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Effect of WASL(N-WASP) gene expression in pterygium

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Master Thesis

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(12 / 01 / 2022)

MOHAMMED RAHMAN

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I present this to my family and my grandfather’s soul (**Jamil Ali Debagai**), who was my most beloved mentor and teacher.

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ABSTRACT

Effect of WASL(N-WASP) gene expression in pterygium,

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Pterygium is a benign growth of the conjunctive tissue that covers the sclera with a high proliferation property which causes intrusive ocular surface and wide matrix remodeling. WASL which also known as n-Wasp (Neural Wiskott-Aldrich syndrome protein) is a nucleation-promoting factor that promotes the formation of branching actin filaments. The WASL gene is located on the q arm (long arm) of chromosome 7, which locate q31.32 and encodes a 505 amino acid sequence. The WASL gene comprises 11 exons, and the encoded protein has a molecular weight of about 54.84 kD.

The WASL gene has several important molecular function, including a actin binding, GTPase regulator activity. SH3 binding motifs with a proline-rich locus, and the C-terminal acidic region, which plays a role in actin cytoskeleton remodeling by stimulating Arp2/3. The WASL gene appears to play a function in signal transduction as well as cytoskeletal remodeling.

Regulation of cortical actin filament rearrangement in response to external stimuli is mediated through the Arp2/3 complex, which links small GTPases (e.g., Rac and Cdc42) with actin polymerization. The aim of this study was to see if the expression of the WASL gene changed in pterygium versus healthy cataract tissue. Tissues were removed with the patient's consent and awareness throughout the procedure. We use the WASL gene expression approach to harvest cDNA from tissue levels determined by real-time PCR. WASL gene expression levels were determined to be statistically significant higher in tissues with pterygium compared to the control group ($p < 0.05$), based on our findings that the increase in WASL gene expression is hypothesized to be linked to the formation and development of pterygium. According to qRT-PCR data, WASL gene expression is pterygium tissue compared to healthy conjunctive tissue (1.320±0,254-foldchange, $p=0.001$).

Key Words: Pterygium, Real-Time PCR, WASL

ÖZET

WASL(N-WASP) gen ekspresyonunun pterjiyumdaki etkisi,

RAHMAN, Mohammed

Yüksek Lisans Tezi, Tıbbi Biyoloji Anabilim Dalı

Tez Danışmanı: Prof. Dr. Hacı Ömer ATEŞ

Pterjium, proliferatif olan ve intruziv oküler yüzey ve geniş matriks yeniden şekillenmesine neden olan sklerayı kaplayan konjonktif dokunun iyi huylu bir büyümesidir.

WASL geni, q31.32'yi yerleştiren ve 505 amino asit dizisini kodlayan kromozom 7'nin q kolunda (uzun kol) bulunur. WASL geni 11 ekzon içerir ve proteinin moleküler ağırlığı yaklaşık 54.84 kD'dir. WASI geni, aktin bağlama, GTPaz düzenleyici aktivite dahil olmak üzere birçok önemli moleküler fonksiyona sahiptir. Prolin açısından zengin bir lokusa sahip SH3 bağlama motifleri ve Arp2/3'ü başlatarak aktin hücre iskeletinin yeniden şekillenmesinde rol oynayan C-terminal asidik bölge. WASL geninin sinyal iletiminde olduğu kadar hücre iskeletinin yeniden şekillenmesinde de bir işlevi olduğu görülüyor.

N-Wasp (Neural Wiskott-Aldrich sendromu proteini), dallanan aktin filamentlerinin oluşumunu destekleyen çekirdeklenmeyi teşvik eden bir faktördür. Dış uyaranlara yanıt olarak kortikal aktin filamentinin yeniden düzenlenmesinin düzenlenmesine, küçük GTPazları (örn., Rac ve Cdc42) aktin polimerizasyonu ile bağlayan Arp2/3 kompleksi aracılık eder. Bu çalışmanın amacı, WASL geninin ekspresyonunun pterjiyumda sağlıklı katarakt dokusuna göre değişip değişmediğini görmekti. İşlem boyunca hastanın onayı ve farkındalığı ile dokular çıkarıldı. Gerçek zamanlı PCR ile belirlenen doku düzeylerinden cDNA'yı toplamak için WASL gen ekspresyonu yaklaşımını kullanıyoruz. WASL gen ekspresyonundaki artışın pterjiyum oluşumu ve gelişimi ile bağlantılı olduğu hipotezimize dayanarak, WASL gen ekspresyon düzeylerinin pterjiyumlu dokularda kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksek olduğu belirlendi ($p < 0.05$). qRT-PCR verilerine göre WASL gen ekspresyonu sağlıklı konjonktif dokuya göre pterjiyum dokusudur (1.320±0.254 kat değişim, $p=0.001$).

Anahtar Kelimeler: Pterjium, Real-Time PCR, WASL

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ABBREVIATION AND SYMBOL LIST

ANG : Angiogenin.
AR : Acid Region.
ARD : Actin Regulatory Domain
ARP : Actin-Related Protein.
BER : Basic Excisional Repair System.
BL : Bowman's layer.
CAT : Catalase.
CDK : Climatic Droplet Keratopathy.
CMV : Stands for Cytomegalovirus.
HPV : Stands for human papillomavirus.
HSV : Stands for herpes simplex virus.
MI : Microsatellite instability.
COX2 : Cyclooxygenase-2.
ECM : Stands for extracellular matrix.
EGF : Epidermal Growth Factor Receptor.
EPO : Erythropoietin.
EPOR : EPO receptor.
ER : Endoplasmic Reticulum.
EVE : Everolimus.
FR : Free Radicals.
G6PD : Glucose-6-Phosphate-Dehydrogenase.
GBD : G Protein-Binding Domain.
GTPase : Guanosine triphosphatase.
GBD : GTPase binding domain.
PH : Pleckstrin homology.
hODG1 : Human 8-Oxoguanine DNA Glycosylase.
HPV : Human papillomavirus
MDA : Malone Dialdehyde.
MMC : Mitomycin C.
NCF : Normal Conjunctival Fibroblasts.
NER : Nucleotide Excision Repair.
NFkb : Nuclear factor kb.

NO : Nitric Oxide.

NPFs : Nucleation-promoting factors.

PCNA : Proliferation cell nuclear antigen.

DNMT3b : DNA methyltransferase 3b.

PDAC : Pancreatic Ductal Adenocarcinomas

PECs : Primary pterygium cells.

LOH : Loss of heterozygosity.

PEDF : Pigment epithelium-derived factor.

PF : Pterygium Fibroblasts.

PF : Pterygial Fibroblast.

PKC : Protein Kinase C.

PPP : Pentose Phosphate.

PRR : Proline-Rich Region.

SNPs : Single nucleotide polymorphisms.

SOD : Superoxide Dismutase.

TCR : T Lymphocyte Receptor Complex.

TCR : T-cell receptor.

TdT : Terminal Deoxynucleotide Transferase.

TF : Tissue Factor.

TGM-2 : Transglutaminase-2.

UV : Ultraviolet.

UVR : Ultraviolet radiation.

VA : Visual acuity.

VCA : Verprolin-homology, cofilin-homology, and acidic.

WH1 : Wiskott Homology 1.

WIP : Wiskott-Aldrich Interactive Protein

1. INTRODUCTION

Pterygium is a non-neoplastic degenerative and proliferative ocular disease (Liu et al., 2017), which characterized by a triangular-shaped growth consisting of fibrotic subconjunctival connective tissue and hypertrophy of the overlying conjunctival epithelium (Kim et al., 2016). Pterygium can impair vision, foreign body sensation, difficulty of eye movements, cause eye irritation, and dryness, Even more In some severe cases, pterygium may lead to irregular corneal astigmatism and corneal stromal scarring with visual impairment (Reid et al., 2010; Clearfield et al., 2016). Due to the proliferation capacity of pterygial cells, they appear to be similar to cancer; yet, despite significant progress in understanding the etiology of the disease, the pathophysiology of pterygium remains unknown (Liu et al., 2017). It's more common in those who live near the equator and in people who work outdoors or in specific factory conditions (Wong et al., 2001).

