göz hastaları ile kontrol grubu bu sitokinlerin ölçülmesi ve karşılaştırılması, sitokinlerin kur göz patogenezinde oynadığı rolün daha iyi anlaşılmasını sağlayacaktır. Gözyaşındaki sitokin düzeylerini ölçmek için çeşitli yöntemler kullanılmıştır, bu yöntemlerden biri de ELISA (Enzyme-Linked ImmunoSorbent Assay)'dır. ELISA, çeşitli biyolojik örneklerde sitokinleri ölçmek için yaygın olarak kullanılan oldukça hassas ve spesifik bir yöntemdir.

İnsan gözyaşı, oküler yüzeyin işlevini ve morfolojisini korumaya yardımcı olan çok çeşitli sitokinler ve kemokinler içermektedir. Oküler yüzey epitel hücreleri hiperosmolar strese maruz kaldığı zaman gözyaşında proinflamatuar sitokinler salınımına neden olmaktadır. Oküler yüzeydeki epitel hücrelerin, enflamasyon olduğunda stresini gösterebilen İL-1β, İL-6, İL-8 ve TNF-α salgıladığı bilinmektedir. Bu, mevsimsel alerjik konjunktivit ve vernal ve atopik keratokonjonktivit dâhil olmak üzere farklı enflamatuar oküler yüzey bozuklukları olan hastaların gözyaşlarında neden bu sitokinlerin bulunduğunu açıklayabilir [306]. Gözyaşı örnekleri elde etmek ve çeşitli proteinlerin seviyesini değerlendirmek için farklı teknikler mevcutur. Bunlardan biri invazif olmayan ve basit olması nedeniyle bu çalışmada kullanılan wash-out yöntemidir. Orta derecede kuru göz hastalığı olan bireylerde çok sayıda sitokinin normal ekspresyon profillerini bulan Narayanan ve arkadaşları dışında, araştırmaların çoğu kuru göz hastalarının gözyaşlarında daha yüksek sitokin seviyeleri tespit etmiştir.

Sitokinler, bağışıklık yanıtının ve iltihaplanmanın düzenlenmesinde çok önemli bir rol oynayan küçük moleküler ağırlığa sahip proteinlerdir. İL-1 sitokin ailesi, doğal ve adaptif bağışıklık sistemleri için anahtar sinyal molekülleridir. İL-1, romatoid artrit, periodontitis ve septik şok dahil olmak üzere insanlarda enflamatuar bozuklukların ve ayrıca kornea ve oküler yüzey hastalıklarının patogenezinde önemli rol oynamaktadır. Oküler irritasyonun yüksek olduğu zaman inflamatuar hasarı belirlenmektedir. Bizim çalışmamızda elde edilen gözyaşı sıvısındaki proinflamatuar İL-1β sitokin düzeyinin glokom grubunda daha yüksek olduğu ve kontrol grupla karşılaştırdığımızda anlamlı bir fark olduğunu bulunmuştur

(P=0,0623). Glokom grubu ile Kuru Göz grubu (P=0,1337) arasında anlamlı bir fark bulunmadığını saptanmıştır. Diğer çalışmalarda, Kuru Göz hastalarında diğer inflamatuar sitokinler ile (İL-1, TNFα) birlikte artan İL-1β seviyeleri gösterilmiştir [164]. İL-1β'nin nöronal ve hormonal mekanizmalar yoluyla gözyaşı oluşumunu azalttığı gösterilmiştir [307].

İL-1Ra, İL-1'in proinflamatuar formlarının İL-1R1 sinyallerine bağlanmasını rekabetçi bir şekilde inhibe ederek çalışır. İL-1β'nın aksine, anti-inflamatuar form, İL-1Ra, yüksek miktarda bulunmaktadır. İL-R1a düzeyi hem glokom hem de kuru göz hastalarında kontrol grubuna göre yüksekti, ancak gruplar arasında istatistikal anlamlı fark yoktu. Gözyaşı sıvısındaki yüksek İL-1Ra konsantrasyonu, oküler yüzeyde İL-1 aracılıyla enflamatuar süreçlerin aktivasyonunu engelleyen fizyolojik bir homeostatik mekanizma olabilir. Bu bulgu, kuru göz hastalığı olan hastaların gözyaşlarında artmış bir İL-1Ra seviyesi gösteren önceki çalışmalarla uyumludur [308].

İL-1β ve İL-1Ra arasındaki denge bozulması, enflamatuar bozuklukların ilerlemesini teşvik etmektedir. Çalışmamız, İL1RA'nın İL-1β'ya oranını çalışmamz olgularında daha yüksek olduğunu gösterilmiştir. Bu sonuç, bu hastlarda pro- ve antiinflamatuar sitokinler arasında bir dengesizlik olduğunu gösterir ve bu sitokin dengesinin yeniden sağlanmasının gelecekteki potansiyel bir tedavi olabileceğini düşündürmektedir. İL-1Ra'nın topikal uygulaması kullanımının, kuru göz hastalığının klinik semptomlarını hafifletmede ve altta yatan iltihabı azaltmada faydalı olduğu gösterilmiştir.

Vasküler endotel faktörü (VEGF), neovaskülarizasyon, drenaj sisteminin yeniden şekillenmesi ve iltihaplanma ile ilgili bir sitokin olarak karakterize edilmiştir. VEGF'nin kornea transplantasyonu, neovasküler glokom, yaşa bağlı maküla dejenerasyonu ve diyabetik retinopati gibi anjiyogenezi içeren bir dizi oküler bozukluktan sorumlu olduğu artık net şekilde belgelenmiştir [309]. Yoo ve arkadaşları [310], VEGF'nin, İL-8 ve MCP-1 gibi kemotaktik faktörler üretmek üzere endotel hücrelerini aktive ederek doğrudan bir proinflamatuar etkiye sahip olduğunu ortaya çıkardı. VEGF ayrıca monositleri, İL-6 ve TNF-α gibi iltihaplanma sürecini şiddetlendiren proinflamatuar molekülleri salgılaması için uyarabilir [311]. Önceki çalışmalarda, kuru göz hastalığı ve vernal keratokonjonktivit gibi oküler kronik inflamatuar bozuklukları olan hastalardan alınan gözyaşı örneklerinde VEGF konsantrasyonlarının arttığı gösterilmiştir [312]. Jiang ve arkadaşları [311], kuru

göz hastalığı olan hastalarda subkonjonktival bevacizumab (VEGF karşıtı) enjeksiyonunun oküler yüzeydeki enflamasyonu giderebileceğini gösterdi.

Çalışmamızda elde dilen veriler, glokom grubunu kontrol grubu ile karşılaştırdığımız zaman daha yüksek ortalama serum VEGF düzeyine sahip olduğunu göstermiştir (P=0.0069). Evaporatif kuru göz hastalığını normal katılımcılarla karşılaştıran bir çalışmada, kuru göz grubunda gözyaşlarındaki VEGF düzeylerinin yüksek olduğunu gösterilmiştir [313]. Bir çalışmada, bizim sonuçlarımızın aksine hem kontrol hem de glokom grubu ile karşılaştırıldığında kuru göz hastalarında VEGF seviyesinde anlamlı bir azalma olduğu fark edilmiştir [314]. Lenfatiklerin, antijen sunan hücreleri, T lenfositlerinin hazırlandığı ve genişletildiği drenaj lenf nodlerine yönlendirerek dokularda enflamatuar yanıtları başlatmada çok önemli bir rol oynadığı iyi bilinmektedir. Kuru göz hastalığı korneasında anjiyojenez olmadan lenf anjiyogenezinin meydana geldiği bildirilmiştir [309]. Sonuç olarak, bu lenfoid organlarda, kuru göz hastalığı immünopatogenezinde rol oynayan otoreaktif Helper T hücresi (TH)17 ve TH1 hücreleri üretilmektedir [315].

İL-1'in, tümörlerin büyümesini destekleyen ve çeşitli hücre tiplerinde VEGF'yi uyaran bir pro-anjiyojenik faktör olduğu gösterilmiştir [316]. Ayrıca, VEGF ekspresyonu prostaglandinler tarafından indüklenmesine yardımcı olmaktadır.

Prostaglandinler (PG'ler), akut enflamasyonun aracıları olarak iyi tanınmaktadır, bunların ayrıca kronik enflamasyonun geçişinde ve sürdürülmesinde de rol oynadıklarını gösterilmiştir. Birkaç çalışma, kuru göz hastalığı ve glokomlu hastalardaki gözyaşlarında PGE2 konsantrasyonlarının arttığını göstermiştir; bu çalışmaların sonuçları, kuru göze sahip farelerin gözyaşı üreten dokusundaki yüksek COX-2 ve PGE sentaz seviyeleri ile daha da desteklenmiştir. Bulgularımız, gözyaşındaki PGE2 seviyesinin glokomlu hastalarda kontrol grubuna göre yüksek olduğunu gösterdi (P=0.0141), bu da PGE2'nin oküler yüzey hastalıklarının enflamatuar süreçlerindeki potansiyel önemini göstermektedir.

Kuru göz hastalığı olan hastalardaki yüksek PGE2 seviyesi, PGE2 sekresyonunu daha da indükleyen diğer inflamatuar molekülleri uyararak oküler yüzey enflamasyonunu artırabilir. Buna ek olarak, oküler yüzeyde prostaglandınler vazodilatasyonu, kan-oküler bariyerin bozulmasını ve lökosit göçünü desteklemektedir [318].

Konjunktival impresyon sitolojisi, oküler yüzey hücre popülasyonlarının araştırılmasını çok önemli bir ölçüde geliştirmiştir. İmpresyon sitolojisi çeşitli oküler yüzey bozukluklarının etiyolojik tanısını, konjonktival ve kornea yüzeyindeki zaman içinde oluşan değişikliklerin belgelenmesini, tedavi etkilerinin izlenmesini ve konjonktival skuamöz metaplazinin evrelenmesini analiz etmek amacıyla sık kullanılan araştırma aracıdır.

Bunlara ek olarak epitel hücre morfolojisi ve konjunktival goblet hücrelerin yoğunluğun, oküler yüzeyin bütünlüğünü değerlendirmek için objektif bir ölçü sağlayabilir.

Skuamöz hücre metaplazisi, çok çeşitli toksik inflamatuar uyaranlara spesifik olmayan bir konjonktival yanıttır [319]. Paschides ve arkadaşları, yaşlanmanın normal gözlerde goblet hücrelerinin miktarı veya nükleer sitoplazmik oran üzerinde hiçbir etkisinin olmadığını bildirmiştir [320]. Ayrıca Herreras ve arkadaşları goblet hücre densitinin ve yapısının 80 yaşın altındaki hastalarda stabil olduğunu göstermiştir [321].

Genel olarak müsinler, vücudun mukozal yüzeylerinin çoğunu kaplayan büyük hücre dışı glikoproteinler olarak tanımlanmaktadır. Goblet hücreleri, gözyaşı filminin mukus tabakasının birincil bileşeni olarak kabul edilen jel oluşturucu müsin MUC5AC'yi salgılar ve bu tabaka, gözyaşı filminin stabilitesini korumaktan sorumludur. Konjonktival goblet hücrelerinin, retinoik asit (RA) ve TGF-β2 dâhil olmak üzere immünomodülatör moleküller salgıladığı da bulunmuştur [322].

Ayrıca, goblet hücreler, önceki yapılan bir çalışmanın bulgularına göre konjonktival immünolojik toleransın indüklenmesinde çok önemli bir rol oynamaktadır. Son zamanlarda, ince bağırsak ve koloniktaki goblet hücreleri lubrikant işlevi yanı sıra dendritik hücrelere de luminal antijen sunumundan sorumlu olduğu keşfedilmiştir. Bu özellik aynı zamanda, oküler yüzeyde altta yatan stromada immün hücrelere antijen taşıma kanalları olarak hizmet eden konjonktival goblet hücrelerde de tanımlanmıştır. Oküler yüzey hastalığının şiddeti ile korelasyona ek olarak, goblet hücrelerinin kaybı oküler yüzeyde bir kronik enflamasyon döngüsünü sürdürür gibi görünmektedir [323].

Bu çalışma, impresyon sitolojisi için PAS boyama sonuçlarında kontrol grubuyla karşılaştırıldığında glokom ve kuru göz gruplarında konjonktival goblet hücre yoğunluğu

önemli ölçüde azaldığını saptanmıştır (P <0.0001). Konjonktival goblet hücredeki azalma, kuru gözde oküler yüzey hasarının önemli bir işaretidir. Artan hastalık şiddeti ile goblet hücre sayısı azalır, bu da skuamöz metaplazi, epitelyal bölgenin hiperplazisi ve bazen oküler yüzeyel keratoz ile sonuçlanır.

Oküler yüzeydeki müsin proteinlerinin saptanması ve miktar tayini, PAS, lektin ve Alcian mavisi boyaları ile müsin histokimyası dahil olmak üzere çeşitli tekniklerle elde edilmiştir. Ayrıca bu boyamalar spesifik olmayabilir. Alınan sonuçlara göre normal kontrol grubuyla karşılaştırıldığında glokom hastalarında MUC5AC ekspresyonunun ciddi ölçüde azaldığını saptanmıştır ve bu farkın istatistiksel olarak anlamlı olduğu göstermiştir (P = 0.0018). Ayrıca kuru göz ve kontrol grubu arasında MUC5AC ekspresyonu belirgin azalma olduğnu bulumuştur (P=0.0301).

Goblet hücre yoğunluğnu ve MUC5AC ekspresyonu, başarılı ve başarısız ameliyatlarda önemli ölçüde ifade edildi: Histoloji ve konfokal mikroskopi, daha yüksek postoperatif GCD'nin daha iyi GİB kontrolü ve daha yüksek başarı oranı ile ilişkili olduğunu ortaya koydu [325].

Bu çalışmada ayrıca enflamatuar sitokinlerin seviyeleri ile kuru göz hastalığının klinik parametreleri arasındaki ilişkiyi de araştırdık. Sonuçlarımız, gözyaşı kırılma zamanı (TBUT) ve Schirmer testi ile VEGF ve PGE2 sitokinleri arasında negatıf bir korelasyon olduğunu görülmüştür. Bu, sitokinlerin artan seviyesinin gözyaşı filmi instabilitiliği ve azalmış gözyaşı üretimiyle ilişkili olduğunu düşündürmektedir.

Hem İL-1β hem de PGE2, kornea lekelenmesi ile pozitif korelasyon göstermiştir. Bu korelasyon, hem İL-1β hem de PGE2'deki artışın, oküler yüzeyde daha fazla hasar ile ilişkili olduğunu göstermektedir. Bu bulgular, İL-1β ile kornea boyaması arasında pozitif bir korelasyon olduğunu gösteren bir diğer çalışma ile uyumludur [308].

Diğer bir çalışmada, bizim bulgularımızın aksine, sadece PGE2 konsantrasyonlarının OSDI skoru ile anlamlı bir şekilde ilişkili olduğu ancak gözyaşı kırılma zamanı, Schirmer testi veya kornea boyama ile ilişkili olmadığı bulunmuştur [326].

Çalışmamızın sonuçları, glokom hastalarının İL-1β, VEGF, PGE2 ve İL-1Ra'nın yüksek ekspresyonunu saptanmıştır. Koruyucu madde içeren topikal ajanların ve birden fazla topikal ilaçların kullanımı, bu ilaçların doza ve zamana bağlı bir şekilde oküler yüzey üzerindeki etkisini yansıtan bu enflamatuar sitokinlerin daha yüksek konsantrasyonlarını açık bir şekilde göstermiştir.

5. SONUÇ

Sonuç olarak, çalışmamızda glokom ve kuru göz hastalarında impresyon stolojisi örneklerinde hem goblet hücrelerinin sayısında hem de MUC5AC ekspresyonunda ciddi bir azalma olduğu bulunmuştur.

Ayrıca glokom hastalarında İL-1β, İL-1Ra, VEGF ve PGE2 sitokinlerinin konsantrasyonunda kuru göz ve normal kontrol gruplarına göre artış olduğu saptanmıştır. Ancak sadece İL-1β, VEGF ve PGE2 sitokinlerin seviyeleri anlamlı bulunmuştur.

Hem VEGF hem de PGE2, gözyaşı kırılma zamanı ve shirmer testi ile negatif korelasyon saptanmıştır. İL-1β ve PGE2 ile kornea boyanması arasında pozitif korelasyon bulunmuştur. Bu korelasyonlar, kuru gözün şiddetini belirlemek amacıyla bu testlerin kullanılmasının yardımcı olabileceğini düşündürtmektedir.

Glokom ve kuru göz olan hastalrında oküler yüzeyindaki değişkenlerin tam temel mekanizmasının ve enflamasyonu üzerindeki etkilerini belirlemek için daha fazla hasta sayısı ile tasarlanmış çalışmaları ihtiyaç olacağını döşünülmektedir. Ayrıca, bu hastalarda oküler yüzey hastalıklarının prevalansını azaltmaya yardımcı olmak için yeni koruyucu madde seçeneklere ihtiyaç vardır.

ACKNOWLEDGEMENTS

Foremost it’s my pleasure to express my deep grateful and sincere thanks to my supervisor Professor Dr. E. Ümit bağrıaçık for his indefinite support and wise guidance in my PhD study, for his motivation and encouragement throughout the journey. His supervision taught me how to be patient, keen, and punctual.

Furthermore, I would like to express my gratitude and appreciation to Prof. Dr. Ayşegül ATAK YÜCEL, Prof. Prof. Dr. Resul Karakuş, Prof. Dr. Vedat BULUT for the invaluable scientific knowledge that they provided me during my Ph.D. education.

I would also like to thank Ass. Prof. Dr. Cüneyt Özmen for his support and help. Further, I would like to thank my colleagues Ass. Prof. Melek Yaman and lecturer Nihan Örüklü for their hleping and support during my project.

Lastly, I would be remiss in not mentioning my family, especially my parents. Their belief in me has kept my spirits and motivation high during this process.

CONTENT

Page

ABSTRACT iv

ÖZET v

GENİŞLETİLMİŞ TÜRKÇE ÖZET vi

ACKNOWLEDGEMENTS xxiv

CONTENT xxv

LIST OF TABLES xxviii

LIST OF FIGURES xxix

LIST OF SYMBOLS AND ABBREVIATIONS xxx

1. INTRODUCTION 1

2. GENERAL INFORMATION 3

2.1. Glaucoma 3

2.1.1. Definition of glaucoma 3

2.1.2. Prevalence and incidence of glaucoma 3

2.1.3. Risk factors for developing glaucoma 3

2.1.4. Classification of glaucoma 6

2.1.5. Primary open angle glaucoma (POAG) 6

2.1.6. Pathogeneis of glaucoma 6

2.1.7. Treatment of glaucoma 8

2.2. Ocular Surface Diseases in Association with Glaucoma 10

2.2.1. Gross anatomy and function of the ocular surface 11

2.2.2. Tear film 13

2.3. Dry Eye Disease 16

2.3.1. Classification of DED 17

2.3.2. The immune regulation of the ocular surface 18

Page

2.3.3. Pathogenesis of dry eye disease 19
2.3.4. The inflammatory response in DED 20
2.3.5. Diagnosis of DED 23
2.4. Cytokines 27
2.4.1. Interleukine-1 cytokine family 28
2.4.2. Prostaglandin E2 (PGE2) 31
2.4.3. Vascular endothelial growth factor (VEGF) 32
2.5. Role of Anti-Inflammatory Therapies in DED 33
2.5.1. Cyclosporine (CSA) 34
2.5.2. Tacrolimus 34
2.5.3. NSAIDs 34
2.5.4. LFA-1 antagonist 35
2.5.5. Thymosin ß4(Tb4) 35
3. MATERIALS AND METHODS 37
3.1. Tools – Equipment 37
3.2. Subjects and Study Design 37
3.3. Clinical Parameters of DED 38
3.3.1. OSDI questionnaire 39
3.3.2. Schirmer I test 39
3.3.3. TBUT test fluorescein strips 39
3.3.4. Corneal fluorescein staining 39
3.4. Tear Collection Method 40
3.5. Conjunctival Impression Cytology Sample Collection 40
3.5.1. Procedure of PAS- hemtoxylin staining 41
3.5.2. Indirect immunofluorescence staining 41
3.6. ELISA 42

Page

3.6.1. IL-1B ELISA kit 42
3.6.2. IL-1ra ELISA 42
3.6.3. VEGF ELISA 43
3.6.4. PGE2 ELISA 43
3.7. Statistical Analysis 44
4. RESULTS 45
4.1. Demographic and Clinical Characteristics of the Study Population 45
4.2. Impression Cytology of the Conjunctiva 46
4.2.1. PAS staining results 46
4.2.2. Assessment of MUC5AC expression by indirect immunofluorescence results 50
4.3. Cytokine Profiles of Tear Fluids 55
4.3.1. IL-1β 56
4.3.2. IL-1Ra 57
4.3.3. VEGF 59
4.3.4. PGE2 60
4.4. Correlation Analysis 61
4.4.1. Correlations between tear cytokines and ocular surface parameters 61
4.4.2. Correlation between inflammatory cytokines and impression cytology results (GCD, MUC5AC Expression) 62
5. DISCUSSION 65
6. CONCLUSION 73
REFERENCES 75
APPENDIX 99
APPENDIX-1. Ethics Committee Permission 100
CURRICULUM VITAE 103

LIST OF TABLES

Table **Page**

Table 3.1. Chemicals that were used in our study are given in table 37

Table 3.2. Kits that were used in our study are given in table 37

Table 3.3. Antibodies that were used in our study are given in table 37

Table 4.1. Distribution of the subjects included in the study by gender 45

Table 4.2. Clinical characteristics of the glaucoma, DE, and control group 46

Table 4.3. Impression cytology scoring in the glaucoma, DE, and control group 47

Table 4.4. MUC5AC expression (Mean, SD) in the glaucoma, DE, and control group 55

Table 4.5. Comparison of IL-1β concentration in the tear between the study groups ... 56

Table 4.6. Comparison of IL-1Ra concentration in the tear between the study groups. 58

Table 4.7. Comparison of VEGF concentration in the tear between the study groups.. 59

Table 4.8. Comparison of PGE2 concentration in the tear between the study groups... 61

Table 4.9. Correlation between cytokines and clinical parameters 62

Table 4.10. Correlation between both GCD and MUC5AC expression and cytoines in the study groups 63

LIST OF FIGURES

Figure **Page**

Figure 2.1. The vicious cycle of the pathology of DED adapted from 20

Figure 3.1. Flowchart demonstrating the sample processing schematic from CIC..... 41

Figure 4.1. A. PAS staining results of impression cytology in normal control group subjects (Magnification 20X). patients were given the same numbers dipected on the left corner of the picture for all figures..... 47

Figure 4.2. Mean GCD levels in glaucoma, DE and healthy control groups 50

Figure 4.3. A. Immunofluorescence image showing numerous MUC5AC positive goblet cells (green) from normal indeviduals. In blue DAPI nuclear staining. Patients were given the same numbers dipected on the left corner of the picture for all figures 52

Figure 4.4. Mean expression of MUC5AC in glaucoma, DE and healthy control groups..... 55

Figure 4.5. Mean tear IL-1β (pg/ml) levels in glaucoma, DE and healthy control groups..... 57

Figure 4.6. Mean tear IL-1Ra (pg/ml) levels in glaucoma, DE and healthy control groups..... 58

Figure 4.7. Mean of VEGF levels (pg/ml) in tears of glaucoma, DE and healthy control groups 60

Figure 4.8. Mean PGE2 levels (pg/ml) in tears of glaucoma, DE and healthy control groups..... 61

LIST OF SYMBOLS AND ABBREVIATIONS

The symbols and abbreviations used in thes thesis are presented in below with explanations.

Abbreviations	Descriptions
ADDE	Aqueus Deficient Dry Eye
APCs	Antigen-Presenting Cells
BAC	Benzalkonium Chloride
CAIs	Carbonic Anhydrase Inhibitors
CALT	Conjunctiva Associated Lymphoid Tissue
cAMP	Cyclic Adenosine Monophosphate
CCCR5	C-C Chemokine Receptor Type 5
COX-2	Cyclooxygenase-2
DCs	Dendritic Cells
DE	Dry Eye
DED	Dry Eye Disease
DEWS II	Dry Eye Workshop
EDED	Evaporative Dry Eye Desease
EGF	Epidermal Growth Factor
Foxp3	Forkhead Box P3 Protein
GC	Goblet Cell
GCD	Goblet Cell Density
GOND	Glaucomatous Optic Nerve Degeneration
HSP60	Heat Shock Protein 60
IFN-γ	Interferon- γ
IgA	Immunoglobulin A
IgG	Immunoglobuling
IgM	Immunoglobulin M
IL- 1β	Interleukin-1 Beta
IL-1Ra	Interleukin-1 Receptor Antagnist
IL-6	Interleukin-6
IL-8	Interleukin-8

Abbreviations	Descriptions
IOP	Intraocular Pressure
LF	Lactoferrin
LNs	Lymph Nodes
MAP Kinases	Mitogen-Activated Protein Kinase
MCP-1	Monocyte Chemoattractant Protein-1
MGD	Meibomian Gland Dysfunction
MHC Class II	Major Histocompatibility Complex Class II
MMP-9	Matrix Metalloproteinase 9
mRNA	Messenger Ribonucleic Acid
NF-Kb	Nuclear Factor-Kb
NTG	Normal Tension Glaucoma
OAG	Open Angle Galucoma
OHTN	Ocular Hypertension
ONH	Optic Nerve Head
OSD	Ocular Surface Desease
OSDI	Ocular Surface Disease Index
PAS	Peri-Iodic Acid
Pgas	Prostaglandin Analogs
PGE2	Prostaglandin E2
POAG	Primary Open Angle Glaucoma
pSS	Primary Sjogren's Syndrome
RA	Retinoic Acid
RA	Retinoic Acid
ROCK	Rho-Associated Protein Kinase
sIgA	Secretory İmmunoglobulin A
SS	Sjogren's Syndrome
sSS	Secondary Sjogren's Syndrome
TBUT	Tear Break Up Time
TF	Tear Film
TGF-2β	Transforming Growth Factor-Beta 2
TH1	T Helper Type 1
TH17	T Helper Type 17

Abbreviations	Descriptions
TIMPS	Tissue İnhibitors Of Metalloproteinases
TM	Trabecular Meshwork
TNF-A	Tumor Necrosis Factor Alpha
TSP-1	Thrombospondin-1
VEGF	Vascular Endothelial Growth Factor
VEGFR-3	Vascular Endothelial Growth Factor Receptor 3

1. INTRODUCTION

Glaucoma is a group of progressive optic neuropathies that share a slow progressive degeneration of retinal ganglion cells and their axons, leading to a characteristic appearance of the optic disc associated with concomitant pattern of vision loss [1]. It's the leading cause of irreversible blindness in the world, and it is associated with a decreased quality of life [2]. Glaucoma affects over 70 million people globally, with around 10% becoming blind bilaterally [3] Glaucoma, in its various types, is distinguished by damage to the optic nerve head (ONH), which is frequently caused by elevated intraocular pressure (IOP) [4].

Topical medication is the most commonly used first-line treatment for glaucoma [5]. Glaucoma and ocular surface diseases (OSD) frequently coexist, and both generally manifest with age [6]. Longterm use of topical drugs with preservatives is one of the important risk factors to deveolp dry eye disease (DED) in glaucoma patients.

The resulting ocular surface disease in glaucoma patients might lead to poor medication compliance due to the related symptoms [7]. Furthermore, OSD has been linked to a higher failure rate in subconjunctival glaucoma surgery [8].

One of the changes that indicate damage at ocular surface is the decrease in goblet cell density (GCD). GCs are specialized secretory cells that produce mucins, which lubricate the ocular surface. Also, conjunctival GC are found to play a crucial role in maintaining homeostasis between tolerogenic and protective immunological responses [9].

Because conjunctival goblet cells are the only cells that secrete MUC5AC into the ocular surface, MUC5AC is one of the best indicators for goblet cell detection [10]. Although the exact mechanism of goblet cell changes in the eye are unknown, data from other mucosal tissues shows that inflammation may play a significant role [11]. MUC5AC, play a protective role in trapping and removing cellular debris and foreign bodies such as pathogens and allergens [12].

Inflammation plays a significant role in the vicious cycle of DED pathophysiology. The components of inflammation in DED include cells such as macrophages and T cells, and

mediators such as proinflammatory cytokines (IL-1β, TNF-α), matrix metalloproteinases (MMPS) [13,14]. A lot of studies have shown significant increase in the levels of numerous inflammatory cytokines in dry eye patients and the relation of these cytokines to the clinical severity of the disease [15,16]. Furthermore, topical anti-inflammatory medications such as corticosteroids and cyclosporin A significantly relieve DED symptoms and signs [17,18].

In this study, we investigated the levels of IL-1β, IL-1Ra, VEGF, and PGE2 in the tears of patients with glaucoma and DED and compared the results with those of normal controls. The conjunctival impression cytology technique was also used to invistigate the changes in the goblet cell density and the expression of MUC5AC at the ocular surface which is considered a significant marker for OSD.

2. GENERAL INFORMATION

2.1. Glaucoma

2.1.1. Definition of glaucoma

Glaucoma is a group of progressive optic neuropathies characterized by the degeneration of retinal ganglion cells and their axons, resulting in changes to the head of the optic nerve, referred to as glaucomatous optic neuropathy (GON), and corresponding visual field loss [2]. It is often but not always accompanied by elevated intraocular pressure (IOP) [19].

2.1.2. Prevalence and incidence of glaucoma

Significantly more individuals are suspected of having glaucoma than are actually diagnosed [20]. Late and underdiagnosis are one of the leading causes of visual impairments in this disease [21]. Therefore, early diagnosis and treatment are the primary means of preventing glaucomatous blindness. Glaucoma will affect approximately 111.8 million individuals worldwide by 2040, as the disease burden is expected to increase [22]. Although glaucoma typically progresses slowly, it is a significant global health concern as the leading cause of irreversible preventable blindness and the second leading cause of blindness worldwide [23]. Over 7 million people worldwide are blind as a result of glaucoma, and the prevalence of bilateral blindness due to this disease ranges from 4% to 16%. [24, 25].

2.1.3. Risk factors for developing glaucoma

Elevated IOP

The aqueous humor produced by the ciliary body is eliminated by the trabecular meshwork and Schlemm's canal, which are located in the angle formed by the cornea and iris. IOP depends on the rate of fluid removal, which is decreased in all types of glaucoma with elevated IOP [26]. High IOP is the primary risk factor for developing glaucoma, which is defined as an IOP of 21 mmHg or greater. It is believed that elevated intraocular pressure damages the lamina cribrosa, resulting in the loss of structural and metabolic support of

retinal ganglion cell axons and impaired axoplasmic transport [27] IOP is the only risk factor that can be modified in glaucoma [28].

Age

Glaucoma is an age-related disease, and there is a direct correlation between increased age and the incidence of glaucoma [29]. According to the findings of the Barbados Eye Research, the incidence of POAG was 2.2% among those aged 40–49 at baseline and 7.9% among those aged 70 and older, with a relative risk of 3.8% for the older age group [30].

Ethnicity

Glaucoma risk and subtypes vary by race and nation. Blacks have a higher prevalence of POAG than whites in the United States [31]. Although POAG is more common in East Asia than PACG, Mongolians and Burmese are more susceptible to PACG than POAG [32].

Gender

The Framingham Eye Study and the Rotterdam Study discovered an increase in prevalence among males, whereas the Blue Mountains Eye Study discovered an increase in prevalence among females [33].

Genetics and family history

Several genes, including myocilin (MYOC, GLC1A) (CCDS1297.1), optineurin (OPTN, GLC1E) (CCDS7094.1), and WD repeat domain 36 (GLC1G) (CCDS4102.1), are associated with a monogenic, autosomal dominant trait. However, these genes account for less than 10% of all glaucoma patients [34]. A family history of glaucoma was found to carry a relative risk of 2.1 times for being linked to at least probable OAG [35].

Myopia

Myopia is a risk factor for the development and progression of glaucomatous optic neuropathy, according to epidemiological studies [36]. With the increased prevalence of

both myopia and glaucoma in older populations, it is expected that the incidence of these two ocular disorders in the same patient will rise [37].

Central corneal thickness (CCT)

The Ocular Hypertension Study found that a thinner CCT is a significant predictor of the development of POAG due to ocular hypertension (OHTN) [38]. Additionally, it has been linked to the severity of the disease [39].

Systemic diseases

Although correlations between numerous systemic disorders and glaucoma have been observed, a causal relationship between these diseases and the development of glaucoma has not yet been established, according to Pache and Flammer [40]. The Beaver Dam Eye Study of Caucasian adults discovered a significant relationship between diabetes and POAG [41]. In addition, clinical research has implicated systemic hypertension, vasospasm, and abrupt hypotension as potential glaucoma risk factors [35]. Other chronic diseases, including cardiovascular disease (CVD) and chronic kidney disease (CKD), have been associated with either elevated IOP or the development of POAG [40].

Smoking

Generally, smoking raises the risk for vascular diseases. Current cigarette smoking was associated with glaucoma presence in multiple logistic regression analyses, according to a cross-sectional study [42].

Drugs

Multiple classes of systemic drugs are known or hypothesized to modulate glaucoma risk, either via their direct effects on IOP or via independent processes [43]. Steroid-induced glaucoma is a form of OAG commonly associated with topical steroid use, but it can also develop in response to inhaled, oral, intravenous, periocular, or intravitreal steroid administration [44]. Several classes of drugs, including adrenergic agonists, cholinergics, selective serotonin reuptake inhibitors, tricyclic and tetracyclic antidepressants, anticholinergics, sulpha-based drugs, anticoagulants, and histamine H1 and H2 receptor

antagonists, have been reported to induce or precipitate acute angle-closure glaucoma, particularly in patients with narrow angles of the anterior chamber [45].

2.1.4. Classification of glaucoma

On the basis of the gonioscopic appearance of the angle, glaucoma can be roughly divided into two categories: open-angle and closed-angle glaucoma. OAG is characterized by increased resistance to aqueous outflow via the TM, whereas ACG is characterized by obstructed drainage pathway access [46].

2.1.5. Primary open angle glaucoma (POAG)

OAG is a chronic, progressive, and irreversible multifactorial optic neuropathy characterized by an open anterior chamber angle, optic nerve head changes, progressive loss of peripheral vision, followed by loss of central visual field [47]. It is the most prevalent form of glaucoma in the majority of global populations. POAG is typically bilateral but often asymmetric [48]. As in other forms of glaucoma, elevated IOP is a key risk factor for POAG; nevertheless, approximately one-third of patients with POAG have optic nerve degeneration with IOP in the range of normal, representing the POAG subgroup, normal tension glaucoma (NTG) [27].

Genetic factors significantly influence the etiology of POAG [49]. Up to fifty percent of POAG patients in cross-sectional studies have a positive family history, suggesting that genetic abnormalities may contribute to the cause of POAG [50]. Several genes, including myocilin (MYOC, GLC1A) (CCDS1297.1), WD repeat domain 36 (GLC1G) (CCDS4102.1), and optineurin (OPTN, GLC1E) (CCDS7094.1), are associated with a monogenic, autosomal dominant trait; however, these genes account for less than 10% of glaucoma cases [34]. MYOC mutations are associated with approximately 4% of adult-onset cases of primary open-angle glaucoma and over 10% of juvenile-onset cases [51].

2.1.6. Pathogeneis of glaucoma

Even though the pathophysiology of glaucoma is not fully known, the level of IOP is related to the death of retinal ganglion cells [34]. Therefore, a variety of hypotheses have

been developed to explain its pathological mechanism. The primary ones are enumerated below.

Mechanical theory

It was hypothesized that glaucomatous optic nerve degeneration (GOND) was caused by elevated IOP pushing the lamina cribrosa backward and compressing nerve fibers within its meshes to disrupt axoplasmic flow [52]. However, this theory fails to explain NTG or the persistence of vision loss in some glaucoma patients despite IOP control with medication or surgery [53].

Ischemic theory

According to the vascular theory of glaucoma, GOND is caused by inadequate blood supply due to elevated IOP and/or other risk factors that reduce ocular blood flow [54]. Moreover, it has been proposed that the ischemic theory of glaucoma plays a crucial role in the pathogenesis of bilateral NTG, based on the observation that patients with systemic vascular disorders, such as atherosclerotic ischemic stroke, have a significantly increased risk of developing glaucomatous cupping of the optic disc and bilateral NTG [55].

Immunological theory

One of the hypothesized pathogenic mechanisms for the neurodegeneration observed in glaucoma is neuroinflammation, which is evident in both the retina and the head of the optic nerve [56]. In 1998, Wax discovered antibodies against endogenous antigens such as heat shock protein 60 (HSP60) in the serum of NTG patients, and this was the first indication that the immune system was involved in the pathogenesis of glaucoma [57]. HSPs are crucial for cellular defense and are elevated in pathophysiological states [58]. Although increased HSP expression in glaucomatous eyes may initially protect cells from further damage and promote repair, they may later recruit immune responses that contribute to disease progression [59].

Additional autoantibodies against ONH proteoglycans have been found in the sera of glaucoma patients [60]. Any immune response against these molecules can weaken the extracellular matrix supporting the lamina cribrosa and cause or exacerbate optic nerve

cupping [61]. The variations in glaucoma patients' serum antibody profiles suggest a role for autoimmune involvement in the etiology of glaucoma in certain individuals, although it remains unclear if the autoantibodies detected in glaucoma constitute an epiphenomenon [62].

2.1.7. Treatment of glaucoma

Currently, lowering IOP is the only objective of glaucoma treatment. Numerous studies have demonstrated that lowering IOP can prevent optic nerve damage and preserve vision in glaucoma patients [63]. The desired IOP must be achieved with the least amount of medication and the fewest adverse effects [34]. The target pressure is defined as the range of IOP sufficient to slow the progression of visual field loss [64]. The optic nerve damage caused by glaucoma cannot be halted or reversed by any medication or surgical procedure [65].

Topical treatment

In most types of glaucoma, topical treatment is regarded as the initial line of defense. Five classes of topical drugs have been shown to be safe and effective in the treatment of glaucoma: cholinergic agents, adrenergic agonists, carbonic anhydrase inhibitors, -adrenoceptor antagonists, and prostaglandin analogs (PGAs) [66]. In general, these drugs reduce intraocular pressure by increasing aqueous outflow and/or decreasing its production [67]. They vary in their mechanisms of action, degree of intraocular pressure reduction, dose, adverse effects, and cost [68].

Topical prostaglandin analogs (PGAs)

Due to their efficacy, minimal systemic adverse effects, and once-daily administration, topical PGAs are commonly considered the first-line treatment for POAG [69]. They reduce IOP by enhancing the uveoscleral outflow of aqueous humor [70]. PGs frequently cause conjunctival allergy, conjunctival hyperemia, corneal epithelial disorders, and blepharitis [71].

Topical β-blockers

β-blockers decrease IOP by decreasing intracellular cyclic adenosine monophosphate (cAMP) production, thereby inhibiting AH production [72]. Due to absorption into the systemic circulation through the eye, they induce systemic adrenergic blocking inhibition despite their topical application [73]. Therefore, topical β-blockers can result in systemic adverse effects, including bronchial smooth muscle contraction, bronchospasm, respiratory failure, and death [74]. Conjunctival allergy, conjunctival injection, blepharitis, corneal epithelium problems, and ocular pemphigoid are the adverse ocular reactions of β-blockers [71].

Topical alpha2-agonists

Brimonidine and apraclonidine are the essential agents in this class, and they reduce IOP through two distinct mechanisms: decreasing aqueous humor production due to vasoconstriction of the ciliary body vasculature and increasing uveoscleral outflow [75]. α2-Agonists is the least well tolerated IOP-lowering drugs due to the high incidence of conjunctival hyperemia and localized allergic changes [76].

Carbonic anhydrase inhibitors

CAIs are particularly useful for treating glaucoma that is resistant to other anti-glaucoma medications, as well as preventing acute glaucoma attacks [77]. Topical CAIs also function by reducing the production of aqueous humor. Locally undesirable side effects in the eye include tearing, burning, and decompensation of the corneal endothelium [68].

Laser therapies to lower IOP and surgical intervention

In order to reduce IOP, laser treatments for glaucoma destroy the function of normal eye tissue to either increase the flow of aqueous out of the eye (laser trabeculoplasty) or decrease the production of aqueous (laser transscleral cyclophotocoagulation) [78].

Glaucoma filtering surgeries with mitomycin C and glaucoma drainage devices remain the surgical treatment gold standard [79]. Incisional surgery has generally been reserved for glaucoma patients who do not respond to medication and laser therapy, with the exception

of congenital and infantile glaucoma [80]. New minimally invasive glaucoma surgery (MIGS) devices have been developed in an effort to reduce IOP in a safer and more effective manner [75].

Novel IOP lowering agents

Rho kinase (ROCK) inhibitors, a new class of topical medications, have been recently evaluated for lowering IOP in glaucoma [81]. The European Medicines Agency's Committee for Medicinal Products for Human Use recently approved the use of 0.02% netarsudil for the treatment of OAG and OHTN [82, 83].

Netarsudil has a unique mechanism of action involving increased trabecular outflow capability, decreased episcleral venous pressure, and increased production of aqueous humor, which may allow its use in traditionally more difficult-to-manage patient populations [84]. Burning and stinging sensations, a foul taste in the mouth, and conjunctivitis are the most frequently reported adverse effects of topical CAIs [85].

It will be necessary, for future personalized medicine based on genetic and other patient characteristics, to identify individuals at high risk of progression and treat them more aggressively [86].

2.2. Ocular Surface Diseases in Association with Glaucoma

Ocular surface diseases (OSD) are a prevalent comorbidity in patients with glaucoma because, like glaucoma, their incidence increases with age. Typically, topical glaucoma medications contain preservatives to prevent bacterial contamination. These preservatives have a toxic effect on the ocular surface, especially in patients receiving multiple drops per day [87]. Benzalkonium chloride (BAK) is one of the most well-defined preservatives with toxic effects on the ocular surface [88]. Chronic use of these topical medications can affect bleb scarring in filtration surgery because it is a risk factor for conjunctival fibrosis, which can result in trabeculectomy failure [89]. According to a number of studies, the prevalence of OSD among glaucoma patients is 30–70%, which is significantly higher than the prevalence of 5–30% among similarly aged individuals without glaucoma [90]. Numerous studies have also demonstrated that preservatives cause neurotoxic effects in the cornea;

for instance, the frequency of instillation of preservative-added therapies correlates with a reduction in corneal sensitivity in glaucoma patients [91].

Modern packaging methods, such as single-dose or preservative-free eye drops (PFED) in multidose bottles, have rendered the use of preservatives obsolete [92]. It is noteworthy that the absence of preservatives in these eye drops is challenging due to the increased risk of contamination [93].

2.2.1. Gross anatomy and function of the ocular surface

Conjunctiva

The conjunctiva is thin, transparent, and vascularized mucous membrane that covers the ocular surface from the limbus to the mucocutaneous junction. It's divided into three parts, the palpebral conjunctiva (covers the inner surface of the eyelid and further subdivided into marginal, tarsal, and orbital regions), the bulbar conjunctiva (covers the anterior portion of the sclera and consists of the scleral and limbal conjunctivas), and the forniceal conjunctiva (forms the junction between the bulbar and palpebral conjunctivas). The conjunctiva is composed of two layers: epithelial and stromal (the substantia propria).

The epithelium of the conjunctiva

The conjunctiva is composed of six or more non-keratinized epithelial cell layers near the limbus and up to twelve layers near the fornix [94]. The epithelial cells at the basal layer are cuboid and become flattened as they reach the surface. In addition, the conjunctiva contains numerous cell types which is distributed throughout the epithelial cells, such as goblet cells, melanocytes, Langerhans cells, and lymphoid cells.

Goblet cells

Goblet cells are plump, rounded, specialized secretory cells that are filled with mucin. These cells extend through the entire thickness of the stratified epithelium to the apical surface [95]. They are surrounded by stratified squamous cells and can exist singly or in clusters [96]. There is regional variation in the distribution of goblet cells throughout the conjunctiva. Inferonasal bulbar conjunctiva, tarsal or palpebral conjunctiva, and temporal

bulbar conjunctiva have the highest goblet cell density, whereas the exposed surface of the eyeball and the sclero-corneal junction have the lowest goblet cell density [97]. The tear's epidermal growth factor (EGF) and the IL-13 cytokine secreted by resident immune cells support goblet cells through stimulating protein synthesis and cell proliferation [98].

Goblet cells secrete the gel forming mucins MUC5AC, MUC2, and MUC19, which are responsible for the stability of the mucous layer in the precorneal tear film. In addition to mucins, the secretory granules of the goblet cell contain other glycoproteins that are released into the mucous layer, such as peroxidase, trefoil peptides (TFF1 and TFF3), and defensins, with conjunctival goblet cells being the only source of MUC5AC at the ocular surface [99]. Goblet cells are capable of releasing their secretory granules in response to stimulation of the surrounding parasympathetic or sympathetic nerves [100]. Researchers discovered that conjunctival goblet cells act as conduits for antigen transport to immune cells in the underlying stroma; consequently, conjunctival goblet cells contribute to the maintenance of immunological tolerance at the ocular surface [101]. Goblet cells can also produce immunoregulatory factors, such as TGF-β2 and retinoic acid (RA), which inhibit the production and maturation of cytokines in mononuclear phagocytic cells [98]. Changes in the secretion of goblet cells result in an unstable tear film and a damaged ocular surface. Several ocular surface inflammatory disorders, including Stevens-Johnson syndrome, ocular mucous membrane pemphigoid, alkali burn, graft-versus-host disease, neutrophilic keratitis, and Sjogren's syndrome, have been linked to goblet cell loss [102].

The stroma of the conjunctiva (substantia propria)

The stroma is a thin, loose, richly vascularized connective tissue full of elastic fibers that facilitates the globe's free movement in all gazes. Conjunctival stroma is composed of a superficial adenoid or lymphoid layer with a mixture of lymphocytes, mast cells, plasma cells, and neutrophils; and a deeper fibrous layer with blood vessels and nerves that are loosely adherent to the underlying Tenon's capsule, especially further from the limbus [103].

The cornea

The cornea is a thin, transparent, and avascular tissue. It provides approximately two-thirds of the total refractive power of the eye [104]. The cornea is one of the most innervated parts of the body [105]. The majority of corneal nerves are sensory nerves that originate from the ophthalmic branch of the trigeminal nerves. The cornea has a horizontal diameter of approximately 11.5 mm and a vertical diameter of approximately 10.5 mm [106]. The tear film covers the anterior surface of the cornea, while the posterior surface is in direct contact with the aqueous humor.

The lacrimal glands

Its multilobular tissue composed of acinar, ductal, and myoepithelial cells [107]. The lacrimal gland contributes to the proper hemostasis of the ocular surface by secreting aqueous tears containing water, electrolytes, proteins, and mucus [108]. In humans, the lacrimal gland consists of the main lacrimal gland and the accessory lacrimal gland.

The meibomian glands

Meibomian glands are large sebaceous glands located in the tarsal plates of the eyelids [109]. The upper and lower eyelids contain approximately 30–40 and 25 glands, respectively [110]. At lid temperature, the lipid secretion of the meibomian glands is liquid and is transported as a transparent fluid called meibum to the lid margin skin [111]. Meibum is essential for preventing tear evaporation, maintaining tear stability, and protecting the ocular surface from desiccating stress.

2.2.2. Tear film

The tear film is a thin layer of fluid that covers the ocular surface and serves as the ocular surface's interface with the environment. The tear film is 2–50 μm thick and has thousands of proteins, peptides, lipids, electrolytes, gases, and metabolites in it [103]. The production of tear is about 1.2 microliters per minute, with a total volume of 6 microliters and a 16% per minute turn-over rate [112]. Tears are distributed into three compartments while the eyes are open: the fornical compartment, which is the narrowest, the tear menisci, and the preocular tear film.

Stability of the preocular tear film is a hallmark of ocular surface health. The tear film serves as the eye's first refractive surface and is an essential optical component for maintaining vision quality [113]. In addition, it nourishes the cornea, provides oxygen to the corneal epithelium, and acts as an antibacterial medium for the cornea and conjunctiva, clears away debris [114]. Therefore, any change to the tear film can cause or contribute to vision-related and ocular surface-related symptoms. Tear film instability plays a crucial role in the etiology of dry eye pathophysiology, as tear stability is diminished in all dry eye forms [115].

There are many factors that affect the tear film, such as blinking, eyelids, the lacrimal system, the outside environment, such as temperature, humidity, and airflow, and internal emotions [113]. Three layers make up tear film: the innermost mucus layer, the middle aqueous layer, and the outermost lipid layer [116].

Mucous layer

The mucous layer, the innermost layer of the tear film, is produced manly by goblet cells. In addition to the mucin produced by goblet cells, corneal and conjunctival epithelia are also known to produce mucin [117]. This layer is composed of mucins, electrolytes, and water. The mucus layer protects and lubricates the cornea, holds the aqueous tear film to the corneal epithelium to protect it from shear forces, and prevents dessication and bacterial contamination [116].

Mucins are a high molecular weight glycoprotein. They are characterized by the presence of several tandem repeats of serine- and threonine-rich amino acids in the central domain of the mucin core peptide, which provide sites for O-glycosylation [118]. They can be classified into secreted (gel-forming) mucins (e.g., MUC2, MUC5AC, and MUC6), soluble (non-gel-forming) mucins (e.g., MUC7, MUC8, MUC9, and MUC20), and transmembrane mucins (e.g., MUC1, MUC4, and MUC16).

MUC5AC

The predominant gel-forming mucin secreted by goblet cells is MUC5AC. MUC5 was formerly thought to be a single mucin; nevertheless, subsequent findings demonstrated that

MUC5 was actually three different mucins; consequently, MUC5A, MUC5B, and MUC5C were identified [119]. MUC5A and MUC5C were subsequently determined to be identical; therefore, the gene was redesignated MUC5AC [119]. MUC5AC is stored as polymers in goblet cells, but secreted as monomers in the tear film [118]. Its secretion is restricted to conjunctival goblet cells at the ocular [120]. MUC5AC is responsible for lubricating ocular surfaces by acting as a surfactant for the ocular surface, allowing an evenly distributed TF to hydrate the hydrophobic epithelium [121]. Additionally, this can aid in reducing the shear stress caused by blinking or rubbing.

MUC5AC is also capable of capturing cellular debris, pathogens, and foreign material and facilitating their removal from the mucosal surface [122]. Mucin production by GCs is normally regulated by a neural reflex originating from corneal and conjunctival sensory nerves that stimulate the parasympathetic nerves surrounding the GCs [123]. Proinflammatory mediators like leukotrienes, histamine, and proinflammatory cytokines, adenine nucleotides (UTP, ATP) are powerful stimulators of MUC5AC secretion [96], [124]. Mucin glycosylation and secretion, as well as the density of goblet cells, may be altered in ocular surface diseases such as dry eye [125, 126].

Lipid layer

This layer serves as the interface between the air and the aqueous in the tear film. This layer is commonly referred to as meibum because it is primarily secreted by Meibomian glands. Complex mixture of non-polar (e.g., wax esters, cholesteryl esters) and polar (e.g., phospholipids, (O-Acyl)-omega-hydroxy fatty acids [OAHFAs]) lipids compose the lipid layer of the tear film [127]. This layer serves multiple purposes, including stabilizing the tear film by reducing the overall surface tension, retarding evaporation, aiding in the respreading of the tear film after blinking, providing a smooth optical surface, acting as a barrier against foreign particles, exhibiting some antimicrobial activity, and sealing the lid margins during prolonged closure [128]

Aqueous layer

In healthy individuals, this layer of TF is produced by the lacrimal glands and comprises the majority of the tear film [129]. Water, electrolytes, proteins, peptide growth factors,

immunoglobulins, and cytokines make up its composition. The aqueous fraction of the tear film lubricates the ocular surface, removes foreign particles and nourishes the avascular cornea [116]. Different impacts, such as hormonal fluctuations and systemic inflammatory disorders, can alter tear film aqueous layer secretion [130].

According to the site that produces them, the major tear proteins can be categorized into two groups.

(1) The lacrimal gland produces the proteins lysozyme, lactoferrin, epidermal growth factor (EGF), lipocalin, and sIgA.
(2) A small proportion of serum proteins, such as albumin, transferrin, IgG, IgM, and complement, that leak from the conjunctival vessels into the tears.

Lactoferrin (LF) and lysozyme play an important role in the ocular surface's innate mucosal immunity [131]. LF possesses antimicrobial and anti-inflammatory properties, while protecting against immune-mediated and hypoxia-induced oxidative stress and UV damage [132]. Lysozyme is a bacteriolytic enzyme that demonstrates antimicrobial activity primarily by destroying the bacterial cell wall [131].

The most abundant antibody in the tear film is secretory immunoglobulin A (sIgA), which is produced by plasma cells residing in the lacrimal gland, accessory lacrimal glands, and conjunctuva associated lymphoid tissue (CALT) [122].

2.3. Dry Eye Disease

Dry eye is a common ocular surface disorder that affects millions of people worldwide. According to the most recent definition of dry eye by the DEWS II, dry eye is a multifactorial disease of the ocular surface characterized by a loss of tear film homeostasis and accompanied by ocular symptoms in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiologic roles [133]. Prevalence rates ranging from 5% to 50%, but reaching 75% in people over the age of 40, with women most frequently affected [134].

DED can have a significant impact on vision and quality of life, as symptoms frequently impede daily activities like reading and writing. In general, the symptoms range from mild

ocular discomfort to sight-threatening corneal complications, including filamentary keratitis, ulceration, and corneal perforation [135]. Age is a known risk factor for DED, as mentioned previously. Other common risk factors for developing DED include women's gender, contact lens wearing, smoking, refractive surgery, extended smartphone use, watching TV, having a high ratio of omega-6 to omega-3 fatty acids in the diet, a deficiency in vitamin A, refractive surgery, radiation therapy [136]. DED can be associated with a lot of diseases such as bone marrow transplantation, hepatitis C, diabetes mellitus, connective tissue diseases, human immunodeficiency virus, HIV and human T cell lymphotropic virus-1 infection, systemic cancer chemotherapy and certain classes of systemic and ocular medications, such as antihistamines, are all risk factors for dry eye [136].

2.3.1. Classification of DED

DED is subdivided into aqueous deficient dry eye (ADDE) and evaporative dry eye (EDDE) according to the disease's underlying mechanism.

Aqueous deficient DED

ADDE is characterized by decreased tear volume and tear secretion due to dysfunctional lacrimal glands. ADDE is further divided into Sjogren's syndrome DED (SS) and non-syndrome Sjogren's DED (NSS).

Sjogren's syndrome DED (SS)

Sjogren's syndrome (SS) is a chronic, systemic autoimmune disease which mostly affect middle aged women [137]. Chronic lymphocytic infiltration of the lacrimal gland, salivary gland, and other exocrine glands, resulting in dry eye, dry mouth, and extra glandular syndrome, characterizes this condition [138]. Primary (pSS) or secondary (sSS) to connective tissue diseases such as lupus erythematosus, rheumatoid arthritis, systemic sclerosis, and, less frequently, multiple sclerosis, thyroiditis, and autoimmune hepatitis [139].

Non-Sjögren DED

Non-SSDE is a form of ADDE in which the systemic autoimmune characteristics of SSDE have been ruled out [115]. Age-related dry eye is the most prevalent form, which is characterized by decreased tear volume and tear film instability, increased osmolarity, and changes in the meibomian lipid composition.

Evaporative dry eye (EDDE)

The most commonly observed form of dry eye disease is EDDE. It is characterized by increased tear film evaporation and is frequently caused by dysfunction of the meibomian glands [140]. Since the lipids layer of the tear film prevents evaporation of the aqueous layer, a deficiency in the lipids layer will lead to increased evaporation and instability of the tear film. Inadequate lid congruity, a low blink rate, and a deficiency of vitamin A are additional potential causes of EDE [141].

2.3.2. The immune regulation of the ocular surface

The complexity and organization of the eye's structure reflect the high degree of specialization required for its function to be maintained. The ocular surface, tear film, lacrimal glands, and eyelids act as a functional unit to protect and prevent injury to the refractive surface. In order to prevent excessive inflammation, which can result in tissue damage and vision impairment, it is necessary to maintain ocular surface homeostasis through a variety of immunoregulatory mechanisms.

The eyelids, eyelashes, eyebrows, and orbit constitute the mechanical barrier that serves as the first line of defense against ocular damage and infection. CALT (conjunctiva associated lymphoid tissue) is the conjunctiva's lymphoid tissue, which serves as an immunological interface between the ocular surface and the external environment [142].

Blinking and reflex tearing continuously push antigens away from the ocular surface, forming a formidable barrier against potentially harmful inflammatory mediators [143]. While the glycocalyx and tight junctions in the apical cell layers of the conjunctival and corneal epithelia act as a formidable physical barrier with immunomodulatory properties, the conjunctival and corneal epithelia's apical cell layers also contain immunomodulatory

properties [144]. The ocular surface epithelium is capable of producing inflammatory cytokines including interleukin IL-1β and α, IL-8, IL-6, and TNF-α [145]. Additionally, tear fluids contain IgA, lactoferrin, and lysozyme, which have antimicrobial properties and are essential for innate mucosal defense. In addition, the ocular surface epithelium expresses membrane receptors that regulate DC and lymphocyte activity [144]. The corneal epithelium constitutively expresses VEGFR-3, which binds to angiogenic VEGF-C and VEGF-D [141]. Thus, it regulates ocular surface immunity by inhibiting both hemangiogenesis and lymphangiogenesis [141]. Moreover, these cells constitutively express programmed death-ligand 1, which regulates T cell-mediated immunity [146]. In addition, it has been demonstrated that ocular surface epithelial cells impart a tolerogenic profile upon DCs, and there is evidence that goblet cells play a significant role in this phenomenon via the production and activation of transforming growth factor TGFβ-2 in a thrombospondin-1 (TSP-1)-dependent manner [143].

Ocular surface immature antigen-presenting cells (APCs) are activated by innate immunity and functional cells' production and release of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases (MMPs) [147]. In a healthy ocular surface, tissue inhibitors of metalloproteinases (TIMPs) tightly regulate MMP activity [148].

Dendritic cells (DCs) at the ocular surface can induce adaptive immune responses by priming naive T cells in the draining lymph node or by stimulating effector T cells in situ [144]. Then, effector T cells will infiltrate the ocular surface, secrete additional pro-inflammatory cytokines, and activate numerous immune cells. In contrast, when Treg differentiation is induced, these cells may inhibit the development of effector T cells in the regional lymph node through contact-dependent and independent mechanisms [149].

2.3.3. Pathogenesis of dry eye disease

The pathogenesis of dry eye has not yet been fully understood, but the immune-inflammatory response on the surface of the eye has become an increasing focus of research [150]. Tear film dysfunction is the primary cause of ocular dryness and inflammation, which occurs when the lacrimal function unit (the ocular surface, the main lacrimal gland and the interconnecting innervation) is unable to maintain a stable precorneal tear film [148].

Various exogenous and endogenous stimuli can induce DED by impairing tear film stability and tear hyperosmolarity; triggering mechanical/osmotic stress pathways [151]. Tear hyperosmolarity stimulates a series of events in the epithelial cells of the ocular surface, including the activation of MAP kinases and NF-κB signaling pathways and the production of the proinflammatory cytokines IL-1 and TNF-α, as well as proteases such as MMP9 [152]. These inflammatory mediators stimulate the maturation of immature antigen-presenting cells (APCs) and induce their migration to lymphatic draining tissues [153]. Mature APCs migrate to regional lymph nodes to stimulate autoreactive T cells, primarily Th1 and Th17 [154]. Autoreactive T cells migrate to the tissues of the ocular surface, where they aggravate the chronic autoimmune response and cause pathology [155]. Thus, the immunopathogenesis of DED can be viewed as a vicious cycle in which a proinflammatory environment promotes the infiltration of pathogenic immune cells, which then damage the ocular surface and attract more inflammatory cells [156].

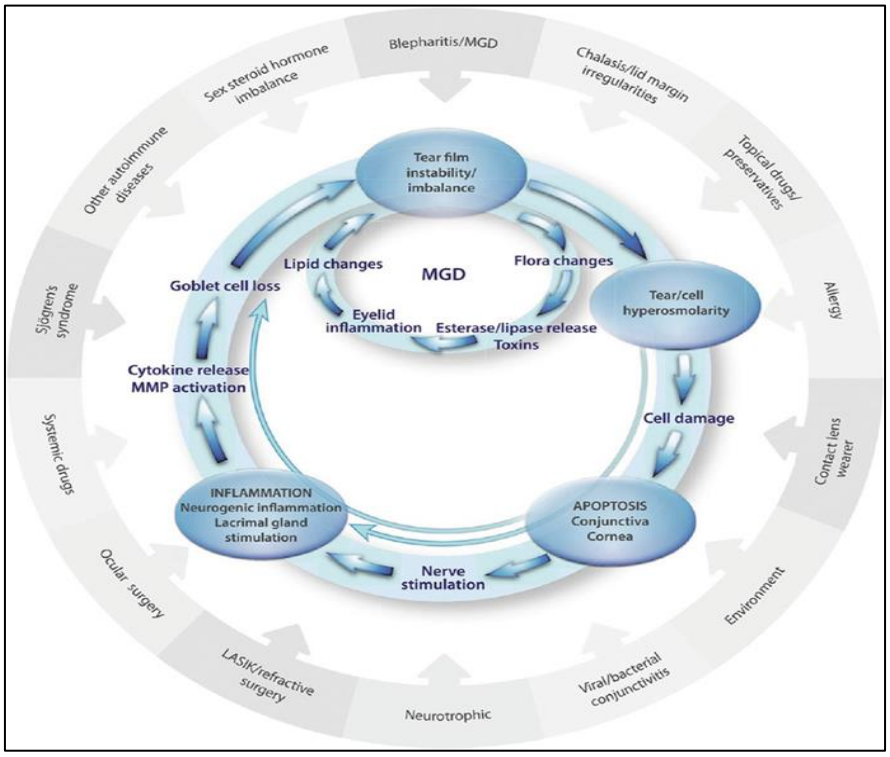


Figure 2.1. The vicious cycle of the pathology of DED adapted fromBaudouin et al. [157]

2.3.4. The inflammatory response in DED

In a strict sense, the inflammatory response can eliminate tissue insults and restore homeostasis. However, improper regulation of the immune system leads to the

development of chronic inflammation and disease [158]. Chronic inflammation caused by the activation of innate immune elements in ocular surface cells, a decrease in tear film stability, and an increase in tear osmolarity are shared by all types of DED [154]. Both cellular and soluble mediators contribute to the inflammatory response at the ocular surface in DED [159].

Molecular mediators in DED

Multiple molecular mediators associated with the pathogenesis of DED have been identified. Numerous studies have documented elevated levels of pro-inflammatory cytokines and chemokines, such as IL-6, IL-1, and TNF-α, in tear fluid [160]. There have been reports of increased concentrations of IL-1β and IL-1α in the tears and conjunctival epithelium of patients with SS and non-SS DE [161]. Other molecules, including IL-17A, EGF, and IL-12, were found to be significantly reduced in DED. Also, numerous studies have reported differences in the cytokine profiles of various types of DED. The conjunctival epithelium of individuals with Sjogren syndrome exhibited decreased expression of the mucin MUC-1 and higher levels of cytokines, including tumor necrosis factor TNF-α, IL-1α, IL-1β, IL-6, IL-8, IL-10, and TGF-1 [162].