Although the cause of pterygium is unknown, its prevalence varies by geographical location and other risk factors for the disease such as rate of UV light exposure, latitude, inflammation, age, certain viruses, and the lifestyle of individuals exposed to the sun outdoors (e.g. farmers, construction workers, welders, etc) are more susceptible than those who work indoors (Džunić et al., 2010; Hossain, 2011). The majority of pterygium cases have been reported in older adults, particularly men over the age of 49, in association with outdoor work (Ma et al., 2007). Pterygium is more prevalent in rural locations than in metropolitan areas, owing to the differences in people's lifestyles and environmental variables (Nemet et al., 2016). UV light has been implicated in the development of pterygia, as it induces the activation of pro-inflammatory proteins and has an effect on the subconjunctival connective tissue. Chronic inflammation and growth factors, as well as UV damage to DNA, result in the formation of dimers, which was observed in pterygium and may indirectly cause it through the formation of UV byproducts, but the factors causing pterygium are still being studied and are not completely understood (Goodsell, 2001; Lu et al., 2017).

Pterygium is typically treated surgically, which has a recurrence rate of up to 61-82 percent (Hirst et al., 2003; Tan et al., 1998). Recurrence rates can be reduced to as low as 2-31 percent when combined with adjunctive treatment methods such as conjunctival autografts, mitomycin C, amniotic membrane grafts, or beta-radiation, depending on factors such as age, geographical area, occupation, pterygium morphology, and surgeon experience. (Hirst et al., 2003; Lewallen et al., 1989; Ma et al., 2005; Ti et al., 2000).

Treatment of pterygium and prevention of pterygium recurrence with 5-FU has also been utilized although MMC is more successful and safer for preventing pterygium recurrence and less of an issue for patients (Altay et al., 2016). Following surgery and before surgery, studies have shown that 20% ethanol can be used as an alternate treatment for pterygium recurrence, with a low rate of recurrence as well as no cosmetic concerns and low hazards (Chen et al., 2006). Bevacizumab and Cyclosporine A (CsA), both of which impede angiogenesis and suppress the immune system, have shown promising results in the prevention of pterygium recurrence and in the treatment of the condition (Kim et al., 2017). Following pterygium removal, radiation therapy has been performed, and beta irradiation has been proven to be beneficial in reducing pterygium recurrence and satisfying the patient's cosmetic concerns (Wilder et al., 1992). Recent research also indicates that pterygium is a stem cell disorder with pre-malignant characteristics (Hirst et al., 2009; Chui et al., 2011). Chronic UV radiation exposure induces cell proliferation, angiogenesis, inflammation, goblet cell hyperplasia, fibrosis, squamous metaplasia, and extracellular matrix degradation (Chui et al., 2008).

Among eye pathologic diseases, human pterygium continues to be one of the most contentious ocular diseases (Eze et al., 2011). Pterygia has been linked to UV radiation exposure, which has been shown to activate pro-inflammatory proteins and affect the subconjunctival connective tissue behind the eyes. UV rays can cause long-term inflammation and growth factor production. Dimerization of DNA, which has been linked to the development of pterygium, may also be caused by UV byproducts, although the underlying reasons of pterygium are still being explored and not completely understood (Goodsell,2001; C.-W. Lu et al., 2017). Human papillomavirus (HPV) is the most often researched virus that can cause pterygium (Gallagher et al., 2001). Additionally, HPV has been associated to the development of conjunctival papilloma, a tumor of the epithelial cells of the conjunctiva (Sjö et al., 2001).

Gene expression is defined as mRNA replicating the genetic information on a DNA strand that serves as a template. This information is subsequently translated into protein, which participates in cellular function and results in the expression of phenotypic features. Increased or decreased activity of this mechanism results in the up- or down-regulation of specific genes, resulting in aberrant protein expression that may be used to identify defects in cells and better understand diseases (Engelsvold et al., 2013).

2. GENERAL INFORMATION

2.1 Pterygium

Although pterygium was explained centuries ago by Hippocrates, Galen, Celcus, it was first described by Winter in 1876. Ptergion means wing in Greek. Pterygium is a lesion characterized by non-cancerous benign growth of the bulbar conjunctiva, On the ocular surface next to the conjunctiva, an epithelial and fibrovascular arrangement is depicted. The tissue migrates towards the cornea's center and invades it, generating a wing-like structure that impairs vision. Pterygium is a vascularized, pathologically proliferative, and invasive tissue. Transformed cells can also be found in the pterygial tissue, which is one of the tumor phenotype's features. It has been confirmed that the proliferation activity in the pterygial epithelium is high compared to the normal conjunctiva (Aslankurt et al., 2003; Kase et al., 2007; Kato et al., 2007; Ando et al., 2011).

Pterygium is made up of three parts: the head, which invades the cornea, the neck, which contains the superficial limbus, and the body, which overlies the sclera. (Seet et al., 2012). (Figure 2.1)

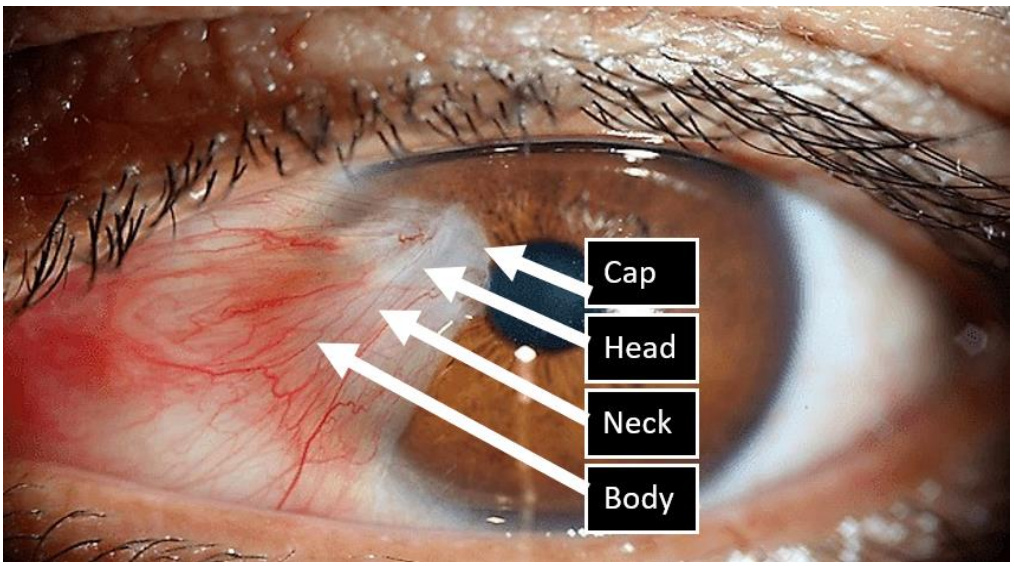


Figure 2.1 : Parts of fully developed Pterygium

Pterygium is a wing-shaped fibrovascular tissue formed by the overgrowth of conjunctival tissue transformed into the cornea and is a member of the family of sunlight-related eye diseases known as ophthalmoheliosis (Kim et al., 2014; Salman and Mansour, 2011). Pterygium, which was thought to be a degenerative lesion due to the degradation and elastosis of Bowman's layer, is now defined as a proliferative disease. Ophthalmologists also consider pterygium a benign lesion because of its slow progression

(Chui et al., 2011). The fact that sunlight itself may be an important risk factor for certain ocular diseases is increasingly accepted, and pterygium has long been included among them (Moran et al, 1984). Although race has already been investigated as a health risk for pterygium development, the precise process is unknown, and the link between race and pterygium recurring is even less understood (Campagna et al., 2018).