In addition, a number of studies suggest that elevated levels of inflammatory cytokines are linked to ocular surface parameters in DED [163]. Neuronal and hormonal effects of proinflammatory cytokines (IL-6, IL-1b, and TNF-a) have been shown to reduce lacrimal gland tear production [164].

Cellular mediators in DED

Antigen-presenting cells, effector T cells, regulatory T cells, and natural killer cells are the primary cellular mediators involved in the inflammatory cascade of DED [165].

Dendritic cells

Antigen-presenting cells (APCs) such as dendritic cells (DCs) play a crucial role in both innate and adaptive immune responses [166]. DCs are typically found in the cornea's anterior stroma and epithelium [167]. In response to inflammation, these cells undergo a number of changes known as activation. These include an increase in cell size, location,

and surface marker expression [168]. Epithelial-derived mediators, such as IL-1β and TNF-a, can activate immature resident corneal dendritic cells and attract dendritic cells into the cornea by increasing CCCR5 and MHC class II antigen expression in DED [169], [170]. Depletion of dendritic cells prevented the onset of dry eye disease in mice exposed to desiccating stress, demonstrating the crucial role of dendritic cells in this disorder [147].

CD4+ T cells

There are resident CD4+ T cells on the ocular surface, including Th1, Th2, Th17, and Treg cells [171]. In DED, the ocular surface inflammation is maintained by the ongoing activation and infiltration of pathogenic immune cells, specifically $CD4^+$ T cells [172].

Th1 cells

Th1 cells secrete IFN-γ, which stimulates macrophages to eliminate intracellular pathogens like mycobacteria [173]. Moreover, Th1 cells promote the development of cytotoxic T cells and delayed type hypersensitivity responses [174, 175]. In DED, both Th1 and Th17 cells have been identified as mediators of ocular surface inflammation [176]. On the ocular surface, IFN-γ is secreted in response to desiccation stress and is known to cause conjunctival goblet cell loss [177]. In addition, significant correlations were discovered between tear IFN-γ concentration and key clinical markers of DED, including tear osmolarity, total ocular surface staining, and Schirmer test score [178].

Th17 cells

The development of Th17 cells is facilitated by TGF-1β in conjunction with IL-6 and IL-21, the growth and stabilization factor (IL-23), and the transcription factors (STAT3, RORγt, and RORα) [179-181]. The cytokines IL-17A, IL-17F, IL-21, and IL-22 are secreted by the Th17 subset [182]. The production of pro-inflammatory molecules (cytokines, chemokines, and MMPs) and the recruitment of neutrophils are stimulated by IL-17 [183] Autoimmunity and tissue inflammation have been linked to Th17 cells, which play a role in the early response to a variety of external pathogens, including bacteria and fungi, and have been implicated in autoimmunity [184]. In addition, it has been

demonstrated that IL-17 increases the mRNA expression of matrix metalloproteinase (MMP-3 and MMP-9) in corneal epithelium [185].

Regulatory T cells (Treg)

Tregs are an immunosuppressive subset of T lymphocytes that express the transcription factor Foxp3. Tregs can originate from the thymus (natural Tregs) or the periphery (inducible Tregs) [184]. Both subtypes of Treg act to maintain peripheral tolerance and immunological balance by inhibiting the priming of naive T cells and dampening the function of effector T cells, respectively [186]. The Treg regulatory functions are impaired in DED, as evidenced by their inability to suppress IL-17 production by effector CD4+ T cells, despite a slight decrease in Treg numbers [187].

Natural killer (NK) cells

NK cells are distinct subsets of innate lymphocytes that play essential roles in microbial pathogen defense by secreting IFN- γ and recognizing and destroying virally or bacterially infected host cells [188]. Immediately after desiccating stress, NK cells are significantly elevated in the conjunctiva [189].They secrete IFN- γ, which increases DED induction by directly damaging the ocular surface and promotes APC maturation in the secondary lymphoid compartment [154].

2.3.5. Diagnosis of DED

Due to the complexity of DED, there is no "gold standard" test or group of tests to diagnose it. It’s crucial to obtain a comprehensive history, including a review of symptoms, medications, and social history, as well as a comprehensive eye examination. In addition, a search for systemic symptoms such as joint pains, dry mouth, and skin rashes can help determine if an underlying systemic disease is contributing to the DED.

<u>Symptoms assessment</u>

Symptoms commonly worsen later in the day and may be exacerbated by factors such as low humidity, smoky environments, frequent contact lens use, or extended periods of time requiring visual focus, such as reading, driving, or computer work. The majority of dry eye

disease symptoms reported by patients include dryness, itching, grittiness, redness, watery eyes, photophobia, and ocular fatigue [190]. In addition, patients complained of vision-related symptoms such as vision that fluctuates with blinking, blurred vision, and glare [191]. All of these symptoms can have a negative impact on the patient's quality of life, preventing them from engaging in daily activities such as work, driving, and socializing.

A lot of questionnaires are available to assess and quantify the severity of DED symptoms and their effect on patients'quality of life; however, a standardized questionnaire for diagnosis has not yet been established.

The Ocular Surface Disease Index (OSDI) is one of the most commonly used questionnaires in the clinic for measuring and quantifying the severity of DED. OSDI is a 12-item questionnaire designed to provide a rapid assessment of the symptoms of ocular irritation associated with dry eye disease and their impact on vision-related functions [192]. There are three subscales: ocular symptoms, vision-related function, and environmental triggers [193]. The OSDI scoring system ranges from 0 to 100 points and is determined by multiplying the total question scores by 25 and dividing by the number of correct responses [194]. 0 to 12 indicates normal, 13 to 22 indicates mild DED, 23 to 32 indicates moderate DED, and ≥ 33indicates severe DED [193] The OSDI aims to make the diagnosis of ocular surface disease easier, faster, and more accurate, as well as provide evidence of variations in ocular impairment caused by dry eye disease [195].

Tear film stability

Tear break up time (TBUT) is a fundamental indicator for measuring tear film stability and detecting evaporative dry eye [196]. During the TBUT test, a fluorescein dye is injected into the conjunctival sac, and the time required for tear breakup is measured while the patient's eye is kept open under the cobalt blue light of the slit-lamp [197]. A brief TBUT indicates an unstable tear film, whereas a longer TBUT indicates a more stable tear film [196].

Schirmer test

Schirmer test is used to evaluate tear production, especially in individuals suspected of suffering from dry eye or excessive tear production [198]. A strip of filter paper is placed in the lower conjunctival cul-de-sac for five minutes to absorb tears produced by basic and reflex tearing [199]. The subject is instructed to close his eyes for five minutes, after which the amount of tear production is measured; values above 15 mm are considered normal.

Assessment of ocular surface staining

Three vital dyes, namely fluorescein, rose-bengal, and lissamine green, can be used to evaluate damage to the ocular surface [200]. Fluorescein is known to penetrate corneal and conjunctival epithelia with disrupted intercellular junctions, whereas rose bengal specifically stains damaged areas of the ocular surface when the tear film itself is discontinuous [201].

Tear osmolarity

According to prior research, an increase in tear osmolarity is a key characteristic of dry eye [202, 203]. There are several methods for determining tear osmolarity, including the TearLab osmometer, which measures osmolarity using a microchip and 0.2µl of tear fluid collected without touching the ocular surface [204].

Matirx metalloprotinease-9 assessment

MMP-9 is an accurate biomarker for ocular surface inflammation and disease severity [205]. The epithelium of the ocular surface secretes MMP-9, and its concentration in tears is typically below 40 ng/ml [206]. The InflammaDry is a recently developed diagnostic instrument capable of detecting MMP-9 in tears at concentrations greater than 40 ng/ml [207].

Conjunctival impression cytology

Impression cytology is a simple, rapid, and noninvasive technique for harvesting the superficial cell layers of the conjunctiva, typically using a cellulose acetate filter

membrane. It was first described by Egbert et al. in 1977 for the study of conjunctival goblet cells [208]. In contrast to smears and excisional biopsy, which make it difficult to preserve cellular morphology and yield few cells, respectively, Impression cytology preserved cellular morphology and allowed for a larger sample of the ocular surface [209].

Impression cytology has several applications, including the etiological diagnosis of various ocular surface disorders, the documentation of sequential changes in the conjunctival and corneal surface over time, the monitoring of treatment effects and staging of conjunctival squamous metaplasia, and as an investigative tool for immunostaining and DNA analysis of ocular surface disease [209, 210].

It is essential to consider the pore size of the filters, as it affects the number of collected cells and facilitates the visualization of the morphological structure of the cells [211]. Staining methods can vary in this technique according to the filter used to collect the cells [212]. The type of filter paper and method of cell collection depend on the objective of the sample collection [210].

Analysis of the tear film biomarkers

Tears are a readily available source of biological material that can be collected with minimal invasiveness, and they have been extensively studied for a variety of DED biomarkers [213]. Tear analysis is an innovative method for diagnosing ocular surface disorders. Tear collection is generally painless and causes no discomfort to the patient [214].

Even though tear samples are small in volume, they contain significant amounts of protein and other molecules. Several techniques can be used to obtain tear samples from human subjects, including capillaries, absorbent techniques (Schirmer strips and sponges), and the eye flush technique [215].

In the flush method; a certain amount of physiological saline is applied to the surface of the eye. This technique can facilitate the tear fluid sampling by increasing the collected volume, thereby making tear fluid collection quicker and easier [216]. Researchers have analyzed biomarkers in tear samples using numerous techniques, including western blot,

mass spectroscopy-based techniques, enzyme-linked immunosorbent assays (ELISA), and protein microarrays [217].

2.4. Cytokines

Cytokines are small, cell-secreted proteins that exert a specific effect on the connections and communications between cells [218]. Initially referred to as lymphokines and monokines to denote their cellular origins. The definition of interleukin (IL) for cytokines was established in 1979 at the second International Lymphokine Conference in Interlaken, Switzerland [219]. Several biological and physiological processes, including immune response, hematopoiesis, homeostasis, inflammation, and tissue repair, are regulated and modulated by cytokines [220].

Expression of cytokine genes and signal transmission through cytokine receptors are tightly regulated on multiple levels, including transcriptional and post-transcriptional levels. Cytokine action can be autocrine, paracrine, or exert endocrine function by acting on distant cells. Cytokine typically stimulates its target cells to synthesize and secrete subsequent cytokines. Cytokines' activity is redundant, meaning that different functions may be stimulated by different cytokines [218].

Some cytokines are proinflammatory and contribute to the progression of disease, while others reduce inflammation and promote healing (anti-inflammatory). IL-1 beta, IL-2, IL-6, IL-8, IL-12, IL-14, IL-15, IL-17, IL-18, IL-20, IL-21, IL-22, IL-23, IL-24, IL-26, IL-25, IL-28, IL-31, IL-32, IL-35, IL-36, and IL-38 are proinflammatory cytokines.

Interleukin (IL)-1 receptor antagonist (IL-1Ra), IL-7, IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-27, IL-28, IL-29, IL-30, IL-34, IL-35, IL37, and TGF-beta are among the most important anti-inflammatory cytokines. Under pathologic circumstances, these anti-inflammatory mediators may provide inappropriate control over proinflammatory activity in immune-mediated disorders or overcompensate and suppress the immune response, leaving the host susceptible to systemic infection [221]. Certain cytokines, including IL-2, IL-3, IL-11, and IL-12, possess both proinflammatory and anti-inflammatory properties.

2.4.1. Interleukine-1 cytokine family

Background on the IL-1

Interleukin-1 was the first cytokine identified as an endogenous pyrogen and a potent proinflammatory cytokine [222]. Macrophages, B cells, large granular lymphocytes, B cells, fibroblasts, endothelium, and astrocytes are the primary producers of IL-1.

The IL-1 cytokine family consists of 11 cytokines, which can be categorized into three subgroups based on their predominant functional effect: I) secreted cytokines with agonistic activity (IL-1α, IL-1β, IL-18, IL-33, IL-36β, IL-36α, and IL-36γ); II) receptor antagonists (IL-1Ra, IL-36Ra); and III) anti-inflammatory (IL-37, IL-38) [223]. This family of cytokines are essential regulators of immunological responses and inflammation, controlling multiple processes in health and disease with extensive effects on immune and nonimmune cells [224].

The signal peptide in IL-1Ra enables the cell to release the antagonist as an active molecule without the need for IL-1Ra precursor processing [225]. In contrast, all other members of the IL-1 family of cytokines lack an N-terminal signal peptide; consequently, they are not released in a conventional manner and must be converted from an inactive precursor to an active form [226].

IL-1 β

The cytokine IL-1β is regarded as a potent proinflammatory cytokine. It is synthesized and secreted by a variety of cell types, but the vast majority of research has focused on its production by innate immune system cells, such as monocytes and macrophages [227]. Pro-IL-1β (31 to 33 kDa) is biologically inactive and is proteolytically converted by caspase-1 into a biologically active secretory protein of 17 kDa [228]. IL-1β expression and secretion are tightly regulated [229]. It’s transcribed in response to the activation of Toll-like receptor (TLR) by pathogen-associated molecular patterns (PAMPs) or cytokine signaling [230].

The pro-inflammatory protease caspase-1 subsequently cleaves pro-IL-1 [231]. Caspase-1 is activated by recruitment to the inflammasome, which is a multi-protein complex

composed of adaptor molecules, a cytosolic pattern recognition receptor, and pro-caspase-1 [227]. The activation of the inflammasome is tightly regulated to protect against pathogenic insults and prevent damage [232].

The NLRP3 inflammasome is the most distinguished inflammasome. The NLRP3 protein of the complex belongs to the family of nucleotide-binding and oligomerization domain-like receptors (NLRs) and is commonly known as "pyrin domain-containing protein 3" [233]. The assembly of inflammasome components activates caspase-1, which in turn converts pro-IL-1 and pro-IL-18 into their mature, biologically active forms [234].

The secretion of IL-1β is still unclear. A simple hypothesis proposes that activated IL-1β is passively released after caspase1-induced pyroptosis, emphasizing the relationship between cell death and inflammation [235]. Pyroptosis is a caspase1-dependent form of cell death that is induced by certain bacteria via the intracellular receptor Ipaf, a member of the NLR family [236]. Exocytosis of the secretory lysosomes, shedding of plasma membrane microvesicles, direct release via transporters, and multivesicular bodies containing exosomes are additional mechanisms for IL-1β release [237].

IL-1β, IL-1α, and IL-1Ra bind to the same receptor, IL-1R1 [238]. IL-1R3 is the coreceptor for creating a trimeric signaling complex with IL-1α or IL-1β [226]. Upon the binding of IL-1β or IL-1α to IL-1R1, a structural change occurs that enables IL-1R3 to bind to IL-1R1 [226]. This trimeric complex promotes the proximity of each receptor chain's TIR domain [238]. MyD88 then binds to TIR domains, and initiates a cascade of kinases that generate a potent pro-inflammatory signal and activate NFkβ [239].

Both IL-1α and IL-1β are pleiotropic cytokines that affect numerous cell types, such as epithelial cells, fibroblasts, NK cells, endothelial cells, mucosal-associated invariant T (MAIT) cells, CD4+ T cells, and CD8+ T cells [240].

IL-1 is a powerful inducer of other inflammatory cytokines, including IL-6, IL-8, TNF-α, and GM-CSF [241]. IL-1 has a variety of biological effects, such as promoting fever, vasodilation, hematopoiesis, lymphocyte activation, leukocyte attraction and extravasation, angiogenesis, and antibody production [242].

IL-1 plays a crucial role in autoimmune, infectious, degenerative, and autoinflammatory disorders [243]. Dry eye was associated with elevated levels of IL- and mature IL-1, as well as matrix metalloproteinase (MMP)9, an enzyme that converts the precursor of IL-1 into its active form [244]. Together, IL-1 and TNF-α exacerbate inflammation on the ocular surface via mechanisms such as upregulation of adhesion molecule expression and activation of APCs [245].

IL-1Ra

IL-1Ra is an anti-inflammatory antagonist of IL-1 and IL-1 that occurs naturally [246]. IL-1Ra binds avidly to the biologically active IL-1R1 but prevents the attachment of the IL-1R accessory protein to this complex, an essential step in the activation of signal transduction pathways, thereby inhibiting the stimulation of the target cell [247]. The rare genetic disorder deficiency of the interleukin-1 antagonist (DIRA), which is associated with neonatal-onset pustular dermatosis, sterile multifocal osteomyelitis, and periostitis, highlights the crucial role of IL-1Ra in the regulation of IL-1 signaling [248]. The majority of IL-1Ra is produced by active monocytes and tissue macrophages [249].

Currently, the IL1Ra molecule family includes one secreted isoform (sIL1Ra) and three intracellular isoforms (icIL1Ra1, 2, and 3) (icIL1Ra1, 2, and 3) [250]. It appears that the physiological function of sIL-1Ra is to prevent the local inflammatory effects of IL-1 through competitive inhibition [251]. Although icIL-1Ra isoforms may be released from dying cells and may function as receptor blockers, icIL-1Ra isoforms may have additional functions within cells [251].

It has been reported that normal tear fluid contains high levels of IL-1Ra that are between 25,000 and 40,000 times greater than both proinflammatory forms of IL-1. It is believed that the concentration of IL-1Ra in tear fluid is a natural homeostatic process for regulating IL-1–mediated functions on the ocular surface [249]. Extremely likely, the high level of IL-1Ra associated with more severe ocular surface damage in DED patients is due to ocular surface inflammation and subsequent production of inflammatory cytokines, which may stimulate increased expression of IL-1Ra in ocular epithelial cells [252]. Moreover, TGF-β and IL-1Ra inhibit resident DC maturation, which is necessary for T-cell activation during experimental dry eye, via direct and indirect mechanisms, respectively [155].

2.4.2. Prostaglandin E2 (PGE2)

PGE2 belongs to the lipid class of eicosanoids, which are produced through the oxidation of arachidonic acid and a related polyunsaturated fatty acid [253]. Phospholipases (PLAs), an enzyme family that catalyzes the hydrolysis of membrane phospholipids and releases free fatty acids, such as arachidonic acid (AA), from membrane lipids, initiate their production [254]. The COX then oxidizes arachidonic acid to generate PGG2, which is then reduced by the same COX to generate the unstable intermediate PGH2 [255]. PGH2 is converted into PGE2 with the aid of the PGE2 sythase enzyme (PGES) [256]. Prostaglandin production is typically quite low in non-inflamed tissues, but increases rapidly in acute inflammation prior to leukocyte recruitment and immune cell infiltration [257]. In response to proinflammatory stimuli such as lipopolysaccharide (LPS), IL-1 β, and TNF- α, the expression of one of three cyclooxygenases promotes PGE2 production [258].

PGE2 exerts its biological activities through the activation of four receptors, namely EP1, 2, 3, and 4. These receptors are seven transmembrane G protein-coupled receptors that activate various downstream effectors and second messengers [259]. PGE2 is able to induce a variety of cellular responses because each subtype of these receptors is associated with the activation of distinct heterotrimeric G proteins [260].

EP3 and EP4 receptors have a high affinity, whereas EP1 and EP2 receptors require significantly higher PGE2 concentrations to become active [261]. Typically, EP2 and EP4 receptors relax smooth muscle by elevating intracellular cAMP levels in response to a Gs protein signal [262]. Additionally, EP2 receptor activation inhibits the release of mast cell mediators [263]. EP1 receptors mediate the contraction of smooth muscle by mobilizing Ca2+ from intracellular storage and, in some cases, by allowing extracellular Ca2+ entry [264]. EP3 is described as an inhibitory receptor because it decreases intracellular cAMP concentration [265].

PGE2 is a key regulator of several biological processes, including immunological responses, blood pressure, gastrointestinal integrity, and fertility, under physiological conditions [257]. PGE2 is generally recognized as a mediator of active inflammation, promoting local vasodilation and the local attraction and activation of neutrophils,

macrophages, and mast cells at early stages of inflammation. However, its ability to facilitate the induction of suppressive IL-10- and to directly suppress the production of multiple pro-inflammatory cytokines allows it to limit nonspecific inflammation, thereby promoting the immune suppression associated with chronic inflammation [266].

Tear PGs remain unchanged throughout the day or while wearing contact lenses, indicating that this biomarker is stable and suitable for monitoring chronic ocular surface disorders [267]. Patients with DE had significantly higher PGE2 levels, which correlated well with the severity of their symptoms, according to a previous study [268].

2.4.3. Vascular endothelial growth factor (VEGF)

VEGF is a major regulator of the formation of new blood vessels and an important inducer of vascular permeability [269]. Multiple cell types, including macrophages, platelets, keratinocytes, renal mesangial cells, and tumor cells, produce VEGF [270]. Depending on the dose, cell type, or tissue type, proinflammatory cytokines such as IL-1, IL-6, IL-8, TGF-, and TNF can increase VEGF expression [271].

The VEGF family proteins consist of VEGF-A/B/C/D and placental growth factor (PGF) [272]. VEGF-A is the most potent stimulator of angiogenic processes and the member with the best characterization [273]. At least four significant VEGF isoforms result from distinct splicing patterns [274]. These isoforms are referred to as VEGF121, VEGF145, VEGF165, and VEGF189 in humans, with the numbers denoting the number of amino acids in each mature isoform [275]. Different isoforms interact differently with heparan sulfate proteoglycans (HSPGs) [276]. VEGF family proteins bind uniquely to three structurally related tyrosine kinase receptors, VEGFR1, -2, and -3 [277]. Signaling by VEGFRs begins with the covalent attachment of a ligand dimer to the extracellular receptor domain [278]. These initiates signal transduction by stimulating receptor phosphorylation and the subsequent recruitment of specific downstream signal transduction mediators [274].

VEGFs perform a wide range of biological functions. VEGFs, in conjunction with their receptors (VEGFRs), control both vasculogenesis and angiogenesis [279]. VEGF is also involved in normal physiological functions such as bone formation, hematopoiesis, wound healing, and development. VEGF inhibits apoptosis and increases cell proliferation in

addition to increasing vascular permeability, vasodilation, and the recruitment of inflammatory cells [280].

2.5. Role of Anti-Inflammatory Therapies in DED

The ultimate objective of DED treatment is to improve tear stability, normalize tear osmolarity, alleviate symptoms, and maintain ocular surface homeostasis. Due to the multifactorial nature of the DED, its management requires a multifaceted approach.

In the past several treatment options are available to patients with DED, the majority of which rely on alleviating the patient's symptoms. Artificial tear substitutes are one type of topical medication. These are utilized to replenish the natural tear film, lubricate the eyes, and temporarily improve symptoms such as blurred vision. Alterations in hygiene and lifestyle, use of autologous serum tears, anti-inflammatory drug therapy, and physical and surgical procedures to increase tear retention are additional treatment options.

Rather than simply treating symptoms by hydrating and lubricating the ocular surface, the current emphasis appears to be on identifying treatments that can reverse the underlying etiology, such as inflammation. Anti-inflammatory agents can reduce the signs and symptoms of DED, according to clinical data [160].

Currently, topical corticosteroids and immunomodulating agents are the most common anti-inflammatory treatments. Topical corticosteroids can effectively interrupt the inflammatory cycle because they are potent inhibitors of multiple inflammatory mediators [281]. They exert their effect via classic glucocorticoid receptor-mediated pathways to directly regulate gene expression, as well as via nonreceptor pathways to inhibit transcriptional regulators of proinflammatory genes, such as NF-kB [160]. In turn, these agents inhibit the production of cytokines and chemokines, decrease the synthesis of matrix metalloproteinases and arachidonic acid derivatives, reduce the expression of cell adhesion molecules, and induce lymphocyte apoptosis [282]. In susceptible patients, prolonged use of topical corticosteroids is associated with cataract formation and increased intraocular pressure; therefore, short-term pulse therapy with corticosteroids is believed to be safer for the treatment of dry eye disease [283].

2.5.1. Cyclosporine (CSA)

Cyclosporine inhibits the secretion of IL-2 by activated T-cells, which is necessary for the activation and proliferation of additional T-cells [284]. Systematically, CSA is administered to patients with autoimmunity and organ transplants as an anti-rejection agent. It is well-established that topical cyclosporine A increases tear production in patients with DED who are resistant to conservative therapies such as ocular lubricants and lid hygiene [285]. In addition, it has been reported that cyclosporine treatment results in the recovery of reduced goblet cell density in the conjunctiva of DED patients [286].

Tetracyclines also have anti-inflammatory effects, such as inhibiting the production of MMPs and IL-1 [287]. Both doxycycline and tetracycline have been shown to be effective in treating rosacea-related dry eyes and chronic posterior blepharitis [288].

2.5.2. Tacrolimus

Tacrolimus, also known as FK506, is a macrolide with immunomodulatory properties that was derived from Streptococcus tsukubaensis fermentation [289]. Tacrolimus has been used to treat a number of ocular inflammatory diseases, such as anterior segment diseases, severe atopic blepharoconjunctivitis, vernal keratoconjunctivitis, atopic keratoconjunctivitis, keratoconjunctivitis sicca (KCS), and giant papillary conjunctivitis [290]. Tacrolimus binds to FK506-binding proteins within T lymphocytes and inhibits calcineurin activity. This decreases the dephosphorylation of the nuclear factor of activated T cells and its translocation into the nucleus, thereby inhibiting T lymphocyte cytokine production [291].

2.5.3. NSAIDs

Numerous topical NSAID formulations are commonly used to treat ocular inflammation [292]. The synthesis and function of various inflammatory mediators, including prostaglandins, coagulation cascade-derived peptides, IL-2, IL-6, and TNF, are altered by NSAIDs [293].

2.5.4. LFA-1 antagonist

The L2-integrin antagonist lifitegrast (lymphocyte function-associated antigen-1, LFA-1) was approved in 2016 for the treatment of DED [294]. Lifitegrast is a direct competitive antagonist of the binding of LFA-1 to ICAM-1, thereby inhibiting T-cell activation, cytokine release, the development of immunological synapses, and thus decreasing the ocular inflammatory cycle [295].

2.5.5. Thymosin ß4(Tb4)

Tb4 is a G-actin-binding protein found in nearly all cells and ocular surface fluid that has been shown to promote epithelial healing and inhibit pro-inflammatory cytokines [296]. In a phase 2 randomized, double-masked, placebo-controlled trial, topical Tb4 significantly decreased ocular pain and total corneal staining in patients with severe DED [297].

Other optional DED treatments include topical vitamin A ointment, auto serum tears for extensive ocular surface damage, and oral immunosuppressants for systemic diseases [298].

In conclusion, the variety of inflammatory molecules found in conjunctival epithelial cells and tears in DED strengthens the case for inflammation's crucial role in disease pathogenesis. In addition, the clinical correlations between clinical parameters and these molecules can aid in the explanation of clinical symptoms and signs. Numerous promising new therapeutic agents are in development, but additional research is necessary to determine their value, efficacy, and safety for treating DED.

3. MATERIALS AND METHODS

3.1. Tools – Equipment

Table 3.1. Chemicals that were used in our study are given in table

Chemical name	Catalog number	Marka
Methanol	67-56-1	MERCK
Ethanol	64-17-5	MERCK
Tween-20	P1379	SİGMA
xylen	1.08685.2500	Merck
DAPI staining solution	ab228549	Abcam
Mounting media	ab128982	Abcam
PBS	PBS404	Bioshop
Bovine serum albumin	A-2153	Sigma

Table 3.2. Kits that were used in our study are given in table

Kit name	Catalog number	Marka
Periodic Acid Schiff (PAS) Stain Kit (Mucin Stain)	ab150680	Abcam
Human IL-1 beta ELISA Kit	ab214025	Abcam
Human IL-1ra ELISA Kit	ab211650	Abcam
Human VEGF ELISA Kit	ab222510	Abcam
Prostaglandin E2 ELISA Kit (High Sensitivity)	ab133055	Abcam

Table 3.3. Antibodies that were used in our study are given in table

Antibody	Catalog number	Marka
Anti-Mucin 5AC antibody [EPR16904] Rabbit Monoclonal IgG	ab198294	Abcam
Goat Anti-Rabbit IgG H&L (FITC)	ab6717	Abcam

3.2. Subjects and Study Design

This study was approved by the Human Research Ethics Committee of Gazi University (Decision No. 57/25.1.2021). A total of 45 volunteers participitated to the study: 15 glaucoma patients, 15 DED patients, and 15 healthy subjects with no ocular or systemic diseases who served as controls. All participants were aged between 22 and 65 years old. Tears were collected over a period of 7 months (June 2021–December 2021). All participants signed a written informed consent following an explanation of the study's

procedures. Following informed consent, subjects completed the baseline OSDI survey, and a detailed ocular examination, including a snellen chart, intraocular pressure, fundus examination, tear film break up time (TBUT), fluorescein staining of the ocular surface, and schirmer's test, was performed on all patients on the same day, following tear collection and impression cytology.The collection was done at the same time between 9:30 a.m. and 12:00 p.m. to minimize diurnal variation, and one eye was selected randomly for both tear collection and impression cytology. The collection of samples was done in the clinics of the ophthalmology department of Gazi University School of Medicine. The analysis of samples was conducted in the laboratory of immunology at Gazi University's Faculty of Medicine.

Subjects were selected to be enrolled in the study according to the following criteria:

Inclusion criteria

1. Patients who have been diagnosed with glaucoma.
2. Patients who have been diagnosed with DED.
3. All participants recruited were over the age of 18.
4. Agree to participate in the study.

Exclusion criteria

1. If there is any other eye disease
2. Not meeting the criteria for the patient group
3. Patients with a chronic illness such as diabetes and rheumatoid arthritis
4. Patients undergoing ocular surgery (within the last year)
5. If they have an active ocular infection

3.3. Clinical Parameters of DED

To evaluate the clinical state of the ocular surface, the following tests were done after the tear samples and impression cytology collection, so that the tests would not interfere with the results of ELISA or immunofluorescence staining.

3.3.1. OSDI questionnaire

OSDI was used to record patients' subjective eye-related discomfort [191]. Questionnaires were filled out first by the participants before any other tests to ensure that they would not influence their responses. According to Grubbs JR Jr et. al., the results were classified based on the patient's final OSDI score as follows: ≤ 12 was taken as normal, 13–32 as mild-to-moderate, and ≥ 33 as severe dry eye [193].

3.3.2. Schirmer I test

Schirmer I test was done according to CHO et.al., sterile strips were placed in the outer one third of the inferior lid margin of both eyes without topical anesthetic, and participants were asked to close their eyes [198]. Wetting length was measured in millimeters after five minutes. A score of more than 10 mm in 5 minutes is considered normal. A score of less than 5 mm in 5 minutes indicates tear insufficiency.

3.3.3. TBUT test fluorescein strips

A fluorescein strip wetted with sterile saline was gently applied to the inferior fornix. Following instillation of fluorescein and a period of blinking, the TBUT was measured, particularly the appearance of the first randomly distributed dry spot occurring in the interval between the last blink and the occurrence of breakup. After that, the subjects were asked to blink for a few seconds several times to ensure proper mixing of the dye. While the cornea was observed with cobalt blue light, the time between the last blink and the appearance of the first corneal dark spot in the stained tear film was measured. According to Bron et. al., a value of less than 10 seconds was considered abnormal [200].

3.3.4. Corneal fluorescein staining

After the evaluation of TBUT, corneal staining was evaluated using cobalt blue light. According to Kaido et. al., the intensity of corneal fluorescein staining was recorded as a score from 0 (absent) to 3 (significant epithelial loss) [199].

3.4. Tear Collection Method

Tear samples were collected using a flushing method. About 50 µl of normal saline was pipetted into the inferior fornix of the eye, and the participant was asked to move his eye up and down and from left to right without closing his eye, and the tears were then collected again by the micropipette tip [215]. To avoid reflex tearing, care was taken to avoid touching the eyelid or bulbar conjunctiva with the pipette tip. Following collection, samples were placed immediately on dry ice. At the laboratory, samples were centrifuged for 4 minutes at 4°C and 3000 rpm to remove cellular debris, and the supernatants of each sample were collected then a total volume of 150µl of PBS was added to each sample to reach the volume of 200µl. The samples then stored in an -80º C freezer at the laboratory until analysis was conducted.