Slit-lamp examination confirms the diagnosis, with grade 1 pterygiums occurring when the fibrovascular tissue reaches the limbus, pterygiums occurring when the fibrovascular tissue covers nearly 2 mm of the cornea in grade 2, grade 3 pterygiums occurring when the fibrovascular tissue reaches the pupil margin, and grade 4 pterygiums occurring when the fibrovascular tissue exceeds the pupil margin (Figure 2.2). Pterygium can be classified as involutive or atrophic when the fibrovascular tissue is fleshy and obscures the structures beneath the lesion, or as inflammatory when the fibrovascular tissue is fleshy and obscures the structures beneath the lesion.

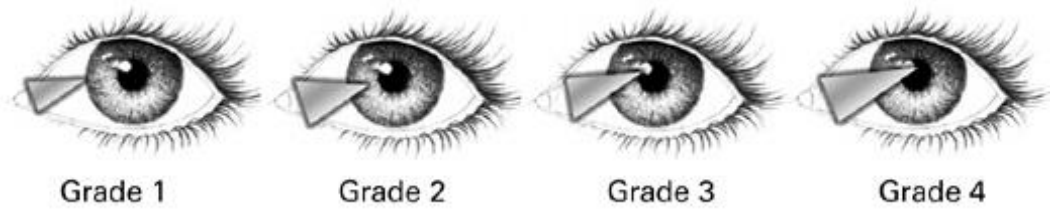


Figure 2.2 : Pterygium classification depends on progression grades.

An early pterygium doesn't require surgery unless it causes visual impairment, inflammation, motility restriction, cosmetic problems and in rare cases blindness (Maheshwari, 2007). Evidence show sometimes after surgery some problems occur to the corneal refraction and with bigger size of the pterygium the greater relation to the corneal refraction involving astigmatism, asymmetry, irregularity and spherical power of the eye (Tomidokoro et al., 2000). But with a successful and good surgery for pterygium excision the eye abnormalities get better such as eye refractive and astigmatism (Stern et al., 1998). Some studies have shown that conjunctival autograft is better than other techniques due its safeness and effectiveness, and regarding recurrence it has lower rates than other techniques (Mahdy et al., 2009). The surgery treatments include first Conjunctival graft which is a technique that the pterygium fibrovascular tissue is excised and then a same size in another part of the bulbar conjunctiva is marked, cut and placed on the site of excised pterygium on conjunctiva (Figure 2.5) (Singh 2017). In addition, using limbal stem cells with 9 conjunctival autograft transplantation has been shown it has very low

rate of recurrence by using thin CAG and LSC may take a long time but it’s very effective and has lowest rate 4.75% of reccurence of pterygium (Mahdy et al., 2009).

2.2 Epidemiology

Pterygium is a common fibrovascular disease in communities living near the equator, which is known as the "pterygium zone" (Coroneo et al., 1993). In several counties within this area, it is believed that approximately 22% of the general population has pterygium (Hilgers et al., 1960). This epidemiologic finding suggests that environmental factors play a significant role in the development of pterygium.

The environmental influence is confirmed by a significantly lower pterygium prevalence rate (less than 2%) in nations outside the pterygium zone (Mackenzie et al., 1992). Sunlight's ultraviolet radiation (UVR) is thought to be a crucial environmental component in the formation of pterygium (Bradley et al., 2010; Detorakis et al., 2009; Mauro et al., 2009). Pterygium is more common in young adults, and it rarely occurs before the age of 15 years. It is thought to have some genetic patterns; however, various risk factors, including dust exposure, heat, inflammation, and ocular surface infection, rural residency, old age, low educational levels, and outdoor activities, have been recorded (Rim et al., 2017). The prevalence of pterygium has been stated as being higher in males (Rim et al., 2017); nevertheless, other research has found that both genders have the same proportions (Viso et al., 2011) or that it occurs more frequently in women (Sreshtha et al.,2014). Its link with smoking has also been explored, however the results are equivocal (Liu et al., 2013). Cameron looked at the worldwide spread of pterygium and discovered that it was more common in dusty, dry, and hot environments. The illness was predominantly seen between 37° north and south equatorial, implying that latitude played a role.

Cameron thought ultraviolet irradiation to be a significant element in the genesis of pterygium, which varies with latitude. Bowman's membrane is damaged by UV light, and the subconjunctival connective tissue thickens and hyperplasia occurs. This condition's occurrence is also influenced by the quantity of time spent in different climates. Although there is no definite link between pterygia and humidity, it is more common among outdoor workers and men. (Droutsas et al., 2010; Hacıoğlu et al., 2017).

Pterygium is more common in men than in women, according to research, which is line with the observations of this meta-analysis (around two times). According to the findings of a meta-analysis, the occurrence of pterygium was higher in rural areas than in urban areas (Liu et al., 2013). Although it has been established that inheritance plays a major role in pterygium formation. Low penetrance is the most common hereditary component, however it looks that the actual lesion is really not inherited, but rather the eye's inclination to respond to environmental stimulation in this way (HILL et al., 1989).

Country	Study	Prevalence	Latitude	Gender %
Brazil (Amazon)	Ribeiro et al., 2011[12]	21.2%	03º 06' S	Male 57.8
	Paula et al., 2006[13]	36.6%		Female 42.2
				Male 15.7
				Female 20.5
Brazil (Southeast)	Shiratori et al., 2010[14]	8.12%	22º 53' S	Male 10.4
				Female 6.5
South Korea	Pyo et al., 2016[8]	8.8%	37° 33' N	Male 3.1
	Rim et al., 2017[16]	1.9%-3.8%		Female 2.5
				Male 0.18
				Female 0.24
Northwest ethiopia	Anbesse et al., 2017[11]	38.7%	12º 31`N	Male 2.20
				Female 1.0
China	Song et al., 2017[9]	9.84%	35° 00' N	Male 10.26
				Female 9.46
Nepal	Shrestha & Shrestha, 2014[18]	12.4%-65.8%	27º 42`N	Male 8.8%-38.5
				Female 14.4%-40.7
USA (Arizona)	West S & Muñoz B, 2009[10]	16%	34° 30' N	Male 23.7
				Female 11.5

Table 2.1 : Epidimiological studies

2.3 Histopathology

Pterygium is an ocular surface disorder featured by a wing-like appearence in the nasal limbus that obstructs vision, causes inflammation, and affects cosmetic appearance. The histopathology of pterygium formation is mainly due to limbal stem cell degeneration and dissolution of Bowman's layer, accompanied by active growth of fibroblast, neovascularization, overgrowth of extracellular matrix, and inflammation (Chui et al., 2008; Sun et al., 2018).

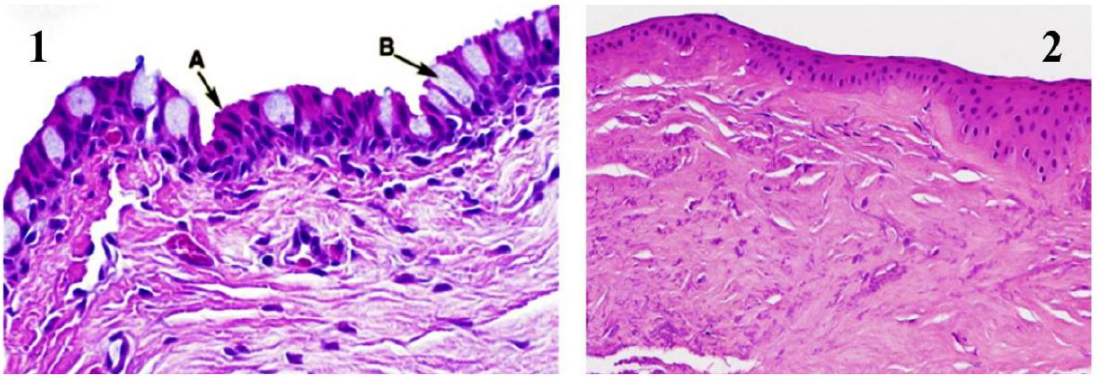


Figure 2.3 : 1-) histopathology of normal conjunctiva 2-) histopathology of pterygium. (A) cylindrical epithelial cells; (B) goblet cells.

In Figure 2.4, it is seen that the morphologies of normal conjunctiva and pterygium tissue are very different from each other. Normal conjunctival histopathology consists of a stratified layer of well-ordered epithelial cells, and goblet cells are regularly located between these cells. A small number of veins are observed under this layer. In the histopathology of the pterygium, conjunctival dysplastic appearance, disorganized increased epithelial cell thickness and loss of polarity, goblet cell loss, intense vascularization, clustering, fibrosis and elastotic degeneration (fibrovascular growth of elastic fibers in Bowman's layer) appearing in the conjunctival stroma are convoluted and segmented. a degenerate amorphous deposit with fibers is observed (Kato et al., 2007; Turan and Turan., 2019; Chui et al,. 2008; Serra et al., 2019; Nassar et al., 2014).

Histologically, Pterygium is distinguished by atrophic conjunctival epithelium and a mass of hypertrophic and elastotic degenerative tissue that is highly vascularized. The pterygium's subepithelial connective tissue exhibits both elastodystrophy (degenerative alterations in elastic fibers) and elastodysplasia (failure of maturation of elastic fibers). These findings suggest that disturbances in elastin metabolism may be involved in the pathogenesis of pterygium (Perez-Rico et al., 2014). Inflammatory cell infiltration consisting of neutrophils, lymphocytes and mast cells in the pterygium tissue draws attention (Girolamo et al., 2006). A fibrovascular stroma is seen in pterygium specimens as evidence of epithelial-mesenchymal change. In some samples, amorphous basophilic materials are encountered, and these materials are often associated with elastic changes in the stroma (Chui et al., 2011). After possessing a pterygium removing, wound healing can cause too much fibrosis., leading to recurrence of this disease. Compared to primary pterygium, fibrovascular growth is more intense in recurrent pterygium (Nuwormegbe et al., 2017).

2.4 Etiology

The etiology of pterygium has a wide scale including many factors such as UV damage, dry eye, environmental dust pollutants (Cimpean et al., 2013). Pterygium is characterized by inflammation, angiogenesis, cell proliferation, fibrosis, disruption of the ECM in the conjunctiva, and increased corneal invasion (Soloman., 2006; He et al., 2019). In particular, the strongest evidence for the pathogenesis of pterygium focuses on UV-mediated limbal damage. In addition, many factors such as abnormal wound healing mechanisms, genetic instability, stem cell disorders, metabolic diseases and neuronal disorders have been shown as other causes of pterygium (Riau et al., 2011).

The pathogenesis of Pterygium has been mostly attributed to UV radiation exposure; nevertheless, this link remains somewhat contentious. Additionally, the complete mechanism of pterygium remains unknown. Causes have been identified as inflammation, viral infection, oxidative stress, DNA methylation, loss of heterozygosity, apoptotic and oncogenic proteins, inflammatory mediators, EM modulators, microsatellite instability, epithelial-mesenchymal cell transition, lymphangiogenesis, and changes in cholesterol metabolism (Wanziler et al., 2019).

In summary, pterygium is a multifactorial disease, a common surface ocular disease with a wide etio-pathogenic scale, influenced by various environmental and genetic factors. In other words, a single mechanism is not responsible for the formation of the disease, but the disease emerges clinically as a result of the common interactions of more than one mechanism with each other (Liu et al., 2013). Even so, the pathogenesis of pterygium induced by various factors but it’s still unknown (Feng et al., 2017). Many influential factors related to the pathogenesis of pterygium have been identified, and they have different functions in the formation and development of the disease.

2.4.1 UV Radiation

The human eye is constantly exposed to UV light, and this type of radiation causes cellular deoxyribonucleic acid (DNA) damage by causing accumulation of reactive oxygen species (ROS) (Ca´rdenas-Cantu´ et al., 2014; Toprak et al., 2019). UV-B radiation is between 280-315 nm and affects the ocular surface. Biologically active small wavelengths, most of which are below 300 nm, are mostly absorbed by the cornea.

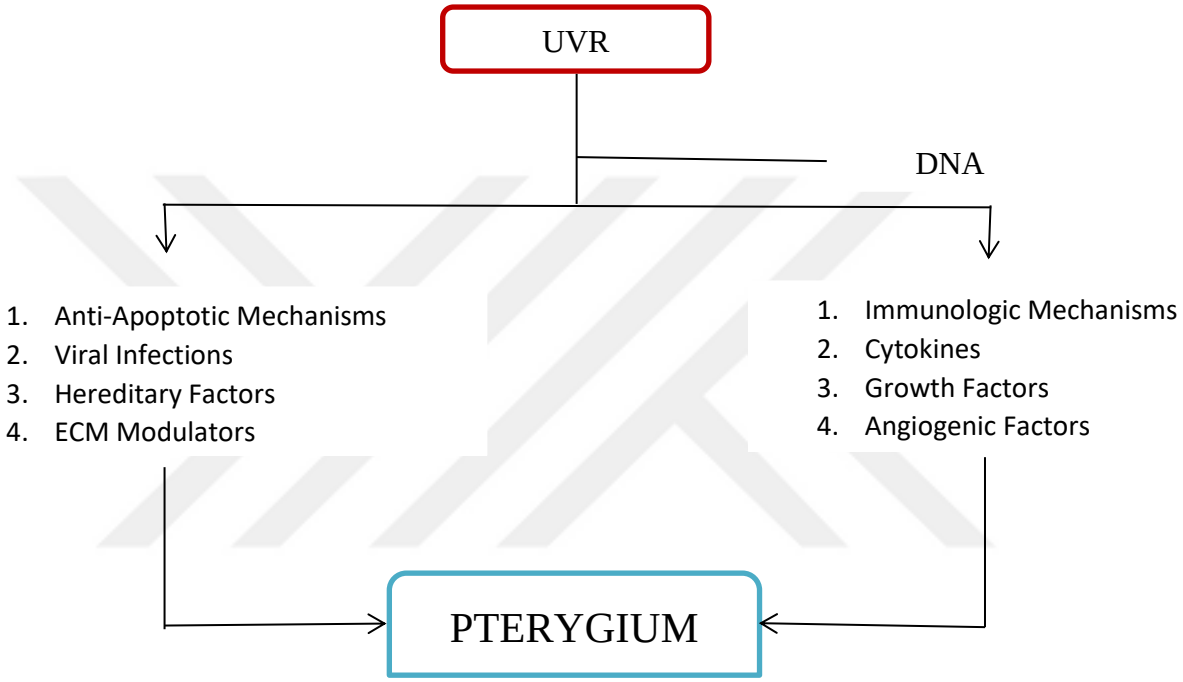
(Ca´rdenas-Cantu´ et al., 2014). UV light causes the formation of pyrimidine dimers and molecular lesions through photochemical reactions in DNA. These lesions are premutagenic and cause changes in DNA structure (Cimpean et al., 2013). Under normal conditions, these damages in healthy cells are eliminated by various defense systems (Toprak et al., 2019).