3.5. Conjunctival Impression Cytology Sample Collection

Impression cytological specimens were obtained by using polyether sulfone (PES) filter papers (1214465, CVS) for the MUC5AC immunofluorescence staining, and for the histochemical staining (PAS-hematoxylin), cellulose acetate (CA) filter papers (GSWG047S6, Millipore) were used [208]. Before use, the filter papers were cut into 5 5 mm pieces and sterilized. The subject was asked to look down and to the opposite side, and the filter paper was held using forceps with a blunt, smooth edge, then palced and gently pressed over the superior temporal bulbar conjunctiva at a 3 mm distance from the limbus. No local anesthesia was used. The peeling motion removed the paper after 15 seconds. There were no complications related to the impression cytology procedure noted in this study. Fixation of the sample was done with cold methanol for PES filters and with 96% ethanol for CA filters.

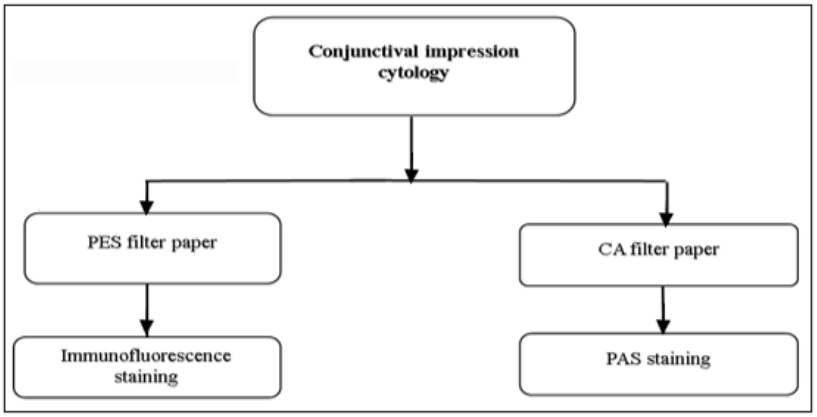


Figure 3.1. Flowchart demonstrating the sample processing schematic from CIC

3.5.1. Procedure of PAS- hemtoxylin staining

After fixation with 96% ethanol for 10 minutes, the sample was placed on a slide with the cells facing up and washed with distilled water for about 5 minutes. The samples were then oxidized in 0.5% periodic acid for 15 minutes. that, samples were rinsed in distilled water for 5 minutes and stained with diluted Schiff reagent (1:1) for about 5 minutes. After that, the sample was rinsed again in DW for 5 minutes and counterstained with hematoxylin for 2 minutes. Samples were then washed three times in DW for 5 minutes. Subsequently, samples were dehydrated in ascending grades of ethanol for 5 minutes before being placed for 3 hours in the xylene, which turned the filter paper transparent. The specimens were then mounted and covered for microscopic observation. Images were taken for each sample, and the goblet cells were counted in five neighboring fields.

3.5.2. Indirect immunofluorescence staining

The indirect immunofluorescence staining was done as follows: The samples were fixed in cold methanol for 10 minutes. Then the cells were washed twice for 5 minutes in 1x PBS and blocked for 1 hour with 10% goat serum to prevent nonspecific binding. Subsequently, samples were incubated in 1:200 rabbit anti-human MUC5AC antibodies diluted in 1% BSA in PBST overnight at 4°C. Decanting the solution was aspirated by micropipette, and samples were washed three times in PBS for 5 minutes each time. After that, samples were incubated in the dark with goat anti-rabbit FITC conjugated for 3 hours at room temperature and diluted 1:250 in PBS. After incubation with the secondary antibody, samples were washed three times for 5 minutes with phosphate buffered saline (PBS) and

counterstained for 10 minutes with 4′,6-diamidino-2-phenylindole (DAPI) DNA binding, diluted to 1:2000. The membranes were mounted with immunofluorescence mounting media. Three photos were obtained at 20X magnification from each membrane using a microscope.

3.6. ELISA

IL-1B, IL-1ra, VEGF, and PGE2 concentrations in the tear fluid were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) test. First, as a point of reference for the selected cytokines in this study, total tear protein concentrations were determined using the quibt protein assay kit (Thermo Fisher, catalog no. Q33211). All assays were performed as recommended by the relevant manufacturer's instructions.

3.6.1. IL-1B ELISA kit

ELISA was perfomed by a commercailly available ELISA kit (Abcam, ab214025). Briefly, the antibody cocktail was prepared by diluting the capture and detection antibodies in antibody diluent as recommended by the manufacturer's instructions. After that, 50 µl of samples and standards were pipetted into appropriate wells, and then 50 µl of an antibody cocktail was added. The plate was sealed and incubated for 2 hours at room temperature on a plate shaker set to 400 rpm. After incubation, the plate was washed three times with a wash buffer using an automated washer (HydroFlex, Tecan, Switzerland). Subsequently, 100 µl tetramethylbenzidine (TMB) substrate was added to each well, and the plate was incubated in the dark for 15 min. Then, 50µl of stop solution was added to each well, and the optical density (OD) at 450 nm was immediately read using a microplate reader (Synergy, Biotek, USA). The accurate sample concentration was estimated from the correlation between standards and their absorpancy.

3.6.2. IL-1ra ELISA

ELISA was perfomed by a commercailly available ELISA kit (Abcam, ab211650). Briefly, an antibody cocktail was prepared by diluting the capture and detection antibodies in antibody diluent. 50 µl of samples and standards were pipetted into the appropriate wells, and then 50 µl of an antibody cocktail was added. The plate was sealed and incubated for 1 hour at room temperature on a plate shaker at 400 rpm. After incubation, the plate was

washed three times with a wash buffer using an automated washer (HydroFlex, Tecan, Switzerland). Subsequently, 100 µl tetramethylbenzidine (TMB) substrate was added to each well and incubated in the dark for 10 min on a plate shaker. Then, 100 µl of stop solution was added to each well, and the optical density (OD) at 450 nm was immediately read using a microplate reader (Synergy, Biotek, USA). The accurate sample concentration was estimated from the correlation between standards and their discrepancy.

3.6.3. VEGF ELISA

ELISA was perfomed by a commercailly available ELISA kit (Abcam, ab222510). Briefly, an antibody cocktail was prepared by diluting the capture and detection antibodies in antibody diluent. 50 µl of samples and standards were pipetted into the appropriate wells, and then an antibody cocktail was added. The plate was sealed and incubated for 1 hour at room temperature on a plate shaker at 400 rpm. After incubation, the plate was washed three times with a wash buffer using an automated washer (HydroFlex, Tecan, Switzerland). Subsequently, 100 µl tetramethylbenzidine (TMB) substrate was added to each well and incubated in the dark for 10 min on a plate shaker. Then, 100 µl of stop solution was added to each well, and the optical density (OD) at 450 nm was immediately read using a microplate reader (Synergy, Biotek, USA). The accurate sample concentration was estimated from the correlation between standards and their discrepancy.

3.6.4. PGE2 ELISA

ELISA was perfomed by a commercailly available ELISA kit (Abcam, ab133055). Briefly, 100 µl of diluted tear samples and standards were pipetted into 96-well goat anti-mouse IgG-coated plates in the appropriate wells, and then 50 µl of the PGE2-AP conjugate was added to each well. The plate was sealed and incubated for 2 hours at room temperature on a plate shaker at 500 rpm. After incubation, the plate was washed three times with a wash buffer using an automated washer (HydroFlex, Tecan, Switzerland). Subsequently, 200 l of pNpp substrate was added to each well and incubated in the dark for 1 hr at 37 °C without shaking. Then, 50 µl of stop solution was added to each well, and the optical density (OD) at 405 nm was immediately read using a microplate reader (Synergy, Biotek, USA). The accurate sample concentration was estimated from the correlation between standards and their discrepancy.

3.7. Statistical Analysis

GraphPad Software Inc.'s Prism 9 and IBM SPSS version 29 software was used for statistical analysis of study data. Every value is presented as the mean and standard deviation SD. Comparisons between groups were performed with t- test and one way ANOVA test. The analysis of correltion between groups clinical parameters, goblet cell density, MUC5AC expression and cytokines concentration was done by Pearson correlation analysis.

A p value of less than 0.05 was considerede as statistically significant.

4. RESULTS

The study was conducted between June 2021 and December 2021 by the immunology department with the contribution of the ophthalmology department of the Gazi university school of medicine. A total of 30 patients (fifteen in DED group and fifteen in glaucoma group) and fifteen healthy participants represented the control group (CG) were included.

4.1. Demographic and Clinical Characteristics of the Study Population

The mean age (mean ± SD) of subjects was 52.87±11.84 years in Glaucoma patients, 47.60±10.58 years in DEG patients, and 46.27±7.066 years in the control gruop. There were no statistically significant differences were found among the three groups concerning the age (P=0.1754). Regarding gender, the percentage of females and males was 60/40% in the glaucoma group, 66.6/33.3% in the DE group, and 66.6/33.3% in the control group, respectively. Table 1 represent the distribution of goups particiants by gender.

Table 4.1. Distribution of the subjects included in the study by gender

	Glaucoma group	Dry eye group	Control group
Gender	N %	N %	N %
Female	9 (60%)	10 (66.6%)	10 (66.6%)
Male	6 (40%)	5 (33.3%)	5 (33.3%)

According to the results of the OSDI questionnaire, seven (46.66%) glaucoma patients had mild to moderate while eight (53.33%) had severe dry eye; regarding the DED group, two (13.33%) subjects had normal ocular surface while eight (53,33%) had mild to moderate and five (33.33%) had severe dry eye. For the control group thirteen subjects (86.66%) with normal score of OSDI, while two subjects (13.33%) have mild score of OSDI. The mean score of the OSDI questionnaire obtained in the Glaucoma group (39.54 ±16.76) was significantly ($p<0.0001$) higher with respect to the DE group (28.37±16.98) and the control group (8.513±4.838).

There was a statistically significant difference between glaucoma, DE, and normal subjects for OSDI, schirmer test, TBUT, and corneal staining (Table 4.2.).

Table 4.2. Clinical characteristics of the glaucoma, DE, and control group

	Glaucoma group	Dry eye group	Control group	P value
OSDI (mean ± SD)	39.54 ±16.76	28.37±16.98	8.513±4.838	<0.0001
Schirmer test (mean ± SD)	7.000±2.360	8.133±3.642	13.73±1.534	<0.0001
TBUT (mean ± SD)	7.733±1.280	7.333±1.952	12.13±1.807	<0.0001
Corneal staining (mean ± SD)	0.8667±0.5164	0.6667±0.4880	0	<0.0001

4.2. Impression Cytology of the Conjunctiva

4.2.1. PAS staining results

Nelson sccaling system

Samples were assessed and graded with the criteria described by Nelson.

Grade 0; the epithelial cells are small and round. The goblet cells are numerous, oval and plump.

Grade 1; the epithelial cells are slightly larger and more polygonal. There is a decrease in goblet cell number, but the same shape can be observed.

Grade 2; the epithelial cells are larger, more polygonal. The goblet cells are significantly reduced in number and are smaller.

Grade 3; the epithelial cells are large and more polygonal with the nuclei small and pyknotic. The goblet cells are completely absent

The results of the impression cytology differed between the three groups. In glaucoma patients, impression cytology was grade 1 in 4 subjects (20.66%), grade 2 in 9 subjects (60%) and grade 3 in 2 subjects (13.33%). In the DE group impression cytology was grade 1 in 9 subjects (60%), and grade II in 6 subjects (40%). In the control group, impression cytology was grade 0 in 13 subjects (86.66%) and grade I in 2 subjects (13.33%) (Table 4.3.). Figure 4.1.A.,B., and C. represents the microscobic feature s of PAS satining of groups subjects.

Table 4.3. Impression cytology scoring in the glaucoma, DE, and control group

Nelson grading system score	Glaucoma group	DE group	Normal control group
Grade 0	(0) 0%	(0) 0%	(13) 86.66%
Grade 1	(4) 26.6%	(9) 60%	(2) 13.33%
Grade 2	(9) 60%	(6) 40%	(0) 0%
Grade 3	(2) 13.33%	(0) 0%	(0) 0%

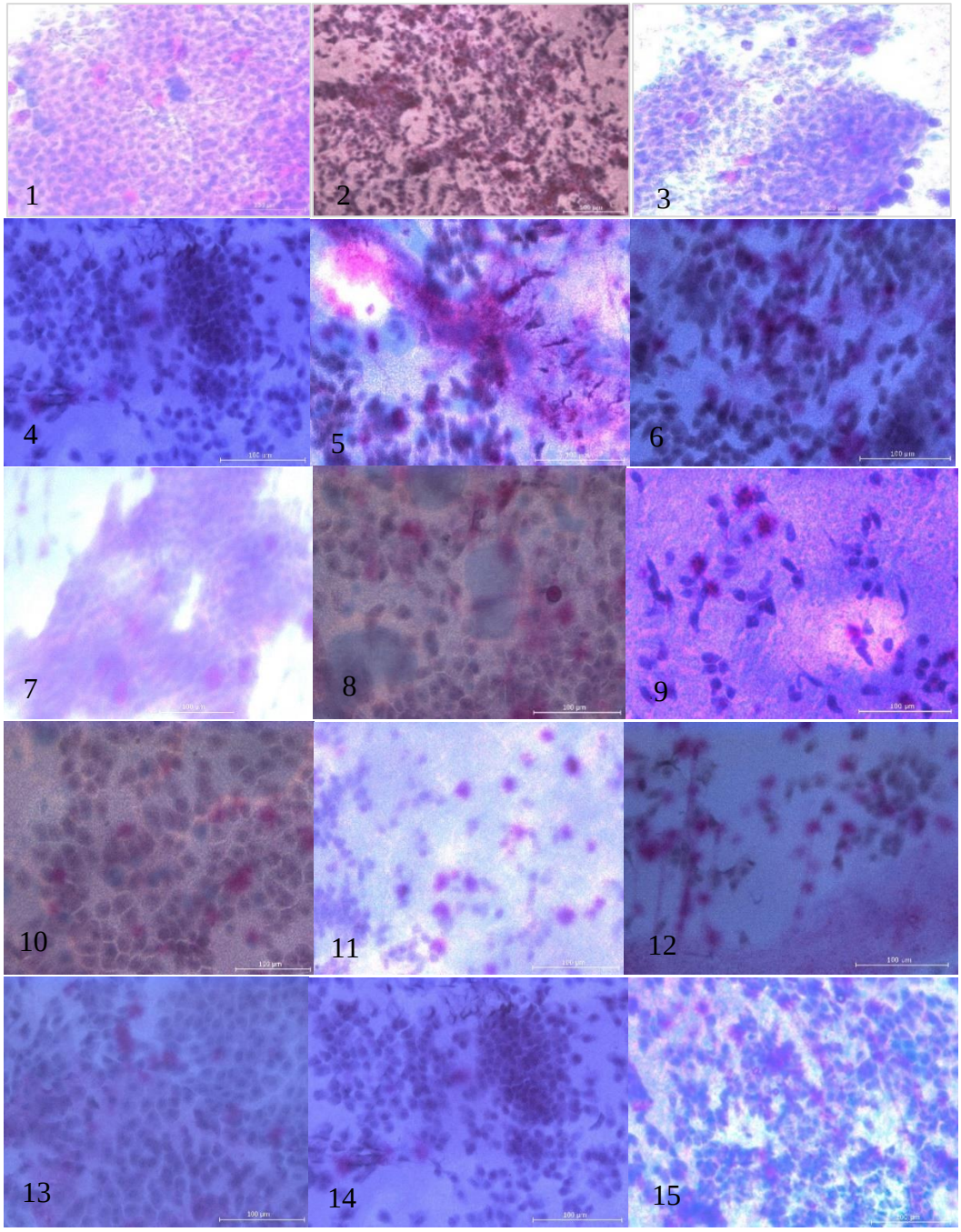


Figure 4.1. A. PAS staining results of impression cytology in normal control group subjects (Magnification 20X). patients were given the same numbers dipected on the left corner of the picture for all figures (Scale bar=100µm)

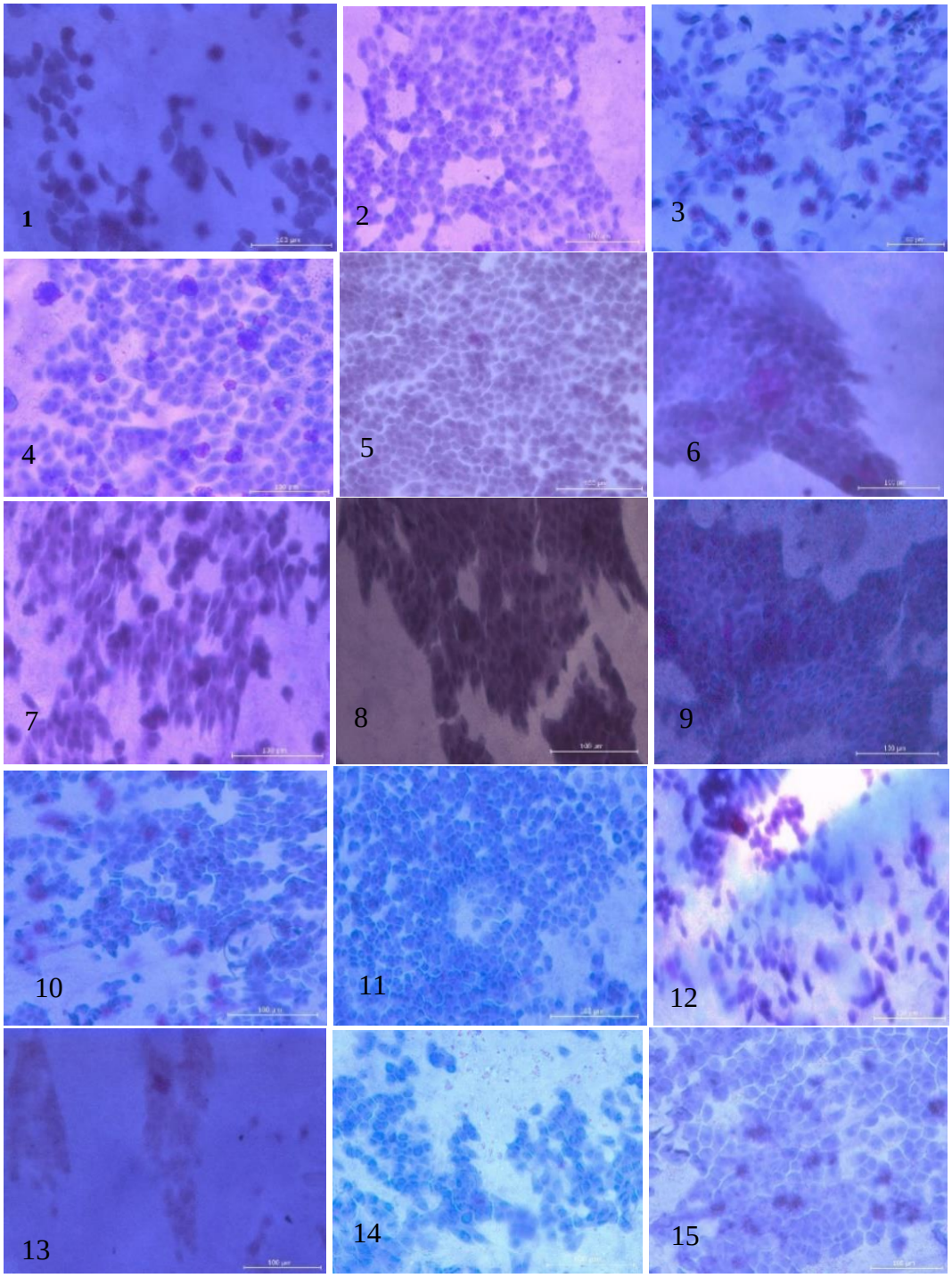


Figure 4.1. (continues) B. PAS staining results of impression cytology in dry eye patients (Magnification 20X). Patients were given the same numbers dipected on the left corner of the picture for all figures(Scale bar=100µm)

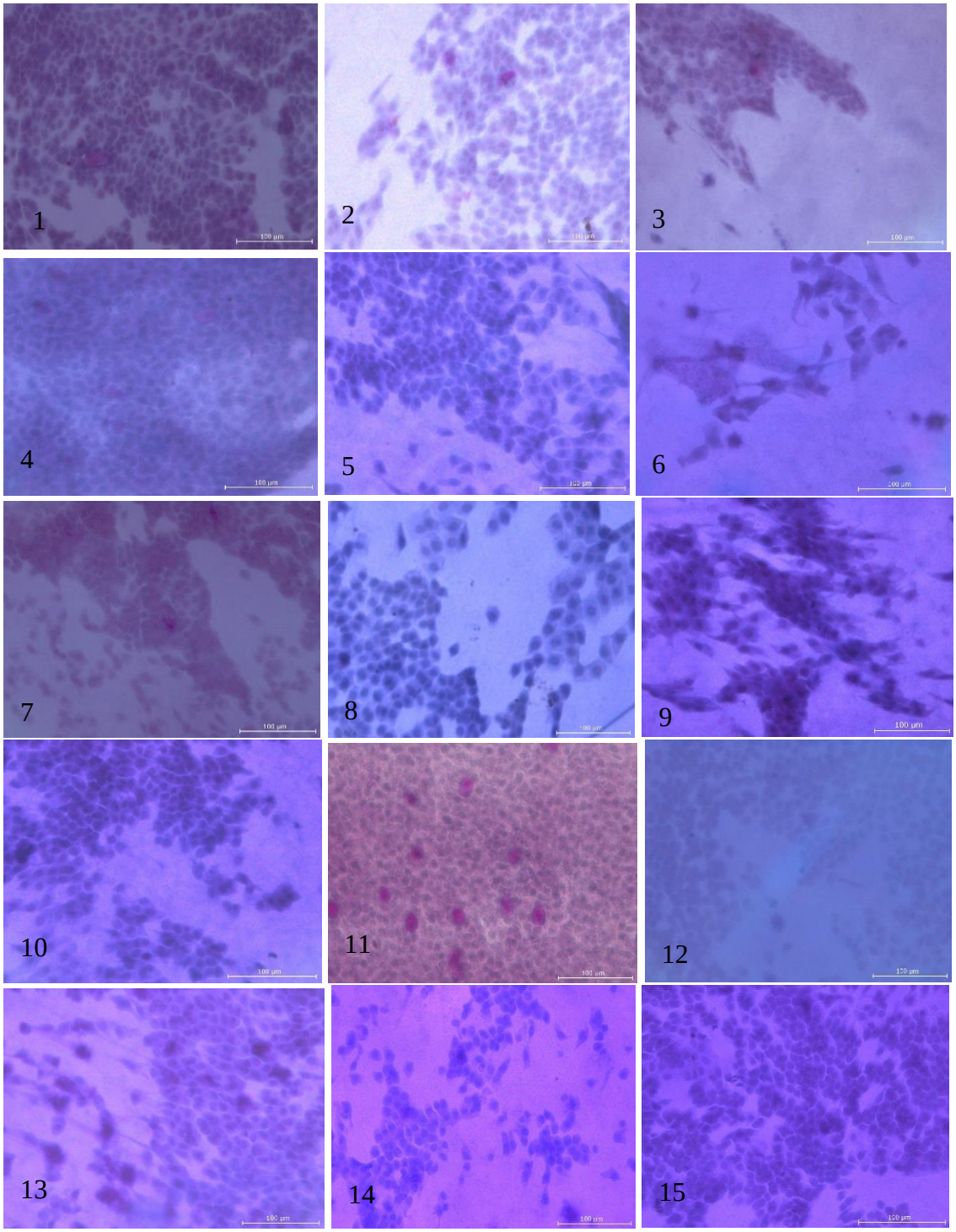


Figure 4.1. (continues) C. PAS staining results of impression cytology in glaucoma patients (Magnification 20X). Patients were given the same numbers dipected on the left corner of the picture for all figures (Scale bar=100µm)

Determination of goblet cell density (GCD) in PAS staining

Mean GCD was determinde by counting goblet cells per 1 mm of conjunctival epithelial. GCD in glaucoma patients was 4.120 ± 4.437. GCD in DE patients and control group were 5.653 ± 2.951, 16.97 ± 5.380, respictively.

A significant difference was estblished between GCD in glaucoma and control group (P<0.0001). Also, there was a significant difference in GCD between the DE and control groups (P<0.0001). No significant difference in GCD between glaucoma and DE group (P= 0.2745).

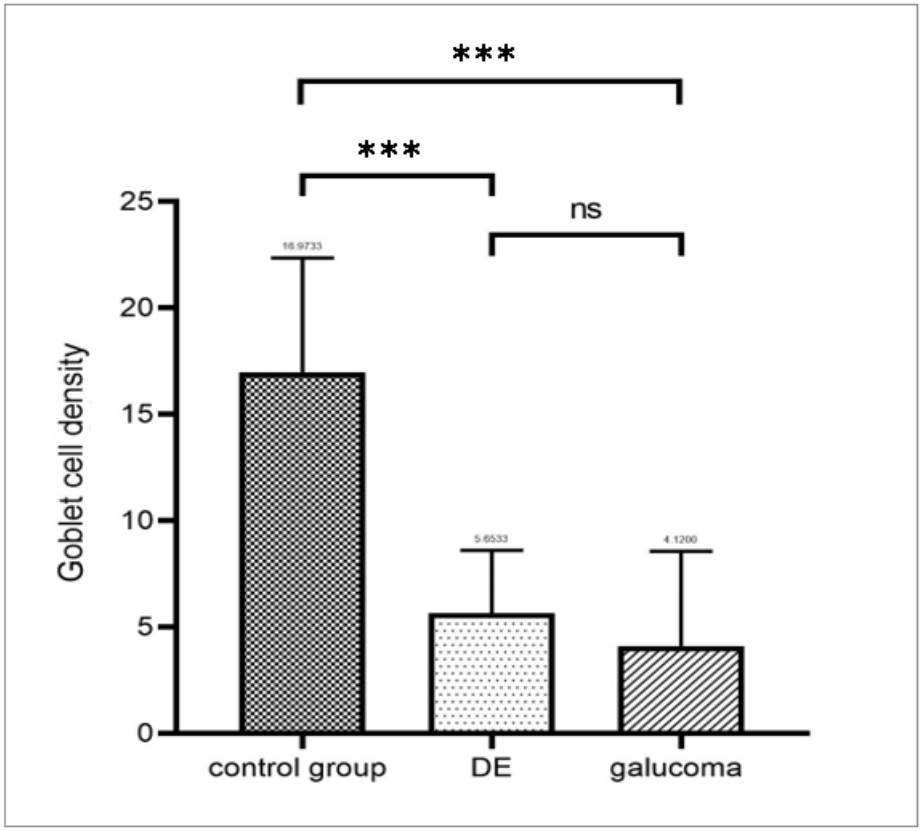


Figure 4.2. Mean GCD levels in glaucoma, DE and healthy control group. (***;P< 0.0001, ns=non-segnificant)

4.2.2. Assessment of MUC5AC expression by indirect immunofluorescence results

Expression of the glycoprtien by indirect immunofluorescence staining of the impression cytology specimens using an anti-MUC5AC antibody. The assessment of the results of indirect immunofluorescence was done by counting the positive MUC5AC cells. Figure 4.3.A.,B., and C. represnts the immunofluorescence results of all subjects of the groups.

Positive immunofluorescent staining for MUC5AC was observed in a significantly greater percentage of conjunctival impression samples from the eyes of the control groups than in those from the glaucoma and DE groups. The mean and standard deviation of MUC5AC expression in normal subjects were 18.42 ± 8.179 and 8.450 ± 7.619 in glaucoma subjects, and there was a significant difference between these two groups (P = 0.0018). Also, there was a significant difference between the control and DE groups (10.89 ± 9.803) (P = 0.0301). No significant difference was noted between the glaucoma and DE groups (P = 0.4532). Table 4.4. and figure 4.4. reprsents the mean expression of MUC5AC in groups subjects.

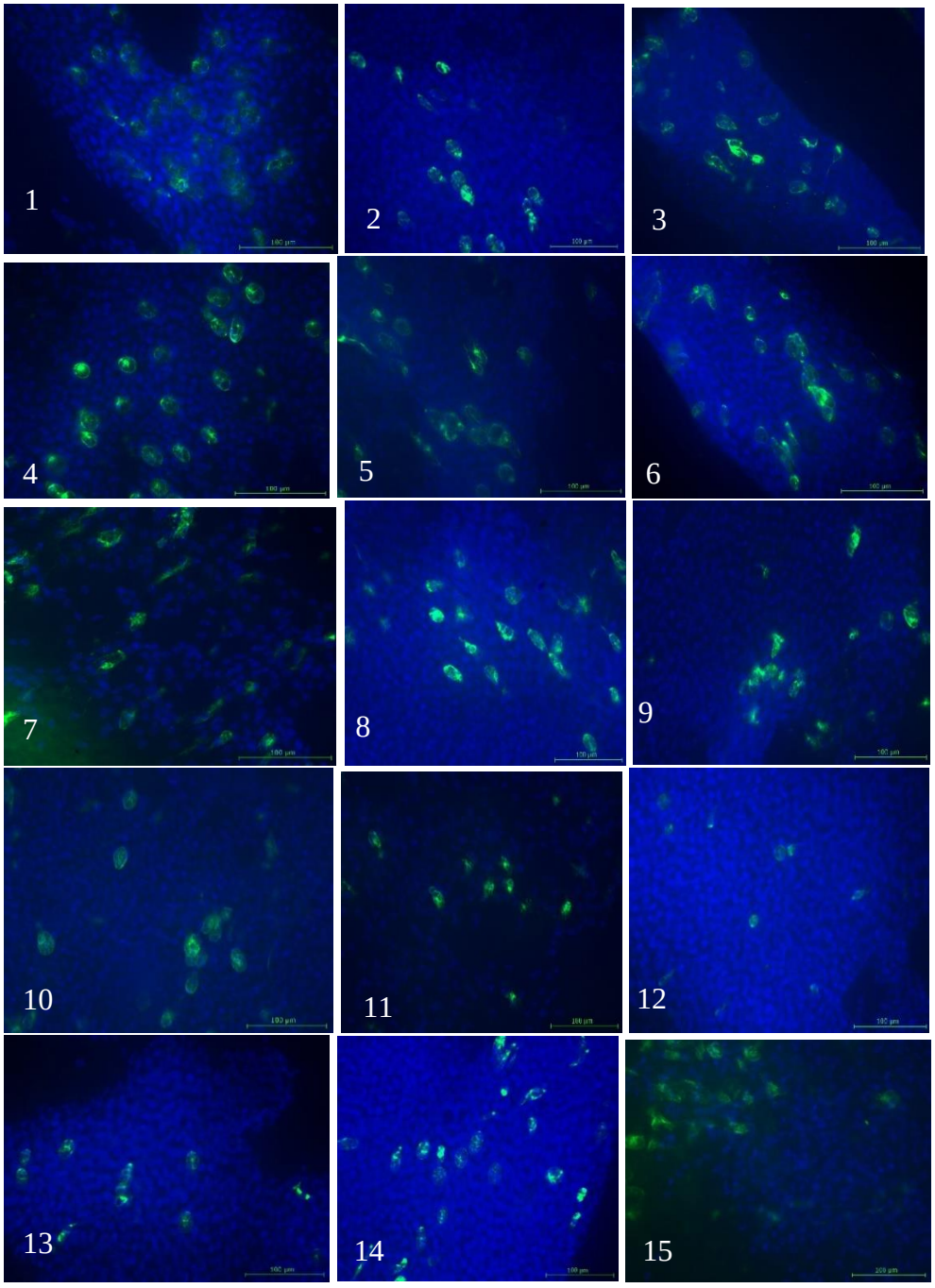


Figure 4.3. A. Immunofluorescence image showing numerous MUC5AC positive goblet cells (green) from normal indeviduals. In blue DAPI nuclear staining. Patients were given the same numbers dipected on the left corner of the picture for all figures (Magnification 20X). (Scale bar=100μm)

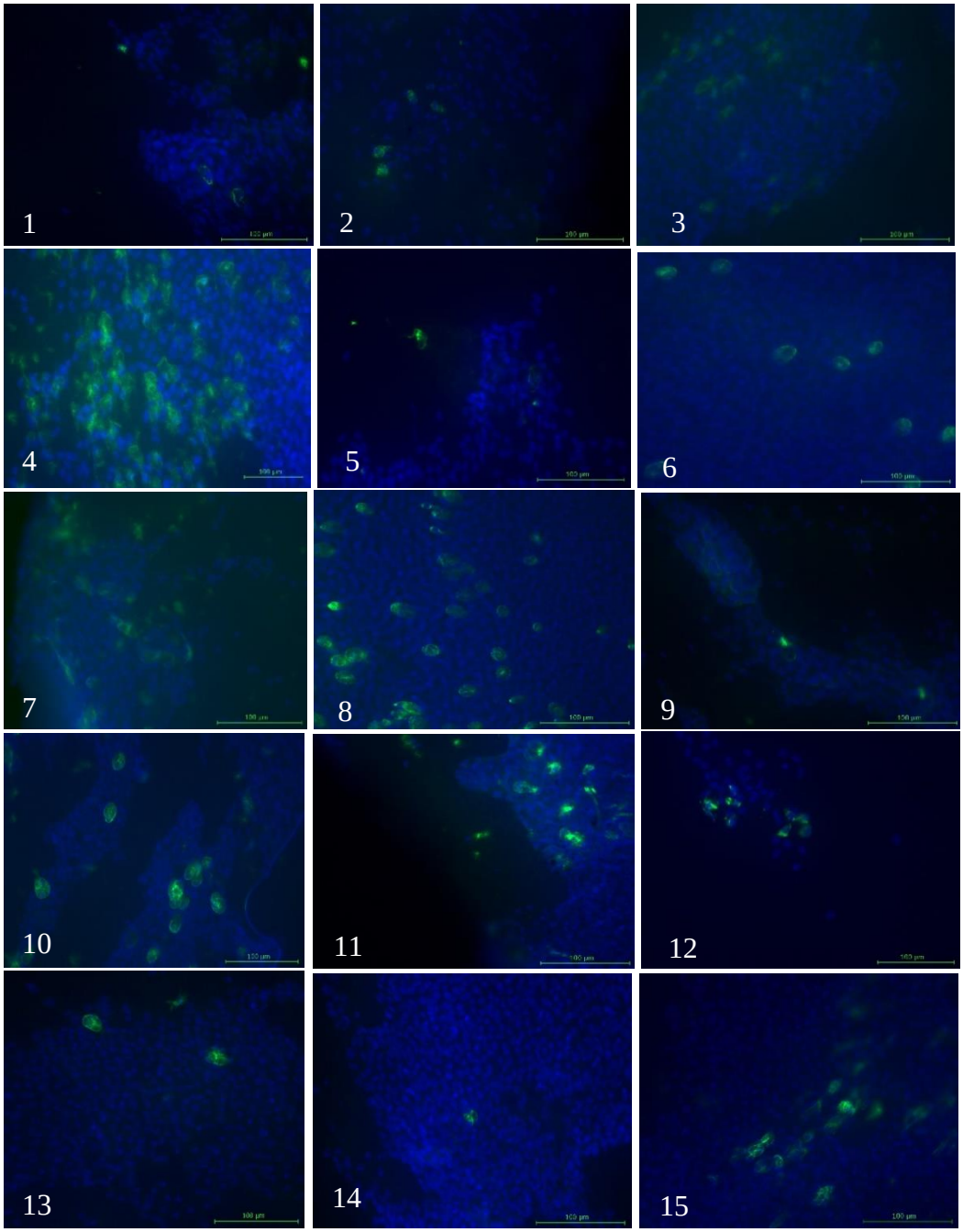


Figure 4.3. (continues) B. Immunofluorescence image showing MUC5AC positive goblet cells(green) from DE patients. In blue DAPI nuclear staining. Patients were given the same numbers dipected on the left corner of the picture for all figures (Magnification 20X). (Scale bar=100μm)

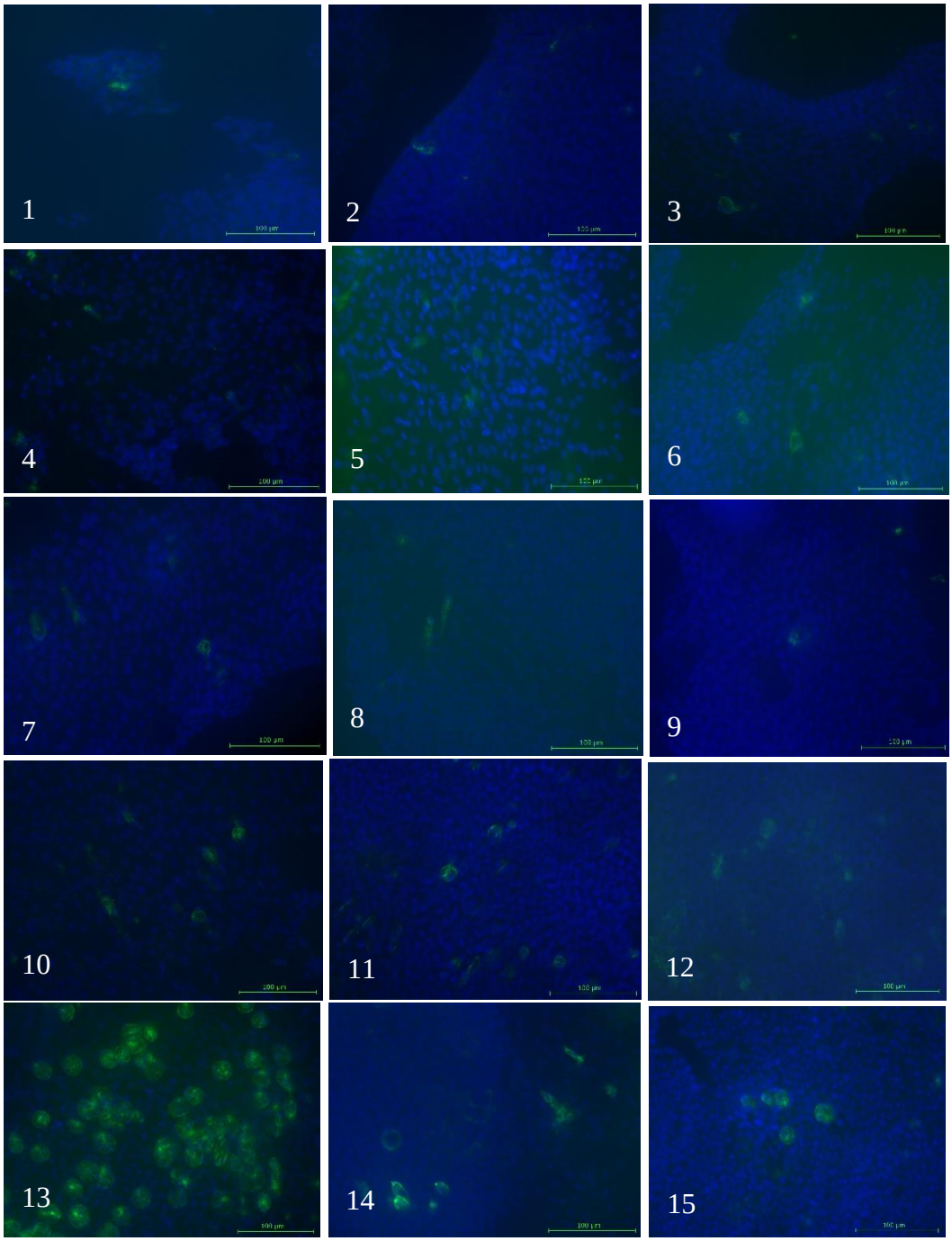


Figure 4.3. (continues) C. Immunofluorescence image showing few MUC5AC positive goblet cells(green) from glaucoma patients. In blue DAPI nuclear staining. Patients were given the same numbers dipected on the left corner of the picture for all figures (Magnification 20X). (Scale bar=100μm)

Table 4.4. MUC5AC expression (Mean, SD) in the glaucoma, DE, and control group

Expression of MUC5AC	Glaucoma	DE	Control group	P*	P**	P***
Mean	8.450	10.89	18.42	0.0018	0.4532	0.0301
SD	7.619	9.803	8.179			

P value:
P* = glaucoma vs control group
P**= glaucoma vs DE group
P***= DE vs control group

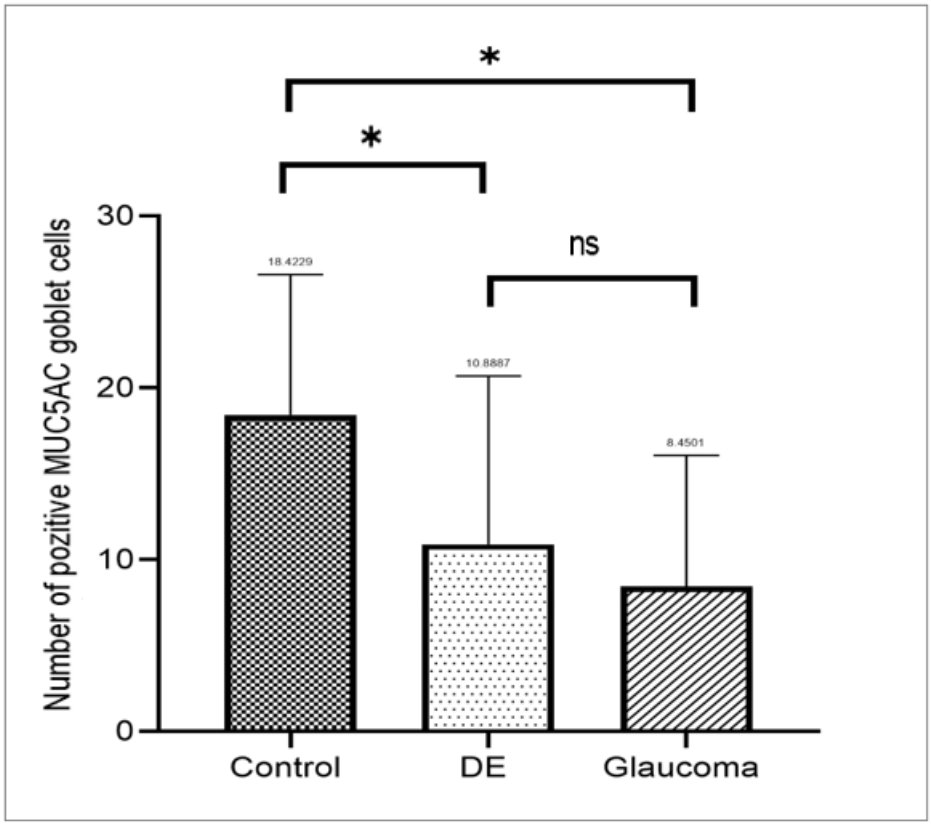


Figure 4.4. Mean expression of MUC5AC in glaucoma, DE and healthy control groups. (*;P< 0.05, ns= non-significant)

4.3. Cytokine Profiles of Tear Fluids

The concentration of IL-1β, IL-1Ra, VEGF, and PGE2 cytokines in tear samples taken from glaucoma, DE, and control groups were determined, and any significant differences were noted.

4.3.1. IL-1β

The concentration of IL-1β was significantly higher in the tear fluid of both the glaucoma and DE groups than in the control group. The mean concentration and standard deviation of IL-1β in tears were 242.4 ± 111.1 pg/ml in the control group, 418.5 ± 318.0 pg/ml in the DE group, and 710.1 ± 621.2 pg/ml in the glaucoma group.

The difference between the glaucoma and control groups was statistically significant (P = 0.0137). By comparing the DE group and the control group, there was no significant difference between the two groups (P = 0.0515). The difference in the concentration of IL-1β higher in the glaucoma group than in the DE group, no significant difference was found between those two groups (P = 0.4509). By using one-way ANOVA test the difference between the groups was statisticaly significant (P = 0.0302).

The concentrations of the IL-1β cytokine in the tear fluid of normal subjects and patients with glaucoma and DED are presented in Table 4.5. and figure 4.9. represents the difference in IL-1β concentrations between the three groups.

Table 4.5. Comparison of IL-1β concentration in the tear between the study groups

Levels of IL-1β (pg/ml)	Glaucoma	DE	Control group	P*	P**	P***	P****
Mean	710.1	418.5	242.4	0.0137	0.4509	0.0515	0.0302
SD	621.2	318.0	111.1				

P value:
P* = glaucoma vs control group
P**= glaucoma vs DE group
P***= DE vs control group
P****= one-way ANOVA test

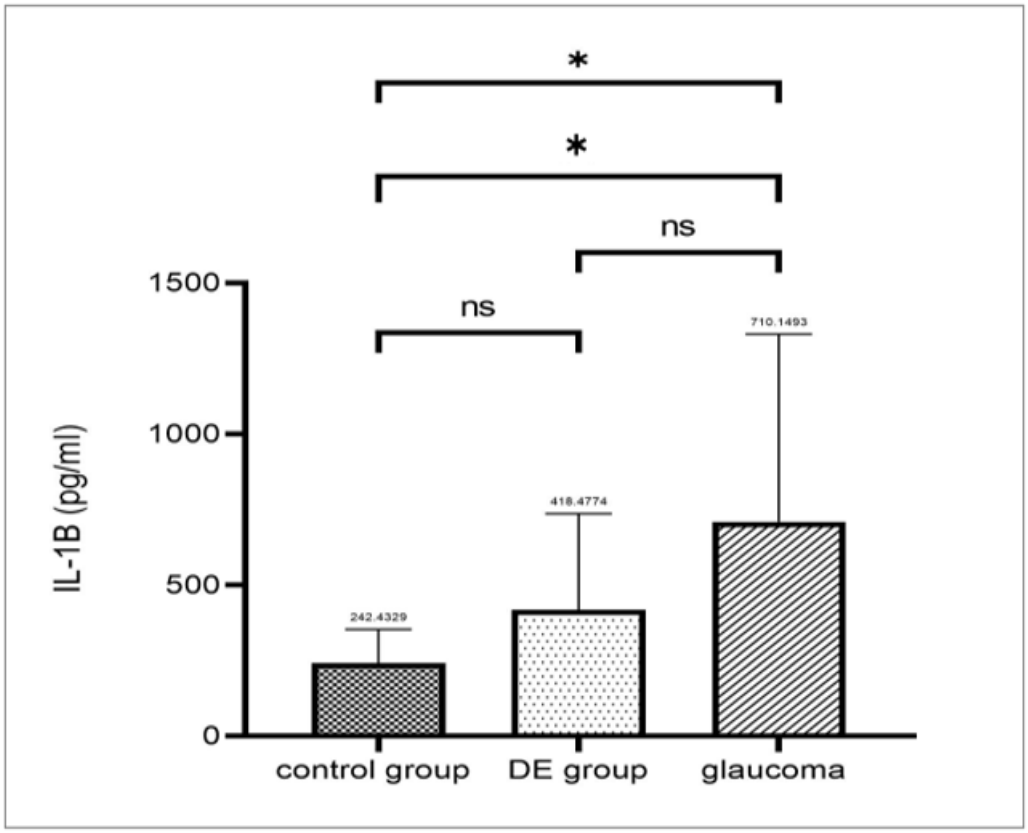


Figure 4.5. Mean tear IL-1β (pg/ml) levels in glaucoma, DE and healthy control groups. (*;P< 0.05, ns= non-significant)

4.3.2. IL-1Ra

The concentration of IL-1Ra was significantly higher in the tear fluid of both the glaucoma and DE groups than in the control group. The mean concentration and standard deviation of IL-1Ra in tears were 3661 ± 3975 pg/ml in the control group, 6056 ±6323 pg/ml in the DE group, and 7617 ± 9022 pg/ml in the glaucoma group.

Despite the fact that the concentration of IL-1Ra was higher in the glaucoma group than the other groups (DE, control group), no significant difference was found between glaucoma and DE (P = 0.7006) or glaucoma and the control group (P = 0.2012). Also, no statistical difference was found between the DE group and the control group (P = 0.5907). By using one-way ANOVA test the difference between the groups was statisticaly significant (P = 0.4776).

The concentrations of the IL-1Ra cytokine in the tear fluid of normal subjects and patients with glaucoma and DED are presented in Table 4.6. and figure 4.10. represents the difference in IL-1R1 concentration between the three groups.

Table 4.6. Comparison of IL-1Ra concentration in the tear between the study groups

Levels of IL-1Ra (pg/ml)	Glaucoma	DE	Control group	P*	P**	P***	P****
Mean	7617	6056	3661	0.2012	0.7006	0.5907	0.4776
SD	9022	6323	3975				

P value:
P* = glaucoma vs control group
P**= glaucoma vs DE group
P***= DE vs control group
P****= one-way ANOVA test

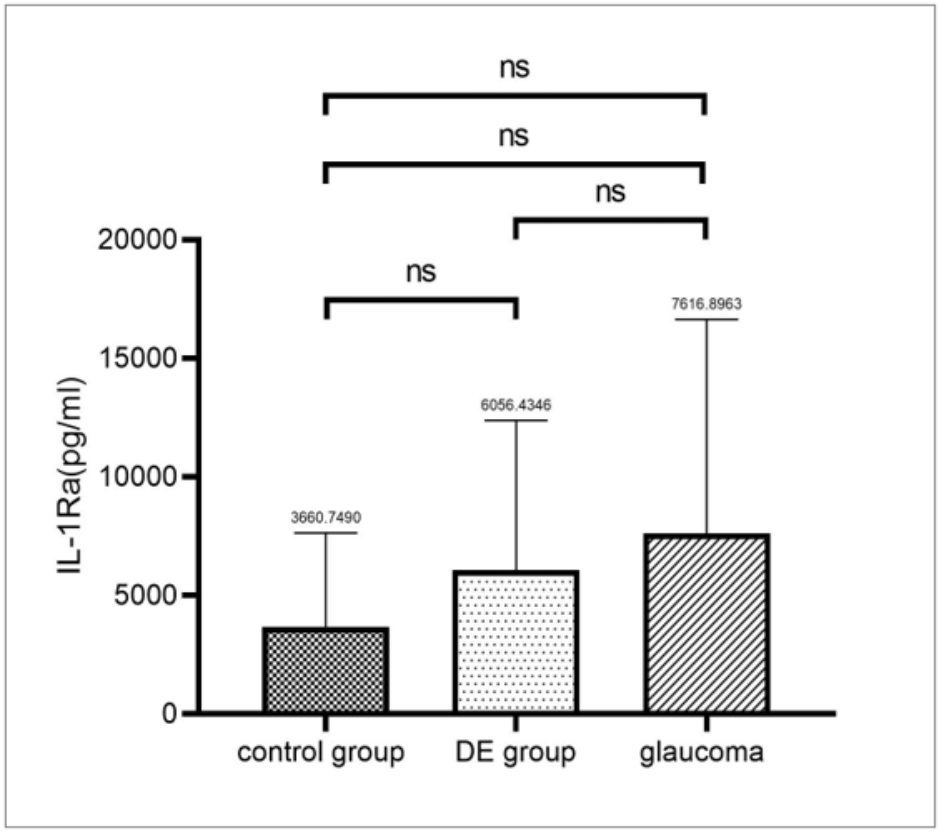


Figure 4.6. Mean tear IL-1Ra (pg/ml) levels in glaucoma, DE and healthy control groups. (ns= non-significant)

4.3.3. VEGF

The table 4.7. shows the mean levels of VEGF in tears. Figure 4.11. shows the differences in VEGF concentration among the 3 groups. The glaucoma group had higher mean concentrations of tear VEGF cytokine than the other two groups. The mean values of VEGF levels in tears were 454 ± 262 pg/ml in the control group, 818.8 ± 642.7 pg/ml in the DE group, and 1276 ± 1060 pg/ml in the glaucoma group.

The level of VEGF in tears were significantly increased in glaucoma group compared to the control group (P= 0.0069). There was no statistically significant difference was found in VEGF concentrations between glaucoma and DE group (P=0.1645). no statisticaly signifacant difference also was been observed between DE and control group (P=0.0513). By using one-way ANOVA test the difference between the groups was statisticaly significant (P = 0.0138).

Table 4.7. Comparison of VEGF concentration in the tear between the study groups

Levels of VEGF (pg/ml)	Glaucoma	DE	Control group	P*	P**	P***	P****
Mean	1276	818.8	454	0.0069	0.1645	0.0513	0.0138
SD	1060	642.7	262				

P value:
P* = glaucoma vs control group
P**= glaucoma vs DE group
P***= DE vs control group
P****= one-way ANOVA test

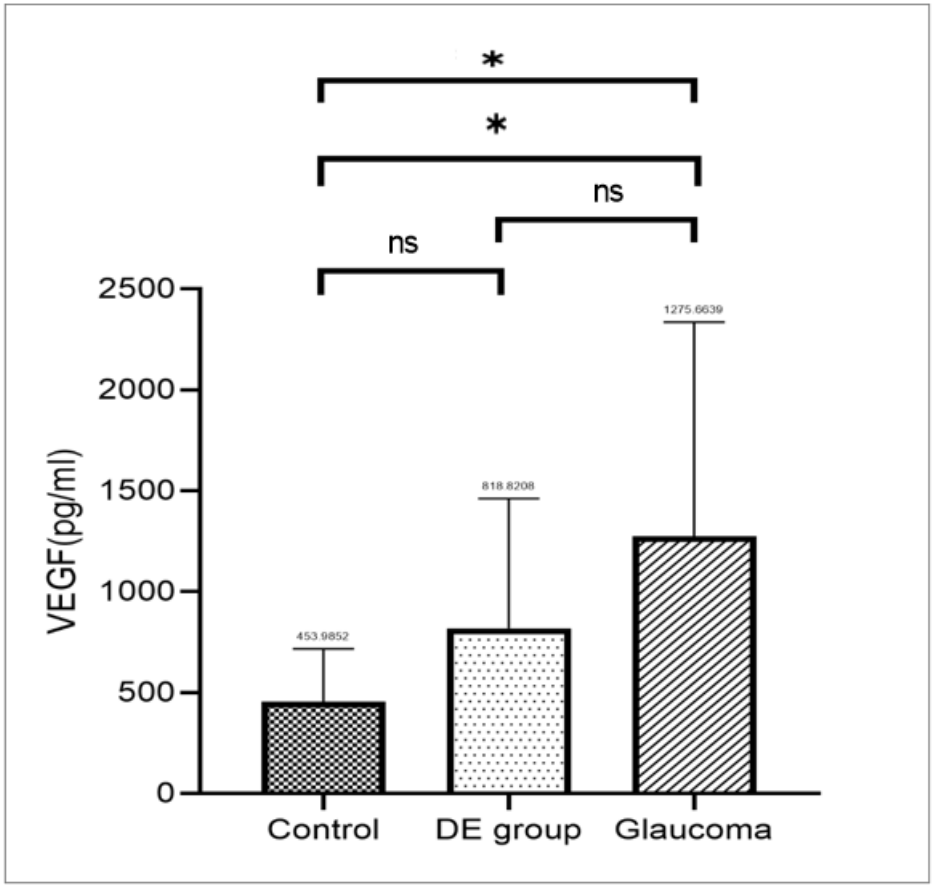


Figure 4.7. Mean of VEGF levels (pg/ml) in tears of glaucoma, DE and healthy control groups. (*; P<0.05, ns= non-significant)

4.3.4. PGE2

A summary of the laboratory analyses for PGE2 concentration among the three groups is appreciated in Table 4.8. and figure 4.12. depicts the differences in PGE2 concentrations between the three groups. The mean values of PGE2 cytokine levels in tears were 325.2 ± 164.2 pg/ml in the control group, 503.3 ± 331.4 pg/ml in the DE group, and 930.9 ± 880.7 pg/ml in the glaucoma group. The level of PGE2 cytokine in tears was significantly higher in the glaucoma group compared with the control group (P = 0.0141). There was no significant difference in PGE2 concentration between the glaucoma and DE groups (P = 0.0894). No statistically significant difference was shown between the DE and control groups (P = 0.0726). By using one-way ANOVA test the difference between the groups was statisticaly significant (P = 0.0135).

Table 4.8. Comparison of PGE2 concentration in the tear between the study groups

Levels of PGE2 (pg/ml)	Glaucoma	DE	Control group	P*	P**	P***	P****
Mean	930.9	503.3	325.2	0.0141	0.0894	0.0726	0.0135
SD	880.7	331.4	164.2				

P value:
P* = glaucoma vs control group
P**= glaucoma vs DE group
P***= DE vs control group
P****= one-way ANOVA test

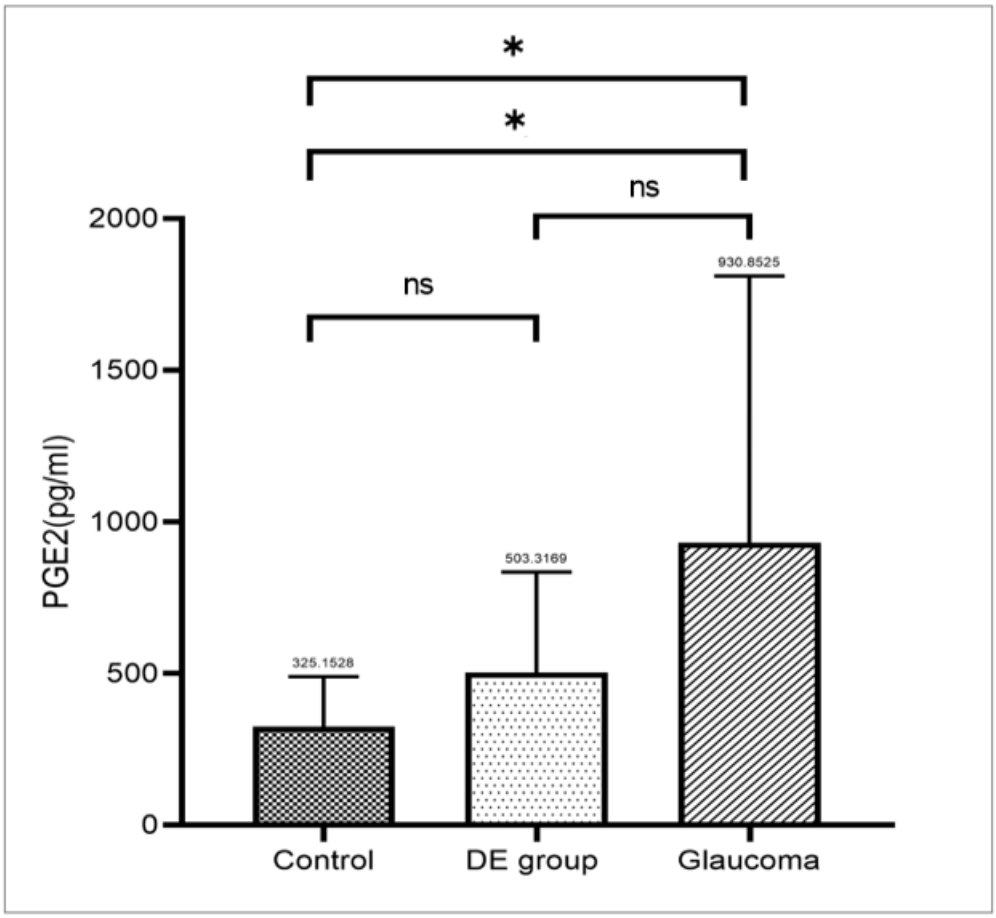


Figure 4.8. Mean PGE2 levels (pg/ml) in tears of glaucoma, DE and healthy control groups. (*; P<0.05, ns= non-significant)

4.4. Correlation Analysis

4.4.1. Correlations between tear cytokines and ocular surface parameters

We next investigated correlations between tear cytokines and parameters of ocular surfaces, including OSDI, TBUT, the Schirmer test, and corneal staining. For the OSDI, there was a weak positive but non-significant correlation found between the OSDI and the

following cytokines: IL-1β (r = 148), VEGF (r = 262), and PGE2 (r = 163). No correlation was found between OSDI and IL-1Ra (r = 0.053).

Strong negative and significant correlations were found between TBUT and two inflammatory mediators: VEGF (r = -0.306, P =0.041) and PGE2 (r = -0.302, P =0.044). A weak negative and non-significant correlation was found between TBUT and IL-1β (r = -0.244, P = 0.106); and IL-1Ra (r = -0.139, P = 0.362).

Both VEGF (r = -0.353, P =0.017) and PGE2 (r = -0.319, P =0.033) levels were negatively and significantly correlated with the schirmer test. IL-1β (r = -0.280, P =0.062) and IL-1Ra (r = -0.116, P =0.448) were negatively correlated with the Shirmer test, but not significantly. The corneal staining score was related to levels of Il-1β (r =0.427, P =0.003) and PGE2 (r = -0.452, P =0.002). No correlation was found between IL-1Ra (r = 0.008, P =0.959) and VEGF (r = 0.259, P =0.086) cytokines.

Table 4.9. Correlation between cytokines and clinical parameters

		IL-1B	IL-1RA	VEGF	PGE2
OSDI	Pearson Correlation	0.148	0.053	0.262	0.163
	Sig. (2-tailed)	0.331	0.728	0.082	0.286
TBUT	Pearson Correlation	-0.244	-0.139	-0.306*	-0.302*
	Sig. (2-tailed)	0.106	0.362	0.041	0.044
Schirmer test	Pearson Correlation	-0.280	-0.116	-0.353*	-0.319*
	Sig. (2-tailed)	0.062	0.448	0.017	0.033
Corneal grade	Pearson Correlation	0.427**	0.008	0.259	0.452**
	Sig. (2-tailed)	0.003	0.959	0.086	0.002

Correlation is significant at the 0.01 level (2-tailed).**
Correlation is significant at the 0.05 level (2-tailed).*
OSDI (Ocular surface desease index), TBTU (Tear break up time)

4.4.2. Correlation between inflammatory cytokines and impression cytology results (GCD, MUC5AC Expression)

The correlation between inflammatory cytokines and both GCD, and MUC5AC expression was also investigated. A negative correlation between inflammatory cytokines and impression cytology results (GCD, MUC5AC) was found, but this correlation was non-significant.

Table 4.10. Correlation between both GCD and MUC5AC expression and cytoines in the study groups

		IL-1B	IL-1Ra	VEGF	PGE2
GCD	Pearson Correlation	-0.185	-0.280	-0.255	-0.270
	Sig. (2-tailed)	0.223	0.063	0.091	0.073
MUC	Pearson Correlation	-0.214	-0.146	-0.191	-0.246
	Sig. (2-tailed)	0.159	0.339	0.208	0.104

5. DISCUSSION

Patients with glaucoma are assumably at a greater risk of developing OSD, as both conditions are more prevalent in older patients. Additionally, chronic use of anti-glaucomatous eyedrops containing preservatives can induce OSD. One of the most common used preservatives in the ophthalmological medications is BAC. The toxicity of BAC has already been revealed on conjunctival cells [299], where BAC was identified, even at a low concentration, as an inducer of cellular death. BAC degrades into hydrogen peroxide, which can induce oxidative stress and inflammatory reactions in ocular and conjunctival epithelial cells [300]. This preservative can remain in numerous ocular tissues for up to one week after administration, making the conjunctival and corneal epithelium susceptible to toxic effects [301].