Epidemiological evidence suggests that exposure to ultraviolet radiation may be a possible trigger for the development of pterygium (Girolamo et al., 2006). patients who have primary pterygium have higher levels of nitric oxide.; It was found that the levels of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase decreased (Toprak et al., 2019). Kim et al. found that the levels of aldehyde dehydrogenase 3A1 (ALDH3A1), protein disulfide isomerase A3 (PDIA3) and peroxiredoxin-2 (PRDX2) enzymes increased as a result of exposure to UV radiation in pterygium tissue. An increase in the level of these enzymes can cause cells to resist UV-induced apoptosis (Kim et al., 2014). The level of 8-OHdG, one of the main markers of oxidative DNA damage, has been found to be elevated in pterygium tissue, and it has also been reported that there are genetic polymorphisms in genes encoding Ku70 and X-ray cross-repair complement (XRCC1) enzymes involved in DNA repair (Toprak et al., 2019).

The p53 gene, which plays an important role in maintaining genomic stability, regulates the response to DNA damage. The frequency of lesions on DNA after exposure to UV light activates p53 and DNA repair is stimulated when the serine residue of this protein is phosphorylated. This process depends on the dose of UV light and the length of its wavelength. Studies have shown that there are mutations in the p53 gene in pterygium epithelial cells that cause abnormal protein production. Abnormal p53 expression slows down apoptosis and increases cellular proliferation, and these mutations are thought to accelerate the development of pterygium (Cimpean et al., 2013).

p53, a major cell stress regulator that operates to integrate signals from a wide range of cellular stressors, is overexpressed and immunohistochemically detectable in many types of UV-damaged cells (Perra et al., 2006). The p53 gene, a well-known tumor suppressor, regulates the cell cycle and is involved in DNA repair and synthesis, cell differentiation, and death (Frezza et al., 2012; Greenblatt et al., 1994). The most prevalent genetic marker of human neoplastic development is p53 mutations (Frezza et al., 2012). Pterygium is seen by many as a neoplastic-like growth condition as opposed to a degenerative malfunction. Two mechanisms are discussed that show that UV radiation has the potential to damage cells. the first is phototoxic effect on cellular DNA; the second

is; It is in the form of the production of Reactive Oxygen species (ROS) that damage cellular DNA, proteins and lipids. Small wavelengths of UV radiation (below 300nm) are the most biologically active form and are known to be mostly absorbed by the cornea (Cárdenas-Cantú et al., 2016).



Fegure 2.4: UV-Related Mechanism in Pterygium Development (Wanziler et al., 2019)

2.4.2 Viral Infection

It is hypothesized that viruses induce tissue proliferation and mutations in DNA that promote tumorigenesis. In some studies, human papilloma virus (HPV) and herpes simplex virus were detected in biopsy specimens from both pterygium and normal conjunctiva (Gallagher et al., 2001; Detorakis et al., 2001). However, subsequent studies have not supported these findings. Another study showed the geographic variability of HPV in pterygium and tested positive for HPV in all samples collected in Italy, only 5 out of 24 cases with pterygium collected from the Equatorial region. These inconsistencies between previous studies suggest that differences in viral infection rates may be related to geographic variations. Because viruses may play a role in the development of pterygium, but this does not constitute significant evidence for the mechanism of pterygium. Since no direct relationship with viruses can be established in

most cases of pterygium, viruses can only increase susceptibility, but it is thought that they are not effective for the pathogenesis of pterygium (Piras et al., 2003; Reid and Dushku., 2003). Several research have looked at the link between HPV infection and pterygium, although their findings are very debatable. According to Rodrigues et al., 58.3 % of Brazilian pterygium samples are positive for HPV DNA, compared to 9.5 % in the control group.

In 42.8 % of HPV-positive samples, type 1 is found, type 2 in 33.3 %, and both types in 23.8 percent. In the control group, however, just 9.52 % are positive, and all HPV in this group is type 16 (Rodrigues et al., 2008). Tsai et al. discovered that HPV is present in 24 % of Taiwanese pterygium samples, all of which are type 16/18. Sjo et al. discovered that just 4.44 % of Danish pterygium samples are HPV positive, with all of them being type 6.

2.4.3 Inheritance Patterns

Although it is accepted that hereditary predisposition plays a role in the development of pterygium, studies are quite limited. Based on this limited number of family studies, the inheritance pattern has been reported as autosomal dominant. However, it is thought that it should be studied with a larger group sample to increase its reliability.

However, an autosomal recessive mode of inheritance may also be possible. It is difficult to compare patients with pterygium to unaffected individuals or to determine how many pedigrees or patients should be considered. It is thought that investigating the heredity information with further studies will facilitate the determination of the possible mechanism of pterygium development (Anguria et al., 2014).

2.5 Pathogenisis

Studies to date show that oxidative stress, apoptotic and oncogenic proteins, extracellular matrix modulators, inflammatory mediators, epithelial-mesenchymal change, angiogenesis and altered limbal stem cells may be effective in the development of pterygium. However, the exact mechanism that causes pterygium development and recurrence is still unknown (Wanzeler et al., 2019).

2.5.1 Inflammation and Immunological Mechanisms

Inflammation is both a normal physiological process, as in wound healing, and a pathological process that can cause diseases, as in cancer (Fang, 2013). Clinically, progression of pterygium is associated with inflammation and neovascularization (Gum et al., 2017). It has been reported that the levels of interleukin 6 (IL-6) and IL-8 were increased after the cultured pterygium epithelial cells were exposed to UV-B rays (Kim et al., 2014). The expression of IL-10 was also found to be elevated in pterygium samples, while the expression of IL-1α was reported to be elevated in both primary and recurrent pterygium tissue. Recently, it has been found that the expression of IL-17 is increased on the ocular surface in inflammatory pathologies such as pterygium (Wanzeler et al., 2019). It has been reported that cyclooxygenase-2, which is effective in stimulating inflammatory cascades, is overexpressed in pterygium (Kim et al., 2014). While there are very few immune cells on the ocular surface under normal conditions, an increase in the number of mast cells, lymphocytes, plasma cells, dendritic cells, CD4+ and CD8+ T cells has been observed in the pterygium tissue (Notara et al., 2018; Kim et al., 2014).

Histological and cytological examinations suggest that the pterygium epithelium is a condition characterized by squamous metaplasia of the ocular surface and goblet cell hyperplasia. Cell cycle-related molecules have recently been shown to play a potential role in proliferation activity in pterygium epithelial cells. These data suggest that cell proliferation and adhesion in the pterygium tissue originate from the epithelium and stroma (Kase et al., 2007). Regardless of origin, inflammatory mediators must be present in significant amounts to be considered a key factor in the development of pterygium. After UV-B radiation, tumor necrosis factor alpha (TNF□) levels were found to be increased as well as interleukin 1 (IL-1), 6 (IL-6) and 8 (IL-8) levels in pterygium. Considering their presence and increased expression, these UV-inducible cytokines may contribute to pterygium formation at various levels (Di Girolamo et al., 2004; Cárdenas-Cantú et al., 2016). Cyclooxygenase-2 (COX-2) is the inducible isoform of

cyclooxygenases and is the key enzyme for inflammatory cytokine-induced angiogenesis. Recently, it has been reported that COX-2 increases vascular endothelial growth factor (VEGF) expression in chronic inflammation and various tumors (Park et al. 2011). COX-2 is also overexpressed in pterygium tissue and is thought to be responsible for potentiation of prostaglandin products and inflammatory cascades.

An increase in the amount of phospholipase D, an enzyme that causes inflammation and cellular differentiation in the pterygium, and cysteine C, an inhibitor of cysteine proteinases, was also found (Tong et al., 2008; Barba-Gallardo et al., 2013; Cárdenas-Cantú et al. et al., 2016). With this; It is thought that both canonical and non-canonical pathways of nuclear factor kb (NF-kb) (related to acute and delayed inflammatory processes, respectively) play an active role in pterygium and may cause numerous biological effects, including recruitment of inflammatory cells into the pterygium tissue. A wide array of growth factors related to cell proliferation, which are effective in pterygium formation, have also been studied.

2.5.2 Cellular Proliferation and Cell Migration

The pterygium tissue shows an abnormal proliferation on the cornea and often invades the Bowman's layer (Konuk et al., 2011). Even short-term exposure to UV light, transforming growth factor (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), connective tissue growth factor (CTGF), as well as heparin-binding, stimulates the synthesis of growth factors such as epidermal growth factor (HB-EGF). These growth factors are necessary stimulants for the proliferation of fibroblasts (Anguria et al., 2014).

Proliferation-related proteins Ki–67, proliferating cell nuclear antigen (PCNA) and cyclin D1 proteins play important roles in the cell cycle, and the Ki–67 protein is an important marker of cellular proliferation. The Ki-67 protein was found to be abnormally expressed when pterygium samples were compared with normal conjunctival samples. PCNA is a non-histone protein required for DNA synthesis, and expression of PCNA is considered a marker for cellular proliferation. Expression of PCNA was significantly increased in pterygium tissue.

Cyclin D1 is an important control gene involved in the transition from G1 phase to S phase in the cell cycle, and in a study, PCNA and cyclin D1 were found to be overexpressed in the limbal part of pterygium epithelial cells, and it is thought that high

expression of these genes may lead to hyperproliferation of epithelial cells. (Wanzeler et al.,2019). Total p63, a potential marker of epithelial cell proliferation, was found to be expressed low in the early stages of pterygium and highly expressed in advanced stages (Xu et al., 2014). E-cadherin and beta-catenin, which are thought to be involved in cell adhesion and proliferation, were found to be intensely present in the pterygium tissue (Wanzeler et al., 2019).

Cell migration is described as the movement of cells toward a particular location and is a fundamental process in the creation of tissues. Transformed basal epithelial cell migration has been found to be a critical feature in pterygium formation (Fang, 2013). Sphingosine 1 phosphate (S1P) is a bioactive lipid that regulates cellular activities such as proliferation, migration and fibrosis, and activation of S1P stimulates RhoA. Rho signals stimulate the production of proinflammatory cytokines and kinases. In their study, Igarashi et al. found that the expression of S1P2, S1P4 and S1P5 receptors was significantly increased in pterygium tissue compared to normal conjunctival tissue. It was also observed that the concentration of S1P increased significantly when normal conjunctival fibroblast cells were exposed to UV (Igarashi et al., 2019).

2.5.3 Angiogenesis

It has been reported that angiogenesis factors have important contributions in the pathogenesis of pterygium, and it is thought that these angiogenic factors facilitate pterygium development by forming new blood vessels (Fang, 2013). TGF-β regulates fibroblast proliferation, angiogenesis, and synthesis and degradation of extracellular matrix (ECM) proteins (Wanzeler et al., 2019). Overexpression of TGF-β gene plays an important role in the pathogenesis of pterygium because it increases angiogenesis (Gum et al., 2017).

VEGF family has been studied intensively in the field of ophthalmology because it increases vascular permeability in ocular diseases such as pterygium and plays a role in pathological angiogenesis. Increased expression of VEGF leads to angiogenesis and lymphangiogenesis, and it has been shown in many studies that VEGF levels are elevated in pterygium samples (Wanzeler et al., 2019). Additionally, several investigations indicated that disordered miRNAs may be involved in angiogenesis, the stimulation of pluripotency genes, and the inhibition of stem cell self-renewal (Lee et al., 2016; Chien et al., 2013). Vascular micro density analysis revealed the presence of angiogenesis at the pterygium level, as well as the overexpression of VEGF, which essentially, in the

proliferating designs of the pterygium, scientific confirmation of this growth factor's detrimental involvement in pterygium growth (Livezeanu et al., 2011). Despite the fact that Wong et al. hypothesized that the angiogenesis factor was a material produced during autolysis, it is also feasible that vascularization is driven by related leukocytes. An immunologic explanation has been proposed based on the discovery of plasma cells, lymphocytes, and immunoglobulins within pterygium in recent articles. According to immunofluorescent tests, IgG was detected mostly in the stroma in areas corresponding to lymphocyte and plasma cell infiltration, whereas IgE was detected in the epithelium, the stroma, and the space between these two issues. Immunofluorescence examinations of normal conjunctiva revealed no (negative) IgE or IgG. (HILL et al., 1989).

2.5.4 Apoptosis

The pro-apoptotic Bax protein was found only in the epithelial basal layer of the pterygium tissue, While the anti-apoptotic protein Bcl-2 was observed in the entire pterygium tissue. It is thought that the absence of these pro-apototic proteins in the head of the pterygium may cause the proliferative behavior of the pterygium tissue (Ca´rdenas-Cantu et al., 2016). Rapamycin complex 1 (mTORC1) is a major regulator of cell growth, proliferation and autophagy. Activation of mTORC1 has been shown to inhibit apoptosis in pterygium, and it has also been found that mTORC1 stimulates cellular proliferation in pterygium by inhibiting the p73 gene. It was found that the survivin protein, which is encoded by the BIRC5 gene, a member of the apoptotic inhibitor gene family, is highly expressed in pterygium tissue and not expressed in normal human conjunctiva (Wanzeler et al., 2019).

In previous studies, it has been reported that this protein is localized extracellularly, in the nucleus, cytoplasm and mitochondria. It also regulates cell division when present in the nucleus and inhibits apoptosis when present in mitochondria. This indicates that different localizations of survivin may be associated with different functions. In their study, Xu et al. found that survivin was mostly located in the cytoplasm in the early stages of the pterygium, while it showed a strong nuclear expression in the advanced stages (Xu et al., 2014).

Although the molecular mechanism of survivin regulation is not fully understood, it is thought that this protein may be related to the p53 protein. It has been shown that oxidative stress, which causes pterygium development, stimulates the activation of survivin protein (Wanzeler et al., 2019).

2.5.5 Altered Limbal Basal Epithelial Stem Cell

The epithelial layer consists of limbal epithelial stem cells in the basal stem cell compartment of the limbus. Limbal epithelial stem cells have a high proliferative potential and are essential for wound healing and regeneration of epithelial cells. In their deficiency, tissue homeostasis changes significantly. UV radiation can cause damage to limbal basal epithelial stem cells and conjunctival fibroblasts (Notara et al., 2018). Dushku et al. reported that changes in limbal basal epithelial stem cells as a result of exposure to UV radiation may cause pterygium development. They also suggested that the pterygium developed from the limbal epithelium, not the conjunctival epithelium, and found that all MMPs were highly expressed in altered limbal basal epithelial stem cells. They also showed that there is abnormal p53 expression in these cells (Dushku et al., 2001).

2.5.6 Epithelial-Mesenchymal Transition

Epithelial mesenchymal transition (EMT) is a process in which epithelial cells assume the characteristics of mesenchymal cells. EMT is involved in development, wound healing and cancerization processes. Recent studies suggest that epithelial mesenchymal transition is involved in the pathogenesis of pterygium. This may be evidence of the origin of pterygium fibroblasts (Hay, 2005; Kase et al., 2007). A series of changes were seen in pterygium EMT compared to normal conjunctival EMT. These include down-regulation of E-cadherin by intranuclear accumulation of beta-catenin and lymphoid enhancer-factor-1, co-expression of cytokeratin 14 and alpha-smooth muscle actin or vimentin in epithelial cells extending into the stroma and absent in normal corneal epithelium. Changes such as snail and leech transcription factors in pterygium epithelial cell nuclei are among the major ones (Kato et al., 2007).