Dry eye disease (DED) is a chronic eye disorder associated with age, corneal inflammation, and hormonal changes, and exacerbated by prolonged use of digital displays. It’s important to mention that due to the prolonged use of face masks by people after the pandemic of COVID 19, the prevalence of dry eye has been increased.

In the present study we sought to compare the expression of some inflammatory cytokines in the tears of glaucoma patients whose taking IOP-lowering topical medications, as well as patients with DED, versus the healthy controls. Also, to assess the ocular surface changes in those patients.

By obtaining medication histories of our group of glaucoma patients, we found out that the majority of patients are receiving more than one medicine and for a period of more than one year. Lavin and colleagues reported that the successful rate of filtering surgery was high in patients who had taken preoperative medical treatment for an average of only two weeks compared with those who had received the medical treatment for at least one year [302].

The OSDI questionnaire has been used in various research studies to determine the prevalence of OSD. In our study OSDI values point out statistically significant differences among the three groups. Most of the participants in the control group presented with a

normal to mild OSDI. Otherwise, glaucoma and dry eye patients had higher OSDI score than the control group (P=0.0001).

Regarding the clinical tests of DED, both patients with glaucoma and DED revealed significant abnormalities, even when glaucoma patients had not previously been diagnosed with DE. Scores of the Schirmer's test were considerably lower in glaucoma patients and DED than in healthy controls (P=0.0001), indicating decreased tear secretion in both disease groups. Moreover, TBUT was significantly shorter in glaucoma patients and DED (P=0.0001) compared to healthy controls, reflecting the instability of the tear film in both glaucoma and DED groups in comparing to healthy control. Our results agree with those of previous studies that showed an increase in the prevalence of OSD in glaucoma patients receiving topical medications [303,304].

The lacrimal function unit (LFU) is composed of the conjunctival goblet cells, surface epithelium, eyelids, lacrimal glands, meibomian glands, lacrimal drainage system, mucosal immune system, and neural network that connects them. These tissues have a functional interaction that act to regulates immune expression levels in the tear film, protects the integrity of the ocular surface, and maintains transparency of the cornea [305].

It's clear now that inflammation has a crucially important role in the etiology of OSD. By measuring and comparing these cytokines in patients with a control group, it will establish a better understanding of the role that cytokines play in the pathogenesis of the DED. Several methods have been used to measure the cytokines levels in tears, one of these methods is enzyme linked immune assay (ELISA). ELISA is a highly sensitive and specific method that has been extensively used to quantify cytokines in several biological samples.

Human tears contain a wide range of cytokines and chemokines that help to maintain the function and morphology of the ocular surface. When ocular surface epithelial cells are exposed to hyperosmolar stress, proinflammatory cytokines are released in the tears. Inflamed epithelial cells are known to release IL-1β, IL-6, IL-8, and TNF-α, which may indicate ocular surface stress. This may explain why those cytokines have been found in the tears of patients with different inflammatory ocular surface disorders, including seasonal allergic conjunctivitis, and vernal and atopic keratoconjunctivitis [306]. There are

several techniques for obtaining tear samples and assessing the level of various proteins; one of them is the flushing method, which was used in this study because it is non invasive and simple. With the exception of Narayanan et al. who found normal expression profiles of numerous cytokines in individuals with moderate DED, the majority of research have identified higher cytokine levels in the tears of DED patients.

Cytokines are small molecular weight proteins which play crucially important role in regulating immune response and inflammation. The family of IL-1 cytokines are key signaling molecules for the innate and adaptive immune systems. IL-1 has been involved in the pathogenesis of inflammatory disorders in human, including rheumatoid arthritis, periodontitis, and septic shock, and also the corneal and ocular surface diseases. The high level of ocular irritation suggests inflammatory damage, our finding revealed that the level of proinflammatory IL-1β cytokine in tear fluid obtained from the glaucoma patients was higher and significant difference was found between the glaucoma and the control group (P=0.0137). Increased levels of IL-1β along with other inflammatory cytokines (IL-1, TNFa) in patients with DE have been demonstrated in other studies [164]. IL-1β has been shown to reduce tear formation via neuronal and hormonal mechanisms [307].

IL-1Ra functions by inhibiting competitively the binding of proinflammatory forms of IL-1 to their signal-transducing IL-1R1. In contrast to IL-1β, the anti-inflammatory form, IL-1Ra, was present in enormous quantities. IL-R1a level was high in both patients with glaucoma and DED than control group, but the difference was non-significant between groups. The high concentration of IL-1Ra in tear fluid may be a physiological homeostatic mechanism that inhibits the activation of IL-1-mediated inflammatory processes on the ocular surface. This fininding is consistent with previous studies demonestrating an increased IL-1Ra level in tears of patients with DED [308].

The disturabance in balance between IL1 and IL-1Ra promotes the progression of inflammatory disorders. Our study revealed that the concentration of IL1Ra was higher than IL-1β in all the subjects of the groups. This result indicates an imbalance between pro- and anti-inflammatory cytokines in these individuals and suggests that restoring this cytokine balance may be a potential future treatment target. It has been shown that the use of topical application of IL-1Ra is beneficial in relieving the clinical symptoms of dry eye disease and decreasing the underlying inflammation.

VEGF has been characterized as a cytokine related to neovascularization, drainage system remodeling, and inflammation. It is well documented now that VEGF is responsible for a number of ocular disorders involving angiogenesis, such as corneal transplantation, neovascular glaucoma, age-related macular degeneration, and diabetic retinopathy [309]. Yoo et al. [310] revealed that VEGF has a direct proinflammatory effect by activating endothelial cells to produce chemotactic factors such as IL-8 and MCP-1. VEGF can also stimulate monocytes to secrete proinflammatory molecules such as IL-6 and TNF-α, which exacerbate the inflammatory process [311]. In previous studies, it has been shown that VEGF concentrations were increased in tear samples from patients with ocular chronic inflammatory disorders, such as DED and vernal keratoconjunctivitis [312]. Jiang et al. [311] demonstrated that the subconjunctival injection of bevacizumab (anti-VEGF) in patients with DED could relieve the inflammation at the ocular surface.

Our data revealed that the glaucoma group had a higher mean serum VEGF level than the control group (P=0.0069). In a study comparing evaporative DED to normal participants, the VEGF levels in tears were shown to be elevated in DE group [313]. In contrast to one study that found a significantly decreased in the VEGF level in DE patients as compared to both control and glaucoma group [314]. It's well established that lymphatics play a crucial role in initiating inflammatory responses in tissues by directing the antigen presenting cells to the draining LNs, where T lymphocytes are primed and expanded. It has been reported that lymphangiogenesis occurs in the DED cornea without concurrent hemangiogenesis [309]. In consequence, autoreactive Helper T cell (TH)17 and TH1 cells, which play a role in DED immunopathogenesis, are produced in these lymphoid organs [315].

It has been shown that IL-1 is a pro-angiogenic factor that promotes the growth of tumors and stimulates VEGF in several types of cells [316]. Also, VEGF expression can be stimulated by prostaglandins [317].

Prostaglandins (PGs) are well recognized as mediators of acute inflammation, and recent animal studies have shown that they also play a role in the transition to and maintenance of chronic inflammation. Several studies demonstrated the increase PGE2 concentrations in tears of DED and patients with glaucoma these studies results were further supported by elevated levels of COX-2 and PGE synthase in the tear-producing tissue of mice with dry eye. Our findings showed the level of PGE2 in tears was reported to be high in patients

with glaucoma compared to the control group(P=0.0141), indicating the potential significance of PGE2 in inflammatory processes of ocular surface diseases.

The high level of PGE2 in patients with DED may increase the ocular surface inflammation by stimulating other inflammatory molecules, which further induce secretion of PGE2. Additionally, at the ocular surface PGs promote vasodilation, disruption of the blood–ocular barrier, and migration of the leukocyte [318].

Impression cytology has significantly improved the research of ocular surface cell populations. Applications of impression cytology include the etiological diagnosis of various ocular surface disorders, the documentation of sequential changes in the conjunctival and corneal surface over time, the monitoring of treatment effects and the staging of conjunctival squamous metaplasia, and as an investigative tool for analyzing ocular surface disease with immunostaining and DNA analysis. Epithelial cell morphology and conjunctival GCD can provide an objective measure to assess the integrity of the ocular surface.

Squamous cell metaplasia is a nonspecific conjunctival response to a wide range of toxic inflammatory stimuli [319]. Paschides et al. reported that aging had no effect on the quantity of goblet cells or the nuclear cytoplasmic ratio in normal eyes [320]. Also, Herreras et al. showed that goblet cell density and structure were stable in patients under the age of 80 [321].

Generally, mucins are defined as large extracellular glycoproteins that cover the majority of the body's mucosal surfaces. Goblet cells secrete the gel forming mucin MUC5AC, which is considered the primary component of the mucous layer of the tear film, and this layer is responsible for preserving the tear film's stability. It has also been found that conjunctival goblet cells secrete immunomodulatory molecules, including retinoic acid (RA) and TGF-β2 [322]. Additionally, GCs play a crucial role in the induction of conjunctival immunological tolerance according to the findings of previous study. Recently, small intestine and colonic goblet cells have been assigned a novel function: luminal antigen presentation to dendritic cells. This characteristic has also been identified in the conjunctival GC, which serve as antigen transport conduits to immune cells in the underlying stroma at the ocular surface. In addition to correlating with the severity of

ocular surface disease, the loss of goblet cells appears to maintain a cycle of chronic inflammation at the ocular surface [323].

In this study, impression cytology PAS staining results revealed that conjunctival GCD was remarkably reduced in the glaucoma and dry eye groups compared to the control group ($P<0.0001$). A decrease in conjunctival GC is an important sign of ocular surface damage in dry eye. As the severity of the disease increases, the number of GC decreases, resulting in squamous metaplasia, epithelial area enlargement, and sometimes ocular surface keratinization.

Detection and quantification of mucin proteins at the ocular surface has been obtained by a variety of techniques including mucin histochemistry with PAS, lectin, and Alcian blue stains. Nevertheless, since these techniques largely depend on recognizing similar mucin carbohydrate moieties, they do not distinguish between members of the mucin family and do not allow for mucin quantification due to the different mucin glycosylation [324]. To overcome the specificity issue, antibodies against specific mucin proteins have been developed. By using indirect immunoflourecsence on impression cytolog samples, we detected markedly decreased expression of MUC5AC in glaucoma patients compared with normal control group ($P=0.0018$). Also, marked decrease of the MUC5AC expression between dry eye and control group ($P=0.0301$).

GCD and MUC5AC were markedly overexpressed in successful versus failed surgeries: histology and confocal microscopy revealed that a higher postoperative GCD was associated with a good IOP control and a higher success rate [325].

In the current study we also investigated the correlation between the levels of the inflammatory cytokines and clinical parameters of DED. Our results indicate significant negative correlation between VEGF and PGE2 concentrations and TBUT and schirmmer test, suggesting that increased level of these cytokines is associated with the tear film instability and decreased tear production.

Both IL-1β and PGE2 were pozitively correlated with corneal staining, which demonstrate that increasing in both IL-1β and PGE2 associated with more damage to the ocular surface.

These findings are consistent with previous study, that revealed direct correlation between IL-1β and corneal staining [308].

In contrast to our finidings, one previous study revealed that PGE2 concentrations alone was significantly correlated with OSDI score, but did not correlate with TBUT, shirmer test or corneal staining [326].

In actuality, the results of our study revealed that glaucoma patients, exhibit significant overexpression of IL-1β, VEGF, PGE2 and IL-1Ra. The use of preservative-containing topical agents and multiple medications reliably showed higher concentrations of these inflammatory cytokines, which reflect the effect of these agents on the ocular surface in a dose and time dependent manner.

6. CONCLUSION

The results of the current study revealed a deterioration in both the number of goblet cells and the expression of MUC5AC in the impression cytology samples of the glaucoma and dry eye groups compared to the normal control group.

Also, we revealed increase in the concentration of the following cytokines: IL-1β, IL-1Ra, VEGF, and PGE2 in glaucoma patients when compared to the dry eye and normal control groups. Despite the fact that only IL-1β, VEGF and PGE2 were found to be significantly different from the control group in glaucoma patients.

Both VEGF and PGE2 were correlated negatively with TBUT and shirmer test. A positive correlation was found between IL-1β and PGE2 and corneal staining. These correlations highlight the importance of using these tests to help determine the severity of dry eye.

This may be considered a further validation of the critically important role that inflammation plays in the pathogenesis of dry eye. A better knowledge of the pathogenesis of dry eye would improve the choice of treatment by developing new agents with specific targeting options like increasing goblet cell number or suppressing some of the inflammatory cytokines that are involved in the development of dry eye disease.

Further studies with a larger number of subjects will help to understand the exact core mechanism of dry eye in glaucoma patients. Also, new preservative options are needed to help decrease the prevalence of ocular surface diseases in those patients.

REFERENCES

1. Weinreb, R.N., Aung, T., and Medeiros, F.A. (2014). The pathophysiology and treatment of glaucoma: A review. *Journal of the American Medical Association*, 311(18), 1901–1911.

2. Allison, K., Patel, D., and Alabi, O. (2020). Epidemiology of glaucoma: the past, present, and predictions for the future. *Cureus*, 12(11), e11686.

3. Liu, W., Guo, R., Huang, D., Ji, J., Gansevoort, R.T., Snieder, H., and Jansonius, N.M. (2022). Co-occurrence of chronic kidney disease and glaucoma: Epidemiology and etiological mechanisms. *Survey of Ophthalmology*, 68(1), 1-16.

4. Orlando, J.I., Fu, H., Breda, J.B., van Keer, K., Bathula, D.R., Diaz-Pinto, A., Fang, R., Heng, P.A., Kim, J., Lee, J.H., Lee, J., Li, X., Liu, P., Lu, S., Murugesan, B., Naranjo, V., Phaye, S.S.R., Shankaranarayana, S.M., Sikka, A., Son, J., van den Hengel, A., Wang, S., Wu, J., Wu, Z., Xu, G., Xu, Y., Yin, P., Li, F., Zhang, X., Xu, Y., and Bogunovic, H. (2020). REFUGE Challenge: A unified framework for evaluating automated methods for glaucoma assessment from fundus photographs. *Medical Image Analysis*, 59, 101570.

5. Banitt, M., and Jung, H. (2018). Ocular surface disease in the glaucoma patient. *International Ophthalmology Clinics*, 58(3), 23-33.

6. Nijm, L.M., de Benito-Llopis, L., Rossi, G.C., Vajaranant, T.S., and Coroneo, M.T. (2020). Understanding the dual dilemma of dry eye and glaucoma: An international review. *Asia-Pacific Journal of Ophthalmology*, 9(6), 481–490.

7. Zhang, X., Vadoothker, S., Munir, W.M., and Saeedi, O. (2019). Ocular surface disease and glaucoma medications: A clinical approach. *Eye and Contact Lens*, 45(1), 11–18.

8. Kaštelan, S. Tomić, M., Metež-Soldo, K., and Salopek-Rabatić, J. (2013). How ocular surface disease impacts the glaucoma treatment outcome. *BioMed Research International*, 2013, ID696328.

9. Logeswaran, L. Contreras-Ruiz, L., and Masli, S. (2021). Conjunctival goblet cell responses to TLR5 engagement promote activation of local antigen-presenting cells. *Frontiers in Immunology*, 12, 716939.

10. García-Posadas, L., Contreras-Ruiz, L., Soriano-Romaní, L. Dartt, D.A., and Diebold, Y. (2016). Conjunctival goblet cell function: effect of contact lens wear and cytokines. *Eye Contact Lens*, 42(2), 83–90.

11. Contreras-Ruiz, L. Ghosh-Mitra, A., Shatos, M. A., Dartt, D. A., and Masli, S. (2013). Modulation of conjunctival goblet cell function by inflammatory cytokines. *Mediators of Inflammation*, 2013, ID636812

12. Baudouin, C., Rolando, M., Del Castillo, J.M.B., Messmer, E.M., Figueiredo, F.C., Irkec, M., Van Setten, G., and Labetoulle, M. (2019). Reconsidering the central role of mucins in dry eye and ocular surface diseases. *Progress in Retinal and Eye Research*, 71, 68–87.

13. Yagci, A., and Gurdal, C. (2014). The role and treatment of inflammation in dry eye disease. *International Ophthalmology*, 34(6), 1291-1301.

14. Perez, V. L., Stern, M. E., and Pflugfelder, S. C. (2020). Inflammatory basis for dry eye disease flares. *Experimental Eye Research*, *201*, 108-294.

15. Mrugacz, M., Ostrowska, L., Bryl, A., Szulc, A., Zelazowska-Rutkowska, B., and Mrugacz, G. (2017). Pro-inflammatory cytokines associated with clinical severity of dry eye disease of patients with depression. *Advances in Medical Sciences*, *62*(2), 338-344.

16. Contreras-Ruiz, L., and Masli, S. (2015). Immunomodulatory cross-talk between conjunctival goblet cells and dendritic cells. *PLoS One*, 10(3), e0120284.

17. de Oliveira, R. C., and Wilson, S. E. (2019). Practical guidance for the use of cyclosporine ophthalmic solutions in the management of dry eye disease. *Clinical Ophthalmology*, 1115-1122.

18. Cutolo, C.A., Barabino, S., Bonzano, C., and Traverso, C.E. (2019). The use of topical corticosteroids for treatment of dry eye syndrome. *Ocular Immunology and Inflammation*, 27(2), 266–275.

19. Scharioth, G.B. (2010). *From deep sclerectomy to canaloplasty Is it possible to re-establish the natural outflow in patients with chronic open-angle glaucoma*. Doctoral Thesis, University of Szeged, Faculty of Medicine, Szeged, 3-6

20. Weinreb, R.N., and Kha, P.T. (2004). Primary open-angle glaucoma. *The Lancet*, 363(9422), 1711-1720.

21. Zhang, S., Sun, J., Liu, S., Liang, Y., Hu, Y., Congdon, N., Pang, C.P., and Wang, H. (2019). Integrating opportunistic glaucoma screening into general health examinations in China: A pilot study. *Clinical and Experimental Ophthalmology*, 47(8), 1000–1008.

22. Tham, Y.C., Li, X. Wong, T.Y., Quigley, H.A., Aung, T., and Cheng, C.Y. (2014). Global prevalence of glaucoma and projections of glaucoma burden through 2040: A systematic review and meta-analysis. *Ophthalmology*, 121(11), 2081–2090.

23. Parihar, J.K.S. (2016). Glaucoma: The 'black hole' of irreversible blindness. *Medical Journal Armed Forces India*, 72(1), 3–4.

24. Kingman, S. (2004). Glaucoma is second leading cause of blindness globally. *Bulleti of the World Health Organization*, 82(11), 887-888.

25. Bambo, M.M., and Gebremariam, M.G. (2022). Statistical analysis on time to blindness of glaucoma patients at Jimma University Specialized Hospital: Application of accelerated failure time model. *Journal of Ophthalmology*, 2022, ID9145921.

26. Moroi, S.E., Reed, D.M., Sanders, D.s.; Almazroa, A., Kagemann, L., Shah, N., Shekhawat, N., and Richards, J.E. (2019). Precision medicine to prevent glaucoma-related blindness. *Current Opinion in Ophthalmology*, 30(3), 187–198.

27. Wiggs, J.L., and Pasquale, L.R. (2017). Genetics of glaucoma. *Human Molecular Genetics*, 26(R1), R21–R27.

28. Hashemi, H., Mohammadi, M., Zandvakil, N., Khabazkhoob, M., Emamian, M. H., Shariati, M., and Fotouhi, A. (2019). Prevalence and risk factors of glaucoma in an adult population from Shahroud, Iran. *Journal of Current Ophthalmology*, *31*(4), 366-372.

29. Allison, K., Patel, D., and Alabi, O. (2020). Epidemiology of glaucoma: the past, present, and predictions for the future. *Cureus*, 12(11), e11686.

30. Leske, M.C., Wu, S.Y., Honkanen, R., Nemesure, B., Schachat, A., Hyman, L., Hennis A., and Barbados Eye Studies Group. (2007). Nine-year incidence of open-angle glaucoma in the barbados eye studies. *Ophthalmology*, 114(6), 1058-1064.

31. Cheng, J. W., Zong, Y., Zeng, Y. Y., and Wei, R. L. (2014). The prevalence of primary angle closure glaucoma in adult Asians: a systematic review and meta-analysis. *PloS One*, *9*(7), e103222.

32. Tham, Y.C., Li, X., Wong, T.Y., Quigley, H.A., Aung, T., and Cheng, C.Y. (2014). Global prevalence of glaucoma and projections of glaucoma burden through 2040: A systematic review and meta-analysis. *Ophthalmology*, 121(11), 2081–2090.

33. Yue, B. Y. (2011). Myocilin and optineurin: differential characteristics and functional consequences. *Taiwan Journal of Ophthalmology*, *1*(1), 6-11.

34. Weinreb, R.N., Aung, T., and Medeiros, F.A. (2014). The pathophysiology and treatment of glaucoma: A review. *Journal of the American Medical Association*, 311(18), 1901–1911.

35. McMonnies, C.W. (2017). Glaucoma history and risk factors. *Journal of Optometry*, 10(2), 71-78.

36. Nitta, K., Sugiyama, K., Wajima, R., and Tachibana, G. (2017). Is high myopia a risk factor for visual field progression or disk hemorrhage in primary open-angle glaucoma?. *Clinical Ophthalmology*, 11, 599–604.

37. Tan, N.Y.Q., Sng, C.C.A., Jonas, J.B., Wong, T.Y., Jansonius, N.M., and Ang, M. (2019). Glaucoma in myopia: Diagnostic dilemmas. *British Journal of Ophthalmology*, 103(10), 1347–1355.

38. Belovay G.W., and Goldberg, I. (2018). The thick and thin of the central corneal thickness in glaucoma. *Eye (Basingstoke)*, 32(5), 915–923.

39. Mansouri, K., Leite, M.T., Weinreb, R.N., Tafreshi, A., Zangwill, L.M., and Medeiros, F.A. (2012). Association between corneal biomechanical properties and glaucoma severity. *American Journal of Ophthalmology*, 153(3), 419-427.

40. Tham, Y.C., and Cheng, C.Y. (2017). Associations between chronic systemic diseases and primary open angle glaucoma: an epidemiological perspective. *Clinical and Experimental Ophthalmology*, 45(1), 24–32.

41. Salim, S., and Shields, M.B. (2010).Glaucoma and systemic diseases. *Survey of Ophthalmology*, 55(1), 64–77.

42. Law, S.M., Lu, X., Yu, F., Tseng, V., Law, S.K., and Coleman, A.L. (2018). Cigarette smoking and glaucoma in the United States population. *Eye (Basingstoke)*, 32(4), 716–725.

43. Wu, A., Khawaja, A.P., Pasquale, L.R., and Stein, J.D. (2020). A review of systemic medications that may modulate the risk of glaucoma. *Eye (Basingstoke)*, 34(1), 12-28.

44. Kersey, J.P., and Broadway, D.C. (2006). Corticosteroid-induced glaucoma: A review of the literature. *Eye*, 20(4), 407–416.

45. Tripathi, R.C., Tripathi, B.J., and Haggerty, C. (2003). Drug-induced glaucomas mechanism and management. *Drug Safety*, 26, 749-767.

46. Rozpędek-Kamińska, W., Wojtczak, R., Szaflik, J.P., Szaflik, J., and Majsterek, I. (2020). The genetic and endoplasmic reticulum-mediated molecular mechanisms of primary open-angle glaucoma. *International Journal of Molecular Sciences*, 21(11), 1–37.

47. Moore, D., Harris, A., Wudunn, D., Kheradiya, N., and Siesky, B. (2008). Dysfunctional regulation of ocular blood fl ow: A risk factor for glaucoma?. *Clinical Ophthalmology*, 2(4), 849-861.

48. Hou, H., Moghimi, S., Zanwill, L.M., Shoji, T., Ghahari, E., Manalastas, P.I.C., Penteado, R.C., and Weinreb, R.N. (2018). Inter-eye asymmetry of optical coherence tomography angiography vessel density in bilateral glaucoma, glaucoma suspect, and healthy eyes. *American Journal of Ophthalmology*, 190, 69–77.

49. Booth, P., Anwar, R., Chen, H., Churchill, A.J., Jay, J., Polansky, J., Nguyen, T., and Markham, A.F. (2000). Genetic screening in a large family with juvenile onset primary open angle glaucoma. *British Journal of Ophthalmology*, 84(7), 722–726.

50. Allingham, R.R., Liu, Y., and Rhee, D.J. (2009). The genetics of primary open-angle glaucoma: A review. *Experimental Eye Research*, 88(4), 837–844.

51. Kwon, Y.H., Fingert, J.H., Kuehn, M.H., and Alward, W.L.M. (2009). Primary open-angle glaucoma. *New England Journal of Medicine*, 360(11), 1113-1124.

52. Ahmad, S.S. (2016). Controversies in the vascular theory of glaucomatous optic nerve degeneration. *Taiwan Journal of Ophthalmology*, 6(4), 182–186.

53. Nicoară, S.D., Brie, I., Jurj, A., and Soriţău, O. (2021). The future of stem cells and their derivates in the treatment of glaucoma. A critical point of view. *International Journal of Molecular Sciences*, 22(20), 11077.

54. Wirostko, B. Ehrlich, R., and Harris, A. (2009). The vascular theory in glaucoma, *Glaucoma Today*, 4, 25-27.

55. Davis, B.M., Crawley, L., Pahlitzsch, M., Javaid, F., and Cordeiro, M.F. (2016). Glaucoma: the retina and beyond. *Acta Neuropathologica*, 132(6), 807–826.

56. Yang, X., Zeng, Q., Göktaş, E., Gopal, K., Al-Aswad, L., Blumberg, D.N., Cioffi, G.A., Liebmann, J.M., and Tezel, G. (2019). T-lymphocyte subset distribution and activity in patients with glaucoma. *Investigative Ophthalmology and Visual Science*, 60(4), 877–888.

57. Rieck, J. (2013). The pathogenesis of glaucoma in the interplay with the immune system. *Investigative Ophthalmology and Visual Science*, 54(3), 2393–2409.

58. Fan-Gaskin, J.C, Shah, M.H., Chan, E.C., Ammendola, R., and Cattaneo, F. (2021). Antioxidants oxidative stress and the role of NADPH oxidase in glaucoma. *Antioxidants*, 10(2), 238.

59. Tezel, G., Hernandez, M.R., and Wax, M.B. (2000). Immunostaining of heat shock proteins in the retina and optic nerve head of normal and glaucomatous eyes. *Archives of Ophthalmology*, 118(4), 511-518.

60. Tezel, G., Edward, D.P., and Wax, M.B. (1999). Serum autoantibodies to optic nerve head glycosaminoglycans in patients with glaucoma. *Archives of Ophthalmology*, 117(7), 917-924.

61. Kuehn, M.H., Fingert, J.H., and Kwon, Y.H. (2005). Retinal ganglion cell death in glaucoma: Mechanisms and neuroprotective strategies. *Ophthalmology Clinics of North America*, 18(3), 383-395.

62. Bell, K., Gramlich, O.W., Hohenstein-Blaul, N.V.T.U., Beck, S., Funke, S., Wilding, C., Pfeiffer, N., and Grus, F.H. (2013). Does autoimmunity play a part in the pathogenesis of glaucoma?. *Progress in Retinal and Eye Research*, 36, 199-216.

63. Al-Humimat, G., Marashdeh, I., Daradkeh, D., and Kooner, K. (2021). Investigational rho kinase inhibitors for the treatment of glaucoma. *Journal of Experimental Pharmacology*, 13, 197–212.

64. Sihota, R., Angmo, D., Ramaswamy, D., and Dada, T. (2018). Simplifying 'target' intraocular pressure for different stages of primary open-angle glaucoma and primary angle-closure glaucoma. *Indian Journal of Ophthalmology*, 66(4), 495–505.

65. Hoy, S. M., Keam, S. J., and Keating, G. M. (2006). Travoprost / timolol. *Drugs and Aging*, *23*, 587-597.

66. Kaštelan, S. Tomić, M., Metež-Soldo, K., and Salopek-Rabatić, J. (2013). How ocular surface disease impacts the glaucoma treatment outcome. *BioMed Research International*, 2013, ID696328.

67. Chae, J.J., Jung, J.H., Zhu, W., Gerberich, B.G., Fard, M.R.B., Grossniklaus, H.E., Ethier, C.R., and Prausnitz, M.R. (2021). Drug-free, nonsurgical reduction of intraocular pressure for four months after suprachoroidal injection of hyaluronic acid hydrogel. *Advanced Science*, 8(2), 2001908.

68. Schuster, K., Erb, C., Hoffmann, E.M., Dietlein, T., and Pfeiffer, N. (2020). The diagnosis and treatment of glaucoma. *Deutsches Ärzteblatt International*, 117(13), 225–234.

69. Eyawo, O., Nachega, J., Lefebvre, P., Meyer, D., Rachlis, B., Lee, C.W., Kelly, S., and Mills, E. (2009). Efficacy and safety of prostaglandin analogues in patients with predominantly primary open-angle glaucoma or ocular hypertension: a meta-analysis. *Clinical Ophthalmology*, 3, 447-456.

70. Alm, A. (2014). Latanoprost in the treatment of glaucoma. *Clinical Ophthalmology*, 8, 1967–1985.

71. Inoue, K. (2014). Managing adverse effects of glaucoma medications. *Clinical Ophthalmology*, 8, 903–913.

72. Wu, X., Yang, X., Liang, Q., Xue, X., Huang, J., Wang, J., Xu, Y., Tong, R., Liu, M., Zhou, Q., and Shi, J. (2021). Drugs for the treatment of glaucoma: Targets, structure-activity relationships and clinical research. *European Journal of Medicinal Chemistry*, 226, 113842.

73. Nieminen, T., Lehtimäki, T., Mäenpää, J., Ropo, A., Uusitalo, H., and Kähönen, M. (2007). Ophthalmic timolol: Plasma concentration and systemic cardiopulmonary effects. *Scandinavian Journal of Clinical and Laboratory Investigation*, 67(2), 237–245.

74. Sidjanin, D.J., McCarty, C.A., Patchett, R., Smith, E., and Wilke, R.A. (2008). Pharmacogenetics of ophthalmic topical β-blockers. *Personalized Medicine*, 5(4), 377–385.

75. Lusthaus, J., and Goldberg, I. (2019). Current management of glaucoma. *Medical Journal of Australia*, 210(4), 180–187.

76. Shalaby, W.S., Shankar, V., Razeghinejad, R., and Katz, L.J. (2020). Current and new pharmacotherapeutic approaches for glaucoma. *Expert Opinion on Pharmacotherapy*, 21(16), 2027–2040.

77. Kaur, P., Smitha, R., Aggarwal, D., and Kapil, M. (2002). Acetazolamide: future perspective in topical glaucoma therapeutics. *International Journal of Pharmaceutics*, 248(1-2), 1-14

78. Ma, Yu, S.W.Y., and Wong, J.K.W. (2019). Micropulse laser for the treatment of glaucoma: A literature review. *Survey of Ophthalmology*, 6(4), 486–497.

79. Lim, R. (2022). The surgical management of glaucoma: A review. *Clinical and Experimental Ophthalmology*, 50(2), 213–231.

80. Schwartz, K., and Budenz, D. (2004). Current management of glaucoma. *Current Opinion in Ophthalmology*, 15(2), 119-126.

81. Kazemi, McLaren, J.W.. Kopczynski, C.C.. Heah, T.G.. Novack, G.D.. and Sit, A.J. (2018). The effects of netarsudil ophthalmic solution on aqueous humor dynamics in a randomized study in humans. *Journal of Ocular Pharmacology and Therapeutics*, 34(5), 380–386.

82. Mehran, N.A., Sinha, S., and Razeghinejad, R. New glaucoma medications: latanoprostene bunod, netarsudil, and fixed combination netarsudil-latanoprost. (2020). *Eye (Basingstoke)*, 34(1), 72–88.