The origin of pterygium fibroblasts remains unclear. Presumably, these fibroblasts originate from the limbal epithelium passing through the EMT. Here, changes in the limbal epithelium are observed as growth factors such as TGF-β or FGF, or EMT-related transcription factors (slag and snail) are induced in keratinocytes as a result of UV exposure. The concept of limbal epithelial cells causing pterygium fibroblasts via EMT is supported by animal models showing that EMT can occur locally during fibrosis. In addition, another phenomenon that supports this concept is the expression of the

mesenchymal marker vimentin in corneal epithelial cells during wound healing (Zavadil and Bottinger., 2005; Hudson et al., 2007; Iwano et al., 2002).

2.5.7 Tumor Suppressor p53 and Related Proteins

p53 is an important cell stress regulator in many cell types damaged by UV radiation, and can be highly expressed and detected immunohistochemically. The p53 gene is a well-known tumor suppressor and regulates the cell cycle. It also functions during DNA repair and synthesis, apoptosis and cell differentiation. The role of one of the checkpoints of cell proliferation in pterygium development is generally controlled by p53. An increased amount of p53 has been reported in the basal epithelium of the pterygium, and p53 mutations are thought to be the most common genetic marker of neoplastic cell growth. Pterygium is also known as a neoplastic growth disorder rather than a degenerative dysfunction.

In one study, positive p53 staining was not observed in any of the control samples, while abnormal levels of p53 expression in pterygium samples were demonstrated immunohistochemically. It is thought that the presence of p53 gene mutation accompanying the abnormal expression of the p53 protein and codon 72 polymorphism may also play a role in the pathogenesis of pterygium. The formation of BPDE-like DNA adduct in pterygium samples is associated with cytochrome p450 polymorphisms. Another tumor suppressor protein, p16, has been found to be down-regulated in pterygium, most likely because to hypermethylation of its promoter region, in any event, mutations in the oncogene KI-Ras codon 12 have been discovered, pterygium has been observed to have a considerable number of them (Cárdenas-Cant et al., 2016). The occurrence of p53 mutations in pterygia's limbal epithelium, limbal cancers, and the majority of pingueculae shows that p53 mutations occurred early in these cells' development (Dushku et al., 1997).

In addition, some studies suggest that p53 mutations may inhibit normal pro-apoptotic function. However, it is thought that there must be a mechanism for p53 resistance in pterygium as opposed to mutations in p53. The p16 gene promoter is hypermethylated in the pterygium and this hypermethylation is said to be strongly associated with its expression. It may also be associated with suppression of positive expression of DNA methyltransferase 3b (DNMT3b) protein and p16 protein.

These data provide molecular evidence that methylation occurs in pterygium and may play a role in their development. However, in pterygium, it can be said that the expression of some proteins related to cell proliferation and apoptosis decreases, while the expression of some importins that participate in cell proliferation increases. Pterygium were also found to have telomerase activity mostly in the epithelium. These findings also suggest that pterygium may be the result of abnormal regulation of apoptosis (Shimmura et al., 2000; Di Girolamo et al., 2004; Chen et al., 2006; Kase et al., 2007; Cárdenas-Cantú et al., 2016) .

UV irradiation stabilizes p53 by post-translational mechanisms that then enter the nucleus, bind to DNA and trigger cell cycle arrest, apoptosis or DNA repair. Stabilization of p53 can lead to interactions between p53 and its regulators and the ratio of bax and bcl-2. The bax-bcl-2 ratio indicates a dose-related relationship, and apoptosis occurs in association with cell cycle arrest with low dose UV and accumulation of bax at high doses (Di Girolamo et al., 2004).

In addition to all these studies with p53 in pterygium give conflicting results. An interaction was found between mutant p53 and the retinoblastoma gene product, leading to increased upregulation of transforming growth factor-b. However, these changes in the pterygium could not be shown as evidence.

Other researchers found an increase in the amount of p53 in the results of their studies using ELISA or immunohistochemical techniques. p53 is normally present in a small proportion in the cell. It has been shown that p53 positive for pterygium initially becomes negative in subsequent repeats. suggesting that accumulation of p53 is not associated with repeats, which contradicts the notion that mutant p53 plays a critical role in the pathogenesis of pterygium. Interestingly, only one study reported the absence of wild-type p53 in p53 mutations in pterygium.

Methodological distinctions may explain for these different findings. Alternatively, p53 accumulation could occur as a result of UV damage and altered cell turnover. In human skin exposed to a single dose of UV-B, p53 accumulation may reach a peak at 24 hours and then revert to baseline levels after 3-4 days. As a result, if individuals with pterygium were exposed to sunlight prior to surgery, the p53 dye in these samples was wild-type.

2.5.8 Microsatellite Instability and Loss of Heterozygosity

In some clinical observations, it has been determined that pterygium in in vitro culture models has some specific features similar to those in pre-malignant diseases. Loss of heterozygosity (LOH) is a common event in tumor tissues due to loss of tumor suppressor genes at mapped loci. In pterygium, LOH has been identified in regions 9q and 17q, associated with postoperative recurrence. It has been reported that these identified regions can be used as a potential prognostic marker for recurrent lesions.

However, these LOH regions were not found in normal phenotype conjunctival tissues taken from protected areas. This suggests that genetic changes may be related to the level of exposure to UV light. A LOH has been identified in the 11q 22.23 region that includes MMP-1 in the pterygium tissue, and this mapped LOH has also been associated with the development of metastatic melanoma (Girolamo et al., 2004).

Microsatellite instability (MSI) results from the inability to repair DNA damage during DNA replication, and MSI is seen as a reflection of the increased mutation rate. Highly polymorphic microsatellite DNA contains dinucleotide and rarely trinucleotide repeat sequences, and these regions are used in genetic linkage analyzes for many diseases (Detorakis et al., 1998). MI has been identified in neurodegenerative diseases, atherosclerotic plaques, spontaneous abortions, and almost all cancer types (Spandidos et al., 1997). In their study, Detorakis et al. examined 15 microsatellite markers on 3 chromosomes in 50 pterygium samples and determined that MI was present in only 3 cases (Detorakis et al., 1998). Spandidos et al., on the other hand, found that 8 of 15 pterygium samples had LOH and 2 had MI (Spandidos et al., 1997).

A study was also conducted on loss of heterozygosity (LOH) and microsatellite instability (MSI) factors, which are known to be associated with cell proliferation. LOH is thought to be associated with cell proliferation and cancer because while MI is an indicator of an impaired DNA mismatch repair mechanism, it usually corresponds to the absence of a functional tumor suppressor gene. In this sense, the results obtained so far are controversial. Pterygium fibroblasts show features of cells that have been altered, including loss of heterozygosity and microsatallite instability. In addition, there are studies showing increased proliferation of the fibrovascular layer in recurrent pterygium, as well as studies not reporting such a difference (Di Girolamo et al. 2004; Cárdenas-Cantú et al., 2016).

2.5.9 Extracellular Matrix Proteins and Remodelization of the Extracellular Matrix

The extracellular matrix consists of a combination of extracellular molecules secreted by the supporting cells surrounding the cells, which contribute structurally and biochemically. ECM proteins include keratin, elastin, collagen, fibrin and some other proteins. Abnormal expression of ECM proteins is thought to be directly related to the proliferative growth of the pterygium (Wanzeler et al., 2019). When Bowman's layer is disrupted, proliferative cells can migrate through the collagen barrier (Ca´rdenas-Cantu, 2016). When pterygium samples were compared with normal conjunctival tissue, tropoelastin expression was found to be higher, Type II collagen expression was positive only in pterygium samples, while collagen I, III and IV were positive in both pterygium and normal conjunctival samples (Wanzeler et al., 2019).