83. Hoy, S.M. (2018). Netarsudil ophthalmic solution 0.02%: first global approval. *Drugs*, 78(3), 389–396.

84. Dasso, L., Al-Khaled, T., Sonty, S., and Aref, A.A. (2018). Profile of netarsudil ophthalmic solution and its potential in the treatment of open-angle glaucoma: Evidence to date. *Clinical Ophthalmology*, 12, 1939-1944.

85. Noecker, R.J. (2006). The management of glaucoma and intraocular hypertension: Current approaches and recent advances. *Therapeutics and Clinical Risk Management*, 2(2), 193-206.

86. Kolko, M., Heegaard, S., and Cvenkel, B. (2021). Novel approaches to optimize treatment strategies in glaucoma. *Journal of Ophthalmology*, 2021, ID9876478.

87. Rosin, L.M., and Bell, N.P. (2013). Preservative toxicity in glaucoma medication: Clinical evaluation of benzalkonium chloride-free 0.5% timolol eye drops. *Clinical Ophthalmology*, 7, 2131–2135.

88. Datta, S., Baudouin, C., Brignole-Baudouin, F., Denoyer, A., and Cortopassi, G.A. (2017). The eye drop preservative benzalkonium chloride potently induces mitochondrial dysfunction and preferentially affects LHON mutant cells. *Investigative Ophthalmology and Visual Science*, 58(4), 2406–2412.

89. Broadway, D., Grierson, I., and Hitchings, R. (1993). Adverse effects of topical antiglaucomatous medications on the conjunctiva. *British Journal of Ophthalmology*, 77(9), 590-596.

90. Goldstein, M.H., Silva, F.Q., Blender, N., Tran, T., and Vantipalli, S. (2022). Ocular benzalkonium chloride exposure: problems and solutions. *Eye (Basingstoke)*, 36(2), 361–368.

91. Thygesen, J. (2018). Glaucoma therapy: Preservative-free for all?. *Clinical Ophthalmology*, 12, 707–717.

92. Alviset, G., Corvis, Y., Hammad, K., Lemut, J., Maury, M., Mignet, N., and Boudy, V. (2022). New Preservative-free formulation for the enhanced ocular bioavailability of prostaglandin analogues in glaucoma. *Pharmaceutics*, 14(2), 453.

93. Rahman, M.Q., Tejwani, D., Wilson, J.A., Butcher, I., and Ramaesh, K. (2006). Microbial contamination of preservative free eye drops in multiple application containers. *British Journal of Ophthalmology*, 90(2), 139–141.

94. Doughty, Michael J. "*Comparative anatomy and physiology of the cornea and conjunctiva."* Ocular surface (2012): 32-78.

95. Gipson, K. (2016). Goblet cells of the conjunctiva: A review of recent findings. *Progress in Retinal and Eye Research*, 54, 49-63.

96. Dartt, D.A., and Masli, S. (2014). Conjunctival epithelial and goblet cell function in chronic inflammation and ocular allergic inflammation. *Current Opinion in Allergy and Clinical Immunology*, 14(5), 464–470.

97. Vujković, V., Mikac, G., and Kozomara, R. (2002). Distribution and density of conjunctival goblet cells. *Medicinski Pregled*, 55(5–6), 195-200.

98. Alam, de Paiva, C.S., and Pflugfelder, S.C. (2020). Immune - Goblet cell interaction in the conjunctiva: Goblet Cell immunomodulatory properties. *Ocular Surface*, 18(2), 326–334.

99. Dartt, D.A. (2004). Control of mucin production by ocular surface epithelial cells. *Experimental Eye Research*, 78(2), 173–185.

100. Ríos, D., Forde, K., Diebold, Y., Lightman, J., Zieske, J.D., and Dartt, D.A. (2000). Development of conjunctival goblet cells and their neuroreceptor subtype expression. *Investigative Ophthalmology and Visual Science*, 41(8), 2127-2137.

101. Galletti, J. G., and de Paiva, C. S. (2021). The ocular surface immune system through the eyes of aging. *The Ocular Surface*, *20*, 139-162.

102. Contreras-Ruiz, Ghosh-Mitra, A., Shatos, M.A., Dartt, D.A., and Masli, S. (2013). Modulation of conjunctival goblet cell function by inflammatory cytokines. *Mediators of Inflammation*, 2013, ID636812.

103. Walker, K., Schornack, M.M., and Vincent, S.J. (2020). Anatomical and physiological considerations in scleral lens wear: Conjunctiva and sclera. *Contact Lens and Anterior Eye*, 43(6), 517–528.

104. Navaratnam, J., Utheim, T., Rajasekhar, V., and Shahdadfar, A. (2015). Substrates for expansion of corneal endothelial cells towards bioengineering of human corneal endothelium. *Journal of Functional Biomaterials*, 6(3), 917–945.

105. Sridhar, S. (2018). Anatomy of cornea and ocular surface. *Indian Journal of Ophthalmology*, 66(2), 190–194.

106. Shafaie, S., Hutter, V., Cook, M.T., Brown, M.B., and Chau, D.Y.S. (2016). In vitro cell models for ophthalmic drug development applications. *BioResearch Open Access*, 5(1), 94–108.

107. Zoukhri, D. (2006). Effect of inflammation on lacrimal gland function. *Experimental Eye Research*, 82(5), 885–898.

108. Zhang, X., Jeyalatha, V., Qu, Y., He, X., Ou, S., Bu, J., Jia, C., Wang, J., Wu, H., Liu, Z., and Li, W. (2017). Dry eye management: Targeting the ocular surface microenvironment. *International Journal of Molecular Sciences*, 18(7), 1398.

109. Nichols, K.K., Foulks, G.N., Bron, A.J., Glasgow, B.J., Dogru, M., Tsubota, K., Lemp, M.A., Sullivan, D.A. (2011). The international workshop on meibomian gland dysfunction: Executive summary. *Investigative Ophthalmology and Visual Science*, 52(4), 1922–1929.

110. Dietrich, J., Garreis, F., and Paulsen, F. (2021). Pathophysiology of Meibomian Glands–An Overview. *Ocular Immunology and Inflammation*, 29(4), 803–810.

111. Asbell, P.A., Stapleton, F.J., Wickström, K., Akpek, E.K., Aragona, P., Dana, R., Lemp, M.A., and Nichols, K.K. (2011). The international workshop on meibomian gland dysfunction: Report of the diagnosis subcommittee. *Investigative Ophthalmology and Visual Science*, 52(4), 2065–2085.

112. Holland, E.J., Mannis, M.J., and Lee, W.B. (2013). *Ocular surface disease: Cornea, conjunctiva and tear film*. Philadelphia: Saunders, 17-21

113. Koh, S., Tung, C.I., Inoue, Y., and Jhanji, V. (2018). Effects of tear film dynamics on quality of vision. *British Journal of Ophthalmology*, 102(12), 1615–1620.

114. Pflugfelder, S.C., and Stern, M.E. (2020). Biological functions of tear film. *Experimental Eye Research*, 197, 108115.

115. Lemp, M.A., and Foulks, G.N. (2008). The definition and classification of dry eye disease – Guidelines from the 2007 International Dry Eye Workshop. *The Ocular Surface*, 5(2), 75-92.

116. Davidson, H.J., and Kuonen, V.J. (2004). The tear film and ocular mucins. *Veterinary Ophthalmology*, 7(2), 71–77.

117. Watanabe, H. (2002). Significance of mucin on the ocular surface. *Cornea*, 21, 17-22.

118. Mantelli, F., and Argüeso, P. (2008). Functions of ocular surface mucins in health and disease. *Current Opinion in Allergy and Clinical Immunology*, 8(5), 477–483.

119. Marko, C.K., Tisdale, A.S., Spurr-Michaud, S., Evans, C., and Gipson, I.K. (2014). The ocular surface phenotype of Muc5ac and Muc5b null mice. *Investigative Ophthalmology and Visual Science*, 55(1), 291–300.

120. Dogru, M., Okada, N., Asano-Kato, N., Tanaka, M., Igarashi, A., Takano, Y., Fukagawa, K., Shimazaki, J., Tsubota, K., and Fujishima, H. (2005). Atopic ocular surface disease implications on tear function and ocular surface mucins. *Cornea*, 24(8), 18-23.

121. Bhattacharya, D., Yu, L., and Wang, M. (2017). Expression patterns of conjunctival mucin 5AC and aquaporin 5 in response to acute dry eye stress. *PLoS One*, 12(11), e0187188.

122. Dartt, D. A. (2002). Regulation of mucin and fluid secretion by conjunctival epithelial cells. *Progress in Retinal and Eye Research*, *21*(6), 555-576.

123. Baudouin, C., Rolando, M., Del Castillo, J.M.B., Messmer, E.M., Figueiredo, F.C., Irkec, M., Van Setten, G., and Labetoulle, M. (2019). Reconsidering the central role of mucins in dry eye and ocular surface diseases. *Progress in Retinal and Eye Research*, 71, 68–87..

124. Jumblatt, J.E., and Jumblatt, M.M. (1998). Regulation of ocular mucin secretion by P2Y 2 nucleotide receptors in rabbit and human conjunctiva. *Experimental Eye Research*, 67(3), 341-346.

125. Argüeso, P., Balaram, M., Spurr-Michaud, S., Keutmann, H.T., Dana, M.R., and Gipson, I.K.(2002). Decreased levels of the goblet cell mucin MUC5AC in tears of patients with sjögren syndrome. *Investigative Ophthalmology and Visual Science*, 43, 1004-1011.

126. Zhao, H., Jumblatt, J.E., Wood, T.O., and Jumblatt, M.M. (2001). Quantification of MUC5AC protein in human tears. *Cornea*, 20(8), 873-877.

127. Khanal, S., Bai, Y., Ngo, W., Nichols, K.K., Wilson, L., Barnes, S., Nichols, J.J. (2021). Human meibum and tear film derived (O-Acyl)-omega-hydroxy fatty acids as biomarkers of tear film dynamics in meibomian gland dysfunction and dry eye disease. *Investigative Ophthalmology and Visual Science*, 62(9), 13.

128. Bron, J., Tiffany, J.M., Gouveia, S.M., Yokoi, N., and Voon, L.W. (2004). Functional aspects of the tear film lipid layer. *Experimental Eye Research*, 78(3), 347–360.

129. Willcox, M.D.P., Argüeso, P., Georgiev, G.A., Holopainen, J.M., Laurie, G.W., Millar, T.J., Papas, E.B., Rolland, J.P., Schmidt, T.A., Stahl, U., Suarez, T., Subbaraman, L.N., Uçakhan, O.Ö., and Jones, L. (2017). TFOS DEWS II tear film report. *Ocular Surface*, 15(3), 366-403.

130. Kimball, S.H., Ewen King-Smith, P.E., and Nichols, J.J. (2010). Evaporation is the primary mechanism of tear film. *Investigative Ophthalmology and Visual Science*, 51(12), 6294-6297.

131. Hanstock, H.G., Edwards, J.P., and Walsh, N.P. (2019). Tear lactoferrin and lysozyme as clinically relevant biomarkers of mucosal immune competence. *Frontiers in Immunology*, 10, 1-11.

132. Flanagan, J.L., and Willcox, M.D.P. (2009). Role of lactoferrin in the tear film. *Biochimie*, 91(1), 35–43.

133. Craig, J.P., Nichols, K.K., Akpek, E.K., Caffery, B., and Dua, H.S. (2017). TFOS DEWS II definition and classification report. *Ocular Surface*, 15(3), 276–283.

134. Rouen, P.A., and White, M.L. (2018). Dry eye disease: Prevalence, assessment, and management. *Home Healthcare Now*, 36(2), 74-83.

135. Messmer, E.M. (2015). The pathophysiology, diagnosis, and treatment of dry eye disease. *Deutsches Ärzteblatt International*, 112(5), 71-82.

136. Kaštelan, S., Tomić, M., Salopek-Rabatić, J., and Novak, B. (2013). Diagnostic procedures and management of dry eye. *BioMed Research International*, 37(1), 199-203.

137. Mavragani, P., and Moutsopoulos, H.M. (2014). Sjögren syndrome. *Canadian Medical Association Journal*, 186(15), 579–586.

138. Ogawa, Y., Shimizu, E., and Tsubota, K. (2018). Interferons and dry eye in sjögren's syndrome. *International Journal of Molecular Sciences*, 19(11), 3548.

139. Roszkowska, M., Oliverio, G.W., Aragona, E., Inferrera, L., Severo, A.A., Alessandrello, F., Spinella, R., Postorino, E.I., and Aragona, P. (2021). Ophthalmologic manifestations of primary sjögren's syndrome. *Genes*, 12(3), 365.

140. Widjaja-Adhi, M.A.K., Chao, K., and Golczak, M. (2022). Mouse models in studies on the etiology of evaporative dry eye disease. *Experimental Eye Research*, 219, 109072.

141. Barabino, S., Chen, Y., Chauhan, S., and Dana, R. (2012). Ocular surface immunity: Homeostatic mechanisms and their disruption in dry eye disease. *Progress in Retinal and Eye Research*, 31(3), 271–285.

142. Mastropasqua, Agnifili, L., Fasanella, V., Nubile, M., Gnama, A.A., Falconio, G., Perri, P., Di Staso, S., and Mariotti, C. (2017). The conjunctiva-associated lymphoid tissue in chronic ocular surface diseases. *Microscopy and Microanalysis*, 23(4), 697–707.

143. Foulsham, W., Coco, G., Amouzegar, A., Chauhan, S.K., and Dana, R. (2018). When clarity is crucial: Regulating ocular surface immunity. *Trends in Immunology*, 39(4), 288-301.

144. Galletti, J.G., Guzmán, M., and Giordano, M.N. (2017). Mucosal immune tolerance at the ocular surface in health and disease. *Immunology*, 150(4), 397–407.

145. Ueta, M., and Kinoshita, S. (2012). Ocular surface inflammation is regulated by innate immunity. *Progress in Retinal and Eye Research*, 31(6), 551–575.

146. El-Annan, J., Goyal, S., Zhang, Q., Freeman, G.J., Sharpe, A.H., and Dana, R. (2010). Regulation of T-cell chemotaxis by programmed Death-Ligand 1 (PD-L1) in dry eye-associated corneal inflammation. *Investigative Ophthalmology and Visual Science*, 51(7), 3418–3423.

147. Pflugfelder, de Paiva, C.S., Li, D.Q., and Stern, M.E. (2008). Epithelial-immune cell interaction in dry eye. *Cornea*, 27(1), 9-11.

148. Joossen, C. (2017). *Dry eye syndrome the establishment of an optimized animal model and the evaluation of novel treatment options: proefschrift*. Doctoral Dissertation, University of Antwerp, Belgium, 23-26

149. Pflugfelder, C., Stern, M.E., and The Symposium Participants. (2009). Immunoregulation on the ocular surface: 2nd Cullen Symposium. *Ocular Surface*, 7(2), 67–77.

150. Yu, L., Yu, C., Dong, H., Mu, Y., Zhang, R., Zhang, Q., Liang, W., Li, W., Wang, X., and Zhang, L. (2021). Recent developments about the pathogenesis of dry eye disease: based on immune inflammatory mechanisms. *Frontiers in Pharmacology*, 12, 1-9.

151. Baudouin, C., Aragona, P., Van Setten, G., Rolando, M., Irkeç, M., del Castillo, J.B., Geerling, G., Lebetoulle, M., and Bonini, S. (2014). Diagnosing the severity of dry eye: A clear and practical algorithm. *British Journal of Ophthalmology*, 98(9), 1168–1176.

152. Ziaragkali, S., Kotsalidou, A., and Trakos, N. (2018). Dry eye disease in routine rheumatology practice. *Mediterranean Journal of Rheumatology*, 29(3), 127–139.

153. Periman, L. M., Perez, V. L., Saban, D. R., Lin, M. C., and Neri, P. (2020). The immunological basis of dry eye disease and current topical treatment options. *Journal of Ocular Pharmacology and Therapeutics*, *36*(3), 137-146.

154. Yagci, A., and Gurdal, C. (2014). The role and treatment of inflammation in dry eye disease. *International Ophthalmology*, 34(6), 1291-1301.

155. Stern, M.E., Schaumburg, C.S., and Pflugfelder, S.C. (2013). Dry eye as a mucosal autoimmune disease. *International Reviews of Immunology*, 32(1), 19–41.

156. Foulsham, W. (2019). *Immunoregulatory strategies in corneal transplantation and dry eye disease*. Doctoral Thesis, University College London, Institute of Ophthalmology, London, 64-72

157. Baudouin, C., Messmer, E.M., Aragona, P., Geerling, G., Akova, Y.A., Benitez-del-Castillo, J., Boboridis, K.G., Merayo-Lloves, J., Rolando, M., and Lavetoulle, M. (2016). Revisiting the vicious circle of dry eye disease: A focus on the pathophysiology of meibomian gland dysfunction. *British Journal of Ophthalmology*, 100(3), 1-7.

158. Na, K.S., Hwang, K.Y., Lee, H.S., Chung, S.H., Mok, J.W., and Joo, C.K. (2015). Wakayama symposium: Interface between innate and adaptive immunity in dry eye disease. *BMC Ophthalmology*, 15(1), 159.

159. Wei, Y., and Asbell, P.A. (2014). The core mechanism of dry eye disease is inflammation. *Eye and Contact Lens*, 40(4), 248–256.

160. De Paiva, C., and Pflugfelder, S.C. (2008). Bases da terapia antiinflamatória em síndrome do olho seco Rationale for anti-inflammatory therapy in dry eye syndrome. *Arquivos Brasileiros de Oftalmologia*, 71(6), 89-95.

161. Chen, Y.T., Nikulina, K., Lazarev, S., Bahrami, A.F., Noble, L.B., Gallup, M., McNamara, N.A. (2010). Interleukin-1 as a phenotypic immunomodulator in keratinizing squamous metaplasia of the ocular surface in Sjögren's syndrome. *American Journal of Pathology*, 177(3), 1333–1343.

162. Narayanan, S., Corrales, R.M., Farley, W., Mcdermott, A.M., and Pflugfelder, S.C. (2008). Interleukin-1 receptor-1-deficient mice show attenuated production of ocular surface inflammatory cytokines in experimental dry eye. *Cornea*, 27(7), 811-817.

163. Na, K.S., Mok, J.W., Kim, J.Y., Rho, C.R., and Joo, C.K. (2012). Correlations between tear cytokines, chemokines, and soluble receptors and clinical severity of dry eye disease. *Investigative Ophthalmology and Visual Science*, 53(9), 5443–5450.

164. Massingale, M.L., Li, X., Vallabhajosyula, M., Chen, D., Wei, Y., and Asbell, P.A. (2009). Analysis of inflammatory cytokines in the tears of dry eye patients. *Cornea*, 28(9), 1023-1027.

165. Ganesalingam, K., Ismail, S., Sherwin, T., and Craig, J.P. (2019). Molecular evidence for the role of inflammation in dry eye disease. *Clinical and Experimental Optometry*, 102(5), 446–454.

166. Oh, J., and Shin, J.S. (2015). The Role of Dendritic Cells in Central Tolerance. *Immune Network*, 15(3), 111-120.

167. Hamrah, P., Liu, Y., Zhang, Q., and Dana, M.R. (2003). The corneal stroma is endowed with a significant number of resident dendritic cells. *Investigative Ophthalmology and Visual Science*, 44(2), 581–589.

168. Levine, H., Hwang, J., Dermer, H., Mehra, D., Feuer, W., and Galor, A. (2021). Relationships between activated dendritic cells and dry eye symptoms and signs. *Ocular Surface*, 21, 186–192.

169. Jiao, H., Naranjo Golborne, C., Dando, S.J., McMenamin, P.G., Downie, L.E., and Chinnery, H.R. (2020). Topographical and morphological differences of corneal dendritic cells during steady state and inflammation. *Ocular Immunology and Inflammation*, 28(6), 898–907.

170. Schaumburg, C.S., Siemasko, K.F., De Paiva, C.S., Wheeler, L.A., Niederkorn, J.Y., Pflugfelder, S.C., and Stern, M.E. (2011). Ocular surface APCs are necessary for autoreactive T cell-mediated experimental autoimmune lacrimal keratoconjunctivitis. *The Journal of Immunology*, 187(7), 3653–3662.

171. Ouyang, W., Wu, Y., Lin, X., Wang, S., Yang, Y., Tang, L., Liu, Z., Wu, J., Huang, C., Zhou, Y., Zhang, X., Hu, J., and Liu, Z. (2021). Role of CD4+ T helper cells in the development of BAC-induced dry eye syndrome in mice. *Investigative Ophthalmology and Visual Science*, 62(1), 25.

172. Chauhan, S.K., El Annan, J., Ecoiffier, T., Goyal, S., Zhang, Q., Saban, D.R., and Dana, R. (2009). Autoimmunity in dry eye is due to resistance of Th17 to treg suppression. *The Journal of Immunology*, 182(3), 1247–1252.

173. Pflugfelder, S.C., Corrales, R.M., and de Paiva, C.S. (2013). T helper cytokines in dry eye disease. *Experimental Eye Research*, 117, 118–125.

174. Mosmann, R., and Coffman, R.L. (1989). TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology*, 7, 145-173.

175. Mosmann, T.R. (1992). T Lymphocyte Subsets, Cytokines, and Effector Functions," *Annals of the New York Academy of Sciences*, 664(1), 89–92.

176. Perez, L., Pflugfelder, S.C., Zhang, S., Shojaei, A., and Haque, R. (2016). Lifitegrast, a novel integrin antagonist for treatment of dry eye disease. *The Ocular Surface*, 14(2), 207-215.

177. Fong, P., Shih, K., Lam, P., Chan, T., Jhanji, V., and Tong, L. (2019). Role of tear film biomarkers in the diagnosis and management of dry eye disease. *Taiwan Journal of Ophthalmology*, 9(3), 150–159.

178. Jackson, D.C., Zeng, W., Wong, C.Y., Mifsud, E.J., Williamson, N.A., Ang, C.S., Vingrys, A.J., and Downie, L.E. (2016). Tear interferon-gamma as a biomarker for evaporative dry eye disease. *Investigative Ophthalmology and Visual Science*, 57(11), 4824–4830.

179. Bettelli, *E.*, Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., and Kuchroo, V.K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*, 441(7090), 235–238.

180. Korn, T., Bettelli, E., Oukka, M., and Kuchroo, V.K. (2009). IL-17 and Th17 cells. *Annual Review of Immunology*, 27, 485–517.

181. Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor rorγt directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*, 126(6), 1121–1133.

182. Spolski, R., and Leonard, W.J. (2009). Cytokine mediators of Th17 function," *European Journal of Immunology*, 39(3), 658–661.

183. Bettelli, E., Kuchroo, V.K., and Oukka, M. (2007). T H-17 cells in the circle of immunity and autoimmunity. *Nature Immunology*, 8(4), 345-350.

184. Chen, Z., and O'Shea, J.J. (2008). Th17 cells: A new fate for differentiating helper T cells. *Immunologic Research*, 41(2), 87–102.

185. Meadows, J.F., Dionne, K., and Nichols, K.K. (2016). Differential profiling of T-cell cytokines as measured by protein microarray across dry eye subgroups. *Cornea*, 35(3), 329-335.

186. Foulsham, W., Marmalidou, A., Amouzegar, A., Coco, G., Chen, Y., and Dana, R. (2017). Review: The function of regulatory T cells at the ocular surface. *Ocular Surface*, 15(4), 652–659.

187. Chen, and Dana, R. (2021). Autoimmunity in dry eye disease – An updated review of evidence on effector and memory Th17 cells in disease pathogenicity. *Autoimmunity Reviews*, 20(11), 102933.

188. Fuchs, A., and Colonna, M. (2011). Natural killer (NK) and NK-like cells at mucosal epithelia: mediators of anti-microbial defense and maintenance of tissue integrity. *European Journal of Microbiology and Immunology*, 1(4), 257-266.

189. Chen, Y., Chauhan, S.K., Saban, D.R., Sadrai, Z., Okanobo, A., and Dana, R. (2011). Interferon-γ-secreting NK cells promote induction of dry eye disease. *Journal of Leukocyte Biology*, 89(6), 965-972.

190. McGinnigle, S., Naroo, S.A., and Eperjesi, F. (2012). Evaluation of dry eye. *Survey of Ophthalmology*, 57(4), 293-316.

191. Koh, S. (2016). Mechanisms of visual disturbance in dry eye. *Cornea*, 35, S83-S88.

192. Schiffman, R.M., Christianson, M.D., Jacobsen, G., Hirsch, J.D., and Reis, B.L. (2000). Reliability and validity of the ocular surface disease index. *Archives of Ophthalmology*, 118(5), 615-621.

193. Grubbs Jr, J.R., Tolleson-Rinehart, S., Huynh, K., and Davis, R.M. (2014). A review of quality of life measures in dry eye questionnaires. *Cornea*, 33(2), 215-218.

194. Okumura, Y., Inomata, T., Iwata, N., Sung, J., Fujimoto, K., Fujio, K., Midorikawa-Inomata, A., Miura, M., Akasaki, Y., and Murakami, A. (2020). A review of dry eye questionnaires: measuring patient-reported outcomes and health-related quality of life. *Diagnostics*, 10(8), 559.

195. Miller, K.L., Walt, J.G., Mink, D.R., Satram-Hoang, S., Wilson, S.E., Perry, H.D., Asbell, P.A., and Pflugfelder, S.C. (2010). Minimal clinically important difference for the ocular surface disease index. *Archives of Ophthalmology*, 128(1), 94-101.

196. Dibajnia, P., Mohammadinia, M., Moghadasin, M., Amiri, M.A. Sq, H., and Damavand, A. (2012). Tear film break-up time in bipolar disorder. *Iranian Journal of Psychiatry*, 7(4), 191-193.

197. Hwang, H.B., Ku, Y.H., Kim, E.C., Kim, H.S., Kim, M.S., and Hwang, H.S. (2020). Easy and effective test to evaluate tear-film stability for self-diagnosis of dry eye syndrome: blinking tolerance time (BTT). *BMC Ophthalmology*, 20(1), 1-7.

198. Cho, P., and Yap, M. (1993). Schirmer Test II: A clinical study of its repeatability. *Optometry and Vision Science*, 70, 157-159.

199. Kaido, Minako, et al. "Corneal fluorescein staining correlates with visual function in dry eye patients." *Investigative Ophthalmology and Visual Science* 52.13 (2011): 9516-9522.

200. Bron, A.J. (2001). Diagnosis of dry eye. *Survey of ophthalmology*, 45(2), 221-226.

201. Ousler, G.W., Gomes, P.J., Welch, D., and Abelson, M.B. (2005). Methodologies for the study of ocular surface disease. *The Ocular Surface*, 3(3), 143-154.

202. Nichols, J. J., Mitchell, G. L., and King-Smith, P. E. (2005). Thinning rate of the precorneal and prelens tear films. *Investigative Ophthalmology and Visual Science*, 46(7), 2353-2361.

203. Lemp, M.A., Bron, A.J., Baudouin, C., Del Castillo, J.M.B., Geffen, D., Tauber, J., Foulks, G.N., Pepose, J.S., and Sullivan, B.D. (2011). Tear osmolarity in the diagnosis and management of dry eye disease. *American Journal of Ophthalmology*, 151(5), 792-798.

204. Tashbayev, B., Utheim, T.P., Utheim, Ø.A., Ræder, S., Jensen, J.L., Yazdani, M., Lagali, N., Vitelli, V., Dartt, D.A., and Chen, X. (2020). Utility of tear osmolarity measurement in diagnosis of dry eye disease. *Scientific Reports*, 10(1), 1-7.

205. Lee, Y.H., Bang, S.P., Shim, K.Y., Son, M.J., Kim, H., and Jun, J.H. (2021). Association of tear matrix metalloproteinase 9 immunoassay with signs and symptoms of dry eye disease: A cross-sectional study using qualitative, semiquantitative, and quantitative strategies. *PLOS One*, 16(10), e0258203.

206. Sambursky, R., Davitt III, W.F., Friedberg, M., and Tauber, S. (2014). Prospective, multicenter, clinical evaluation of point-of-care matrix metalloproteinase-9 test for confirming dry eye disease. *Cornea*, 33(8), 812-818.

207. Lanza, N.L., McClellan, A.L., Batawi, H., Felix, E.R., Sarantopoulos, K.D., Levitt, R.C., and Galor, A. (2016). Dry eye profiles in patients with a positive elevated surface matrix metalloproteinase 9 point-of-care test versus negative patients. *The Ocular Surface*, 14(2), 216-223.

208. Alp, B.N., Karabaş, L., Bilen, N., Talu, H., Kaur, A., and Yanyali, A. (1999). Evaluation of ocular surface changes usingimpression cytology in acne vulgaris patients. *Turkish Journal of Medical Sciences*, 29(6), 693-696.

209. Lopin, E., Deveney, T., and Asbell, P. A. (2009). Impression cytology: recent advances and applications in dry eye disease. *The Ocular Surface*, 7(2), 93-110.

210. Singh, R., Joseph, A., Umapathy, T., Tint, N.L., and Dua, H.S. (2005). Impression cytology of the ocular surface. *British Journal of Ophthalmology*, 89(12), 1655-1659.

211. Martinez, A.J., Mills, M.B., Jaceldo, K.B., Tio, F.O., Aigbivbalu, I.B., Hilsenbeck, S.B., and Yee, R.W. (1995). Standardization of conjunctival impression cytology. *Cornea*, 14(5), 515-522.

212. Colorado, L.H., Alzahrani, Y., Pritchard, N., and Efron, N. (2016). Assessment of conjunctival goblet cell density using laser scanning confocal microscopy versus impression cytology. *Contact Lens and Anterior Eye*, 39(3), 221-226..

213. Roy, N.S., Wei, Y., Kuklinski, E., and Asbell, P.A. (2017). The growing need for validated biomarkers and endpoints for dry eye clinical research. *Investigative Ophthalmology and Visual Science*, 58(6), 1-19.

214. Di Zazzo, A., Micera, A., De Piano, M., Cortes, M., and Bonini, S. (2019). Tears and ocular surface disorders: Usefulness of biomarkers. *Journal of Cellular Physiology*, 234(7), 9982-9993.

215. Rentka, A., Koroskenyi, K., Harsfalvi, J., Szekanecz, Z., Szucs, G., Szodoray, P., and Kemeny-Beke, A. (2017). Evaluation of commonly used tear sampling methods and their relevance in subsequent biochemical analysis. *Annals of Clinical Biochemistry*, 54(5), 521-529.

216. Nättinen, J., Aapola, U., Nukareddy, P., and Uusitalo, H. (2022). Looking deeper into ocular surface health: an introduction to clinical tear proteomics analysis. *Acta Ophthalmologica*, 100(5), 486-498.

217. Benitez-del-Castillo, J.M., Soria, J., Acera, A., Muñoz, A.M., Rodríguez, S., and Suárez, T. (2021). Quantification of a panel for dry-eye protein biomarkers in tears: A comparative pilot study using standard ELISA and customized microarrays. *Molecular Vision*, 27, 243.

218. Zhang, J.M., and An, J. (2007). Cytokines, inflammation, and pain. *International Anesthesiology Clinics*, 45(2), 27–37.

219. Lissoni, P., Messina, G., Pelizzoni, F., Rovelli, F., Brivio, F., Monzon, A., Crivelli, N., Lissoni, A., Tassoni, S., Sassola, A., Pensato, S., and Di Fede, G. (.2020). The fascination of cytokine immunological science. *Journal of Infectiology*, 3(1), 18-28.

220. Louis, I.V.S., and Bohjanen, P.R. (2017). Post-transcriptional regulation of cytokine and growth factor signaling in cancer. *Cytokine and Growth Factor Reviews*, 33, 83-93.

221. Opal, S.M., and DePalo, V. A. (2000). Anti-inflammatory cytokines. *Chest*, 117(4), 1162-1172.

222. Rothwell, N. (2003). Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic potential. *Brain, Behavior, and Immunity*, 17(3), 152-157.

223. Cavalli, G., Colafrancesco, S., Emmi, G., Imazio, M., Lopalco, G., Maggio, M. C., Sota, J., and Dinarello, C. A. (2021). Interleukin 1α: a comprehensive review on the role of IL-1α in the pathogenesis and treatment of autoimmune and inflammatory diseases. *Autoimmunity Reviews*, 20(3), 102763.

224. Muñoz-Wolf, N., and Lavelle, E.C. (2018). A Guide to IL-1 family cytokines in adjuvanticity. *The FEBS Journal*, 285(13), 2377-2401.

225. Kim, B., Lee, Y., Kim, E., Kwak, A., Ryoo, S., Bae, S.H., Azam, T., Kim, S., and Dinarello, C.A. (2013). The interleukin-1α precursor is biologically active and is likely a key alarmin in the IL-1 family of cytokines. *Frontiers in Immunology*, 4, 391.

226. Dinarello, C.A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Reviews*, 281(1), 8-27.

227. Lopez-Castejon, G., and Brough, D. (2011). Understanding the mechanism of IL-1β secretion. *Cytokine and Growth Factor Reviews*, 22(4), 189-195.

228. Leal, M.C., Casabona, J.C., Puntel, M., and Pitossi, F.J. (2013). Interleukin-1β and tumor necrosis factor-α: reliable targets for protective therapies in Parkinson's disease?. *Frontiers in Cellular Neuroscience*, 7, 53.

229. Weinert, C. (2013). *Structural and functional investigations on pyrin/TRIM20, a modulator of the innate immune response*. Doctoral Dissertation, University of Zurich, Switzerland, 15-35

230. Fields, J.K., Günther, S., and Sundberg, E.J. (2019). Structural basis of IL-1 family cytokine signaling. *Frontiers in Immunology*, 10, 1412.

231. Brough, D., and Rothwell, N.J. (2007). Caspase-1-dependent processing of pro-interlukin-1β is cytosolic and precedes cell death. *Journal of Cell Science*, 120(5), 772–781.

232. Sharma, D., and Kanneganti, T. D. (2016). The cell biology of inflammasomes: Mechanisms of inflammasome activation and regulation. *Journal of Cell Biology*, 213(6), 617-629.

233. Shao, B.Z., Xu, Z.Q., Han, B.Z., Su, D.F., and Liu, C. (2015). NLRP3 inflammasome and its inhibitors: a review. *Frontiers in Pharmacology*, 6, 262.

234. Kelley, N., Jeltema, D., Duan, Y., and He, Y. (2019). The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *International Journal of Molecular Sciences*, 20(13), 3328.

235. Tulotta, C., and Ottewell, P. (2018). The role of IL-1B in breast cancer bone metastasis. *Endocrine-Related Cancer*, 25(7), 421-434.

236. Broz, P., and Monack, D.M. (2011). Molecular mechanisms of inflammasome activation during microbial infections. *Immunological Reviews*, 243(1), 174-190.

237. Qu, Y., Franchi, L., Nunez, G., and Dubyak, G.R. (2007). Nonclassical IL-1β secretion stimulated by P2X7 receptors is dependent on inflammasome activation and correlated with exosome release in murine macrophages. *The Journal of Immunology*, 179(3), 1913-1925.

238. Weber, A., Wasiliew, P., and Kracht, M. (2010). Interleukin-1β (IL-1β) processing pathway. *Science Signaling*, 3(105), p.cm2.

239. Arend, W.P., Palmer, G., and Gabay, C. (2008). IL-1, IL-18, and IL-33 families of cytokines. *Immunological Reviews*, 223(1), 20–38.

240. Sun, R., Gao, D.S., Shoush, J., and Lu, B. (2022). The IL-1 family in tumorigenesis and antitumor immunity. *Seminars in Cancer Biology*, 86, 280–295.

241. Bankers-Fulbright, J.L., Kalli, K.R., and McKean, D.J. (1996). Interleukin-1 signal transduction. *Life Sciences*, 59(2), 61-83.

242. Afonina, I.S., Müller, C., Martin, S.J., and Beyaert, R. (2015). Proteolytic processing of interleukin-1 family cytokines: variations on a common theme. *Immunity*, 42(6), 991-1004.

243. Dinarello, C.A. (2011). Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood, *The Journal of the American Society of Hematology*, 117(14), 3720-3732.

244. Deschamps, N., and Baudouin, C. (2013). Dry eye and biomarkers: present and future. *Current Ophthalmology Reports*, 1(2), 65-74.

245. Stern, E., Schaumburg, C.S., Dana, R., Calonge, M., Niederkorn, J.Y., and Pflugfelder, S.C. (2010). Autoimmunity at the ocular surface: Pathogenesis and regulation," *Mucosal Immunology*, 3(5), 425–442.

246. Akash, M.S.H., Rehman, K., and Chen, S. (2013). IL-1Ra and its delivery strategies: inserting the association in perspective. *Pharmaceutical Research*, 30(11), 2951-2966.

247. Banda, N.K., Guthridge, C., Sheppard, D., Cairns, K.S., Muggli, M., Bech-Otschir, D., Dubiel, W., and Arend, W.P. (2005). Intracellular IL-1 receptor antagonist type 1 inhibits IL-1-induced cytokine production in keratinocytes through binding to the third component of the COP9 signalosome. *The Journal of Immunology*, 174(6), 3608-3616.

248. Powers, N.E., Swartzwelter, B., Marchetti, C., de Graaf, D.M., Lerchner, A., Schlapschy, M., Datar, R., Binder, U., Carl K. III, E., Skerra, A., and Dinarello, C. A. (2020). PASylation of IL-1 receptor antagonist (IL-1Ra) retains IL-1 blockade and extends its duration in mouse urate crystal-induced peritonitis. *Journal of Biological Chemistry*, 295(3), 868-882.

249. Okanobo, A., Chauhan, S.K., Dastjerdi, M.H., Kodati, S., and Dana, R. (2012). Efficacy of topical blockade of interleukin-1 in experimental dry eye disease. *American Journal of Ophthalmology*, 154(1), 63-71.

250. Arend, W.P., and Guthridge, C.J. (2000). Biological role of interleukin 1 receptor antagonist isoforms. *Annals of the Rheumatic Diseases*, 59(1), 60-64.

251. Arend, W.P., and Gabay, C. (2000). Physiologic role of interleukin-1 receptor antagonist. *Arthritis Research and Therapy*, 2(4), 1-4.

252. Huang, J.F., Zhang, Y., Rittenhouse, K.D., Pickering, E.H., and McDowell, M.T. (2012). Evaluations of tear protein markers in dry eye disease: repeatability of measurement and correlation with disease. *Investigative Ophthalmology and Visual Science*, 53(8), 4556-4564.

253. Elwakeel, E., Bruene, B., and Weigert, A. (2019). PGE2 in fibrosis and cancer: Insights into fibroblast activation. *Prostaglandins and Other Lipid Mediators*, 143, 106339.

254. Nakanishi, M., and Rosenberg, D.W. (2013). Multifaceted roles of PGE2 in inflammation and cance. *Seminars in Immunopathology*, 35(2), 123–137.

255. Park, Y., Pillinger, M.H., and Abramson, S.B. (2006). Prostaglandin E2 synthesis and secretion: The role of PGE2 synthases. *Clinical Immunology*, 119(3), 229–240.

256. Murakami, M., Nakatani, Y., Tanioka, T., and Kudo, I. (2002). Prostaglandin E synthase. *Prostaglandins and Other Lipid Mediators*, 68, 383-399.

257. Fitzgerald, G.A., and Ricciotti, E. (2011). Prostaglandins and inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 31(5), 986-1000.

258. Agard, M., Asakrah, S., and Morici, L.A. (2013). PGE2 suppression of innate immunity during mucosal bacterial infection. *Frontiers in Cellular and Infection Microbiology*, 3, 45.

259. Tian, M., and Schiemann, W.P. (2010). PGE2 receptor EP2 mediates the antagonistic effect of COX-2 on TGF-β signaling during mammary tumorigenesis. *The FASEB Journal*, 24(4), 1105-1116.

260. Kandola, M.K., Sykes, L., Lee, Y.S., Johnson, M.R., Hanyaloglu, A.C., and Bennett, P.R. (2014). EP2 receptor activates dual G protein signaling pathways that mediate contrasting proinflammatory and relaxatory responses in term pregnant human myometrium. *Endocrinology*, 155(2), 605-617.

261. O'Callaghan, P., and Houston, A. (2015). Prostaglandin E2 and the EP receptors in malignancy: Possible therapeutic targets?. *British Journal of Pharmacology*, 172(22), 5239–5250.

262. Sheller, J., Dworski, R., Hagaman, D., Oates, J., and Murray, J. (2002). The prostaglandin E agonist, misoprostol, inhibits airway IL-5 production in atopic asthmatics. *Prostaglandins and Other Lipid Mediators*, 70(1-2), 185-193.

263. Kay, L.J., Yeo, W.W., and Peachell, P.T. (2006). Prostaglandin E2 activates EP2 receptors to inhibit human lung mast cell degranulation. *British Journal of Pharmacology*, 147(7), 707.

264. Watts, S.W., Kanagy, N.L., and Lombard, J.H. (2008). Receptor-mediated events in the microcirculation. In Tuma, R.F., Ley, K., and Duran, W.N. (Eds.), *Microcirculation*. USA: Academic Press, 285-348.

265. Narumiya, S. (2007). Physiology and pathophysiology of prostanoid receptors. *Proceedings of the Japan Academy, Series B*, 83(9-10), 296-319.

266. Kalinski, P. (2012). Regulation of immune responses by prostaglandin E2. *The Journal of Immunology*, 188(1), 21-28.

267. Ambaw, Y.A., Timbadia, D.P., Raida, M., Torta, F., Wenk, M.R., and Tong, L. (2020). Profile of tear lipid mediator as a biomarker of inflammation for meibomian gland dysfunction and ocular surface diseases: Standard operating procedures. *The Ocular Surface*, 26, 318-327.

268. Shim, J., Park, C., Lee, H.S., Park, M.S., Lim, H.T., Chauhan, S., Dana, R., Lee, H., and Lee, H. K. (2012). Change in prostaglandin expression levels and synthesizing activities in dry eye disease. *Ophthalmology*, 119(11), 2211-2219.

269. Melincovici, C.S., Boşca, A.B., Şuşman, S., Mărginean, M., Mihu, C., Istrate, M., Moldovan, I.M., Roman, A.L., and Mihu, C.M. (2018). Vascular endothelial growth factor (VEGF)-key factor in normal and pathological angiogenesis. *Romanian Journal of Morphology and Embryology*, 59(2), 455-467.

270. Dulak, J., and Józkowicz, A. (2003). Regulation of vascular endothelial growth factor synthesis by nitric oxide: facts and controversies. *Antioxidants and Redox Signaling*, 5(1), 123-132.

271. Maloney, J. P., and Gao, L. (2015). Proinflammatory cytokines increase vascular endothelial growth factor expression in alveolar epithelial cells. *Mediators of Inflammation*, 2015, ID 387842.

272. Rakic, J.M., Lambert, V., Devy, L., Luttun, A., Carmeliet, P., Claes, C., Nguyen, L., Foidart J.M., Noel, A., and Munaut, C. (2003). Placental growth factor, a member of the VEGF family, contributes to the development of choroidal neovascularization. *Investigative Ophthalmology and Visual Science*, 44(7), 3186-3193.

273. Peach, C.J., Mignone, V.W., Arruda, M.A., Alcobia, D.C., Hill, S.J., Kilpatrick, L.E., and Woolard, J. (2018). Molecular pharmacology of VEGF-A isoforms: binding and signalling at VEGFR2. *International Journal of Molecular Sciences*, 19(4), 1264.

274. Carmeliet, P. (2005). VEGF as a key mediator of angiogenesis in cancer. *Oncology*, 69(3), 4-10.

275. Wiszniak, S., and Schwarz, Q. (2021). Exploring the intracrine functions of VEGF-A. *Biomolecules*, 11(1), 128-140.

276. Goerges, A.L., and Nugent, M.A. (2003). Regulation of vascular endothelial growth factor binding and activity by extracellular pH. *Journal of Biological Chemistry*, 278(21), 19518-19525.

277. Koch, S., and Claesson-Welsh, L. (2018). Signal transduction by vascular endothelial growth factor receptors. *Cold Spring Harbor Perspectives in Medicine*, 2(7), a006502.

278. Mac Gabhann, F., and Popel, A. S. (2007). Dimerization of VEGF receptors and implications for signal transduction: a computational study. *Biophysical Chemistry*, 128(2-3), 125–139.

279. Shibuya, M. (2011). Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti-and pro-angiogenic therapies. *Genes and Cancer*, 2(12), 1097-1105.

280. Aguilar-Cazares, D., Chavez-Dominguez, R., Carlos-Reyes, A., Lopez-Camarillo, C., Hernadez de la Cruz, O.N., and Lopez-Gonzalez, J.S. (2019). Contribution of angiogenesis to inflammation and cancer. *Frontiers in Oncology*, 9, 1399.

281. Pflugfelder, S.C. (2004). Antiinflammatory therapy for dry eye. *American Journal of Ophthalmology*, 137(2), 337-342.

282. McCabe, E., and Narayanan, S. (2009). Advancements in anti-inflammatory therapy for dry eye syndrome. *Optometry-Journal of the American Optometric Association*, 80(10), 555-566.

283. Foulks, G.N. (2008). Pharmacological management of dry eye in the elderly patient. *Drugs and Aging*, 25(2), 105-118.

284. Zdanowicz, M.M. (2009). The pharmacology of immunosuppression. *American Journal of Pharmaceutical Education*, 73(8), 144.

285. Periman, L.M., Mah, F.S., and Karpecki, P.M. (2020). A review of the mechanism of action of cyclosporine A: the role of cyclosporine A in dry eye disease and recent formulation developments. *Clinical Ophthalmology (Auckland, NZ)*, 14, 4187-4200.

286. Lee, H., Kim, C.E., Ahn, B.N., and Yang, J. (2017). Anti-inflammatory effect of hydroxyproline-GQDGLAGPK in desiccation stress-induced experimental dry eye mouse. *Scientific Reports*, 7(1), 1-12.

287. Kymionis, G.D., Bouzoukis, D.I., Diakonis, V.F., and Siganos, C. (2008). Treatment of chronic dry eye: focus on cyclosporine. *Clinical Ophthalmology*, 2(4), 829-836.

288. Dogru, M., and Tsubota, K. (2011). Pharmacotherapy of dry eye. *Expert Opinion on Pharmacotherapy*, 12(3), 325-334.

289. Moscovici, B.K., Holzchuh, R., Chiacchio, B.B., Santo, R.M., Shimazaki, J., and Hida, R.Y. (2012). Clinical treatment of dry eye using 0.03% tacrolimus eye drops. *Cornea*, 31(8), 945-949.

290. Mahmoudi, A., Malaekeh-Nikouei, B., Hanafi-Bojd, M. Y., Toloei, M., Hosseini, M., and Nikandish, M. (2020). Preliminary in vivo safety evaluation of a tacrolimus eye drop formulation using hydroxypropyl beta cyclodextrin after ocular administration in NZW rabbits. *Clinical Ophthalmology (Auckland, NZ)*, 14, 947–953.

291. Shoughy, S.S. (2017). Topical tacrolimus in anterior segment inflammatory disorders. *Eye and Vision*, 4(1), 1-7.

292. Hoffman, R.S., Braga-Mele, R., Donaldson, K., Emerick, G., Henderson, B., Kahook, M., Mamalis, N., Miller, K.M., Realini, T., Shorstein, N.H., Stiverson, R.K., and Wirostko, B. (2016).Cataract surgery and nonsteroidal antiinflammatory drugs. *Journal of Cataract and Refractive Surgery*, 42(9), 1368–1379.

293. Gunaydin, C., and Bilge, S.S. (2018). Effects of nonsteroidal anti-inflammatory drugs at the molecular level. *Eurasian Journal of Medicine*, 50(2), 116–121.

294. Paton, D.M. (2016). Lifitegrast: First LFA-1/ICAM-1 antagonist for treatment of dry eye disease. *Drugs Today*, 52(9), 485-493.

295. Abidi, A., Shukla, P., and Ahmad, A. (2016). Lifitegrast: a novel drug for treatment of dry eye disease. *Journal of Pharmacology and Pharmacotherapeutics*, 7(4), 194-198.

296. O'Neil, E.C., Henderson, M., Massaro-Giordano, M., and Bunya, V.Y. (2019). Advances in dry eye disease treatment. *Current Opinion in Ophthalmology*, 30(3), 166–178.

297. Messmer, E.M. (2022). Pathophysiology of dry eye disease and novel therapeutic targets. *Experimental Eye Research*, 217, 108944.

298. Rao, S.K., Mohan, R., Gokhale, N., Matalia, H., and Mehta, P. (2022). Inflammation and dry eye disease—where are we?. *International Journal of Ophthalmology*, 15(5), 820–827.

299. De Saint Jean, M., Brignole, F., Bringuier, A.F., Bauchet, A., Feldmann, G., and Baudouin, C. (1999). Effects of benzalkonium chloride on growth and survival of Chang conjunctival cells. *Investigative Ophthalmology and Visual Science*, 40(3), 619-630.

300. Cha, S.H., Lee, J.S., Oum, B.S., and Kim, C.D. (2004). Corneal epithelial cellular dysfunction from benzalkonium chloride (BAC) in vitro. *Clinical and Experimental Ophthalmology*, 32(2), 180-184.

301. Andole, S., and Senthil, S. (2022). Ocular surface disease and anti-glaucoma medications: various features, diagnosis, and management guidelines. *Seminars in Ophthalmology*, 35915557, 1-9.

302. Lavin, M.J., Wormald, R.P., Migdal, C.S., and Hitchings, R.A. (1990). The influence of prior therapy on the success of trabeculectomy. *Archives of Ophthalmology*, 108(11), 1543-1548.

303. Sahlu, M., and Giorgis, A.T. (2021). Dry eye disease among Glaucoma patients on topical hypotensive medications, in a tertiary hospital, Ethiopia. *BMC Ophthalmology*, 21(1), 1-8.

304. Mathews, P.M., Ramulu, P.Y., Friedman, D.S., Utine, C.A., and Akpek, E.K. (2013). Evaluation of ocular surface disease in patients with glaucoma. *Ophthalmology*, 120(11), 2241-2248.

305. Zierhut, M., Dana, M.R., Stern, M.E., and Sullivan, D.A. (2002). Immunology of the lacrimal gland and ocular tear film. *Trends in Immunology*, 23(7), 333-335.

306. Lam, H., Bleiden, L., De Paiva, C.S., Farley, W., Stern, M.E., and Pflugfelder, S.C. (2009). Tear cytokine profiles in dysfunctional tear syndrome. *American Journal of Ophthalmology*, 147(2), 198-205.

307. Zoukhri, D., Hodges, R.R., Byon, D., and Kublin, C.L. (2002). Role of proinflammatory cytokines in the impaired lacrimation associated with autoimmune xerophthalmia. *Investigative Ophthalmology and Visual Science*, 43(5), 1429-1436.

308. Solomon, A., Dursun, D., Liu, Z., Xie, Y., Macri, A., and Pflugfelder, S.C. (2001). Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. *Investigative Ophthalmology and Visual Science*, 42(10), 2283-2292.

309. Goyal, S., Chauhan, S.K., and Dana, R. (2012). Blockade of prolymphangiogenic vascular endothelial growth factor C in dry eye disease. *Archives of Ophthalmology*, 130(1), 84-89.

310. Yoo, S.A., Kwok, S.K., and Kim, W.U. (2008). Proinflammatory role of vascular endothelial growth factor in the pathogenesis of rheumatoid arthritis: prospects for therapeutic intervention. *Mediators of inflammation*, 2008, ID 129873.

311. Jiang, X., Lv, H., Qiu, W., Liu, Z., Li, X., and Wang, W. (2015). Efficiency and safety of subconjunctival injection of anti-VEGF agent–bevacizumab–in treating dry eye. *Drug Design, Development and Therapy*, 9, 3043.

312. Abu El-Asrar, A.M., Al-Mansouri, S., Tabbara, K.F., Missotten, L., and Geboes, K. (2006). Immunopathogenesis of conjunctival remodelling in vernal keratoconjunctivitis. *Eye*, 20(1), 71-79.

313. D'Souza, S., and Tong, L. (2014). Practical issues concerning tear protein assays in dry eye. *Eye and Vision*, 1(1), 1-12.

314. Sánchez, J.B.D.C., Morillo-Rojas, M.D., Galbis-Estrada, C., and Pinazo-Duran, M.D. (2017). Determination of inmune response and inflammation mediators in tears: Changes in dry eye and glaucoma as compared to healthy controls. *Archivos de la Sociedad Española de Oftalmologia (English Edition)*, 92(5), 210-217.

315. Kasetsuwan, N., Chantaralawan, K., Reinprayoon, U., and Uthaithammarat, L. (2020). Efficacy of topical bevacizumab 0.05% eye drops in dry eye disease: A double-masked, randomized trial. *PloS One*, 15(6), e0234186.

316. Sola-Villa, D., Camacho, M., Sola, R., Soler, M., Diaz, J.M., and Vila, L. (2006). IL-1β induces VEGF, independently of PGE2 induction, mainly through the PI3-K/mTOR pathway in renal mesangial cells. *Kidney International*, 70(11), 1935-1941.

317. Ayalasomayajula, S.P., and Kompella, U.B. (2003). Celecoxib, a selective cyclooxygenase-2 inhibitor, inhibits retinal vascular endothelial growth factor expression and vascular leakage in a streptozotocin-induced diabetic rat model. *European Journal of Pharmacology*, 458(3), 283-289.

318. Kim, S.J., Flach, A.J., and Jampol, L.M. (2010). Nonsteroidal anti-inflammatory drugs in ophthalmology. *Survey of Ophthalmology*, 55(2), 108-133.

319. Cvenkel, B., Štunf, Š., Srebotnik Kirbiš, I., and Strojan Fležar, M. (2015). Symptoms and signs of ocular surface disease related to topical medication in patients with glaucoma. *Clinical Ophthalmology*, 9, 625-631.

320. Paschides, C.A., Petroutsos, G., and Psilas, K. (1991). Correlation of conjunctival impression cytology results with lacrimal function and age. *Acta Ophthalmologica*, 69(4), 422-425.

321. Herreras, J.M., Pastor, J.C., Calonge, M., and Asensio, V.M. (1992). Ocular surface alteration after long-term treatment with an antiglaucomatous drug. *Ophthalmology*, 99(7), 1082-1088.

322. Xiao, Y., De Paiva, C.S., Yu, Z., de Souza, R.G., Li, D.Q., and Pflugfelder, S.C. (2018). Goblet cell-produced retinoic acid suppresses CD86 expression and IL-12 production in bone marrow-derived cells. *International Immunology*, 30(10), 457-470.

323. Pflugfelder, S.C., and de Paiva, C.S. (2020). Goblet cells promote tolerance induction in the conjunctiva. *Mucosal Immunology*, 13(5), 717-718.

324. Walsh, M.D., and Jass, J.R. (2000). Histologically based methods for detection of mucin. *Glycoprotein Methods and Protocols*, 125, 29-44.

325. Agnifili, L., Fasanella, V., Mastropasqua, R., Frezzotti, P., Curcio, C., Brescia, L., and Marchini, G. (2016). In vivo goblet cell density as a potential indicator of glaucoma filtration surgery outcome. *Investigative Ophthalmology and Visual Science*, 57(7), 2928-2935.

326. Lekhanont, K., Sathianvichitr, K., Pisitpayat, P., Anothaisintawee, T., Soontrapa, K., and Udomsubpayakul, U. (2019). Association between the levels of prostaglandin E2 in tears and severity of dry eye. *International Journal of Ophthalmology*, 12(7), 1127.

APPENDIX

APPENDIX-1. Ethics Committee Permission

Sayı 02

Tarih: 01.02.2021

Konu: Toplantı Kararları

Sayın Prof. Dr. E. Ümit Bağırsak

Proje Yürütücüsü

Gazi Üniversitesi Klinik Araştırmalar Etik Kurulu'nun 25 Ocak 2021 tarihinde yapmış olduğu toplantı kararı ekte sunulmuştur.

Bilgilerinizi rica ederim

Prof. Dr. D. Berrin GÜNAYDIN
GÜ Klinik Araştırmalar Etik Kurulu
Başkanı

APPENDIX-1. (continue) Ethics Committee Permission

GAZİ ÜNİVERSİTESİ KLİNİK ARAŞTIRMALAR ETİK KURULU
GİRİŞİMSEL OLMAYAN ARAŞTIRMALAR KARAR FORMU

ETİK KURUL İLETİŞİM BİLGİLERİ	**ETİK KURULUNUN ADI**	**Gazi Üniversitesi (GÜ) Klinik Araştırmalar Etik Kurulu**
	AÇIK ADRES	**Gazi Üniversitesi Tıp Fakültesi Dekanlık (GÜTF) Binası 06500 Beşevler/Ankara**
	TELEFON	**0312 202 69 58**
	FAKS	**0312 202 46 73**
	E-POSTA	**tipetikkurul@gazi.edu.tr**

BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	**Glokom Hastalarında Oküler Yüzey Hasarının Ve InflamatuarSitokinlerin Değerlendirmesi**			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI ÜNVANI/ADI/SOYADI	**Prof. Dr. E. Ümit BAĞRIAÇIK**			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI /UZMANLIK ALANI/ BULUNDUĞU MERKEZ	İmmünoloji Anabilim Dalı / GÜTF			
	DESTEKLEYİCİ (Varsa)				
	ARAŞTIRMANIN TÜRÜ	Kan, idrar, doku, radyolojik görüntü gibi biyokimya, mikrobiyoloji, patoloji ve radyoloji koleksiyon materyalleriyle veya rutin muayene tetkik tahlil ve tedavi işlemleri sırasında (önceden) elde edilmiş materyallerle yapılacak araştırmalar-Doktora Tezi			
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ☒	ÇOK MERKEZLİ ☐	ULUSAL ☒	ULUSLARARASI ☐

	Belge Adı	Tarihi	Ver.No	Dili
DEĞERLENDİRİLEN BELGELER	ARAŞTIRMA PROTOKOLÜ	26.12.2021	1	Türkçe ☒ İngilizce ☐ Diğer ☐
	AYDINLATILMIŞ ONAM FORMU	26.12.2021	1	Türkçe ☐ İngilizce ☐ Diğer ☐

	Belge Adı		Açıklama
DEĞERLENDİRİLEN DİĞER BELGELER	ARAŞTIRMA BÜTÇESİ	☐	
	BİYOLOJİK MATERYAL TRANSFER FORMU	☐	
	DİĞER	☐	

KARAR BİLGİLERİ

Karar No: 57 **Toplantı tarihi: 25.01.2021**

Glokom Hastalarında Oküler Yüzey Hasarının Ve Inflamatuar Sitokinlerin Değerlendirmesi başlıklı başvuru dosyası ile ilgili belgeler araştırmanın gerekçe amaç, yaklaşım ve yöntemleri ile incelenerek uygun bulunduğuna GÜ Klinik Araştırmalar Etik Kurulu üyelerinin oybirliği ile karar verilmiştir.

KLİNİK ARAŞTIRMALAR ETİK KURULU

ETİK KURULUN ÇALIŞMA ESASI	İlaç ve Biyolojik Ürünlerin Klinik Araştırmaları Hakkında Yönetmelik İyi Klinik Uygulamaları Kılavuzu
BAŞKANIN UNVANI / ADI / SOYADI	Prof. Dr. D. Berrin GÜNAYDIN

Unvanı/Adı/Soyadı	Uzmanlık Alanı	Kurumu	Cinsiyet		Araştırmayla İlişkisi		Katılım		İmza
Prof. Dr. D. Berrin GÜNAYDIN BAŞKAN	Anesteziyoloji ve Reanimasyon Anabilim Dalı Farmakoloji Bilim Dr.	GÜTF	E☐	K☒	E☐	H☒	E☒	H☐	
Prof. Dr. Gülten TAÇOY BAŞKAN YARD.	Kardiyoloji Anabilim Dalı	GÜTF	E☐	K☒	E☐	H☒	E☒	H☐	
Doç. Dr. Murat UÇAR BİLDİRİMDEN SORUMLU ÜYE	Radyoloji Anabilim Dalı	GÜTF	E☒	K☐	E☐	H☒	E☒	H☐	

APPENDIX-1. (continue) Ethics Committee Permission

Prof. Dr. Nevzat YÜKSEL ÜYE	Psikiyatri Anabilim Dalı	GÜTF	E☒	K☐	E☐	H☒	E☐	H☒
Prof. Dr. Nesrin ÇOBANOĞLU ÜYE	Tıp Tarihi ve Etik Anabilim Dalı	GÜTF	E☐	K☒	E☐	H☒	E☒	H☐
Prof. Dr. Mehmet Ali ERGÜN ÜYE	Tıbbi Genetik Anabilim Dalı	GÜTF	E☒	K☐	E☐	H☒	E☒	H☐
Prof. Dr. Ö. Sezai LEVENTOĞLU ÜYE	Genel Cerrahi Anabilim Dalı	GÜTF	E☒	K☐	E☒	H☐	E☒	H☐
Prof. Dr. Nuriye ÖZDEMİR ÜYE	İç Hastalıkları Anabilim Dalı Tıbbi Onkoloji Bilim Dalı	GÜTF	E☐	K☒	E☐	H☒	E☒	H☐
Prof. Dr. Aylin SEPİCİ DİNÇEL ÜYE	Tıbbi Biyokimya Anabilim Dalı	GÜTF	E☐	K☒	E☐	H☒	E☒	H☐
Prof. Dr. Ebru ARHAN ÜYE	Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Çocuk Nöroloji Bilim Dalı	GÜTF	E☐	K☒	E☐	H☒	E☒	H☐
Doç. Dr. Gökçe S. ÖZTÜRK FİNCAN ÜYE	Tıbbi Farmakoloji Anabilim Dalı	GÜTF	E☐	K☒	E☐	H☒	E☒	H☐
Doç. Dr. Elçin ÖZGÜR BÜYÜKATALAY ÜYE	Biyofizik Anabilim Dalı	GÜTF	E☐	K☒	E☐	H☒	E☒	H☐
Uzm. Dr. Emine AVCI ÜYE	Halk Sağlığı	Halk Sağlığı Genel Müd.	E☐	K☒	E☐	H☒	E☒	H☐
Araş. Gör. Dr. Fahri Erdem KAŞAK ÜYE	Hukukçu	Hacı Bayram Veli Üniv.	E☒	K☐	E☐	H☒	E☐	H☒
İ. Nükhet EKŞİ ÜYE	Sivil Temsilci	-	E☐	K☒	E☐	H☒	E☐	H☐

CURRICULUM VITAE

Personal Information

Surname, name : MUSTAFA ALHARDODE, Eman

Nationality : Libya

Education

Degree	Education Unit	Date of Graduation
Ph.D.	Student in Gazi University / Health Sciences Institute, Immunology	Continues
License	Omar Almukhtar University, School of Medicine	2007-2008
High school	Al-Zahaf High School	2000

Work experience

Year	Location	Task
2008-2013	AL-Wahda Hospital	Assistant

Foreign Language

Arabic, English, Turkish,

Hobby

Camping, Drawing, Reading,

Pupliacations

1. Alhardode, E. ve Bağrıaçık, E. Ü. (2022). The Balance of Th17/Treg Cells at The Ocular Surface in Dry Eye Disease. *Journal of Gazi University Health Sciences Institute* , 4 (3) , 158-166 .



GAZİLİ OLMAK AYRICALIKTIR...