Matrix metalloproteinases (MMP), known as matrixins, are proteinases involved in tissue remodelization, and metalloproteinase tissue inhibitors (TIMP) bind MMPs and inhibit their activities. In studies on the pathogenesis of pterygium, these two protein groups have been extensively discussed (Wanzeler et al., 2019). It has been found that there is an increase in the expression levels of MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 in the head of the pterygium, which is the main site of proliferation and migration. Hypomethylation of the MMP-2 gene, which is associated with matrix remodeling, has been reported to be highly associated with pterygium invasion (Ca´rdenas-Cantu et al., 2016). In pterygium fibroblast culture, it was found that cyclosporine A decreased the expressions of MMP-3 and MMP-13. It has been shown that there are changes in the expression of MMP and TIMP at different stages of pterygium. Disruption of the balance between MMPs and TIMPs, It is thought that it may be responsible for its progression and recurrence (Wanzeler et al., 2019).

2.5.10 Molecular Genetics Changes

In gene analysis studies on pterygium, it has been reported that genes encoding proteins involved in wound healing, proteins involved in cellular adhesion and mitotic proteins are highly expressed (Pérez-Rico et al., 2014). Proteins belonging to the claudin family are involved in cell-cell adhesion in epithelial and endothelial cell layers. Immunohistochemical analyzes showed that healthy corneal and conjunctival tissues were positive for claudin-1 and claudin-4, and the expression of claudin-1 protein was significantly reduced in pterygium samples. While the tumor suppressor genes p63 and

p16 were rarely expressed in normal conjunctival tissue, it was found that protein expression of both p63 and p16 was increased in pterygium samples (Wanzeler et al., 2019). In studies, it has been reported that a heat shock protein (Hsp90) responsible for the folding and degradation of proteins is overexpressed in the pterygium epithelium, while the expression of Hsp27 and Hsp70 proteins is increased. Overexpression of these proteins can be considered as a natural response of cells to modified proteins resulting from oxidative stress (Ca´rdenas-Cantu´ et al., 2014).

Large tumor suppressor kinase 1 (LATS1) and large tumor suppressor kinase 2 (LATS2) are tumor suppressor genes involved in the response to UV-induced DNA damage. Methylation in the promoter region of these genes can lead to silencing of these genes and tumor development. In a study, it was found that decreased expression of LATS1 and LATS2 genes as a result of methylation may pose a risk for the development of pterygium. In another study, it was shown that E-cadherin and MMP-2 genes were hypermethylated in pterygium tissue (Ginger-Eke et al., 2018).

2.5.11 Changes in Cholesterol Metabolism

In pterygium-derived fibroblasts, a shift in cholesterol metabolism was detected. Additionally, elevated mRNA levels of hydroxyl-methylglutaryl-coenzyme A-reductase (the rate-limiting enzyme in cholesterol production) and low-density lipoprotein receptor (containing cholesterol-rich lipoproteins) were detected in human pterygium fibroblasts. Everolimus and pioglitazone were found to inhibit pterygium fibroblast growth and intracellular cholesterol homeostasis, as well as the levels of acyl-coenzyme A cholesterol acyltransferase and molecules implicated in multidrug resistance protein. These findings indicate that tropical medicines may be utilized to inhibit pterygium proliferation by modulating cholesterol ester metabolism (Cárdenas-Cantú et al., 2016).

2.6 WASL (nWASP) Gene

One of the Wiskott-Aldrich syndrome (WAS) protein family members is encoded by this gene. Wiskott-Aldrich syndrome proteins have a similar domain structure and interact with a range of signaling molecules to modify the actin cytoskeleton. The encoded protein is widely expressed in brain tissues and interacts with a number of cytoskeletal proteins, including cell division control protein 42 (CDC42) and the actin-related protein-2/3 (ARP2/3) complex. The encoded protein could participate in the production of lengthy actin microspikes and neurite extension.

The WASL gene is located on the q arm (long arm) of chromosome 7, which locate q31.32 and encodes a 505 amino acid sequence. The WASL gene comprises 11 exons, and the protein has a molecular weight of about 54.84 kD. WASL gene regulates Actin polymerization by boosting Arp2/3 complex actin nucleating activity (Ginger-Eke et al., 2018; Wu et al., 2006). Through its role in the regulation of actin polymerization, it is involved in a variety of activities, including mitosis and cytokinesis (Miki et al., 1998; Zhang et al., 2009). Involved in the extension and maintenance of the creation of thin, actin-rich surface projections known as filopodia in collaboration with CDC42. Also regulates gene transcription, perhaps via encouraging nuclear actin polymerization, in the nucleus as well as in the cytoplasm (Wu et al., 2006). Forms a compound with HSF1/HSTF1 that inhibits HSP90 expression by binding to heat shock promoter elements (HSE) (By similarity). Plays a function in the formation of dendritic spines (By similarity).

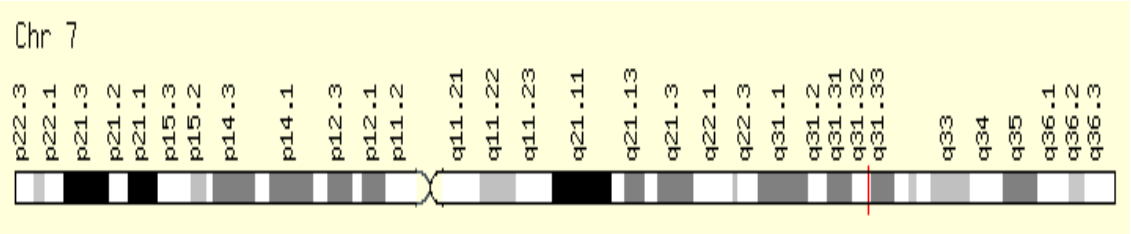


Figure 2.5: WASL gene location on the chromosome 7.

N-Wasp (Neural Wiskott-Aldrich syndrome protein) is a nucleation-promoting factor that promotes the formation of branching actin filaments. (Alekhina et al., 2017). Regulation of cortical actin filament rearrangement in response to external stimuli is mediated through the Arp2/3 complex, which links small GTPases (e.g., Rac and Cdc42) with actin polymerization (Takenawa and Miki, 2001). A number of studies have shown that WASL(N-Wasp) and Wasp family verprolin homologous protein-1 (WAVE1) are essential for myelination in both the PNS and CNS, respectively, and that

they govern the differentiation of oligodendrocytes. Myelinating Schwann cells as well as oligodendrocytes express the N-Wasp molecule, which is found in both cell types (Tsuchiya and colleagues 2006, Novak and colleagues 2011; Zhang and colleagues 2014; Marques et al., 2016). The actin cytoskeleton is most likely regulated by it, as it is critical for Schwann cell membrane wrapping and longitudinal extension of the myelin unit in the PNS (Jin et al., 2011; Novak et al., 2011). Mutant N-Wasp mouse schwann cells lacked distinct F-actin-rich cones and shorter radial lamellipodia (Jin et al., 2011). Schwann cells could sort and sheath axons appropriately without N-Wasp, but they couldn't wrap myelin (Jin et al., 2011; Novak et al., 2011). N-Wasp and Arp2/3 components were found in newly produced oligodendrocytes and purified myelin fractions in the central nervous system (Bacon et al., 2007). Pharmacological suppression of N-Wasp also hindered process extension and produced filopodium retraction in cultured oligodendrocyte precursor cells. N-Wasp and Arp2/3-driven actin polymerization appear to control the expansion of oligodendrocyte precursor cells' biological processes. The loss of N-Wasp in oligodendrocytes causes hypomyelination, a variety of myelin abnormalities, including outfoldings that enwrap neuronal cell bodies, and myelin and axonal degradation.

2.6.1 Role of WASL Gene in Actin Polymerization

The three-dimensional structures of individual actin molecules and actin filaments were determined in 1990 by Kenneth Holmes, Wolfgang Kabsch, and colleagues. Actin molecules are globular proteins containing 375 amino acids (43 kd). Each actin monomer (globular [G] actin) contains tightly packed binding sites that facilitate head-to-tail contacts with two other actin monomers, resulting in the polymerization of actin monomers to create filaments. (filamentous [F] actin) figure 2.5.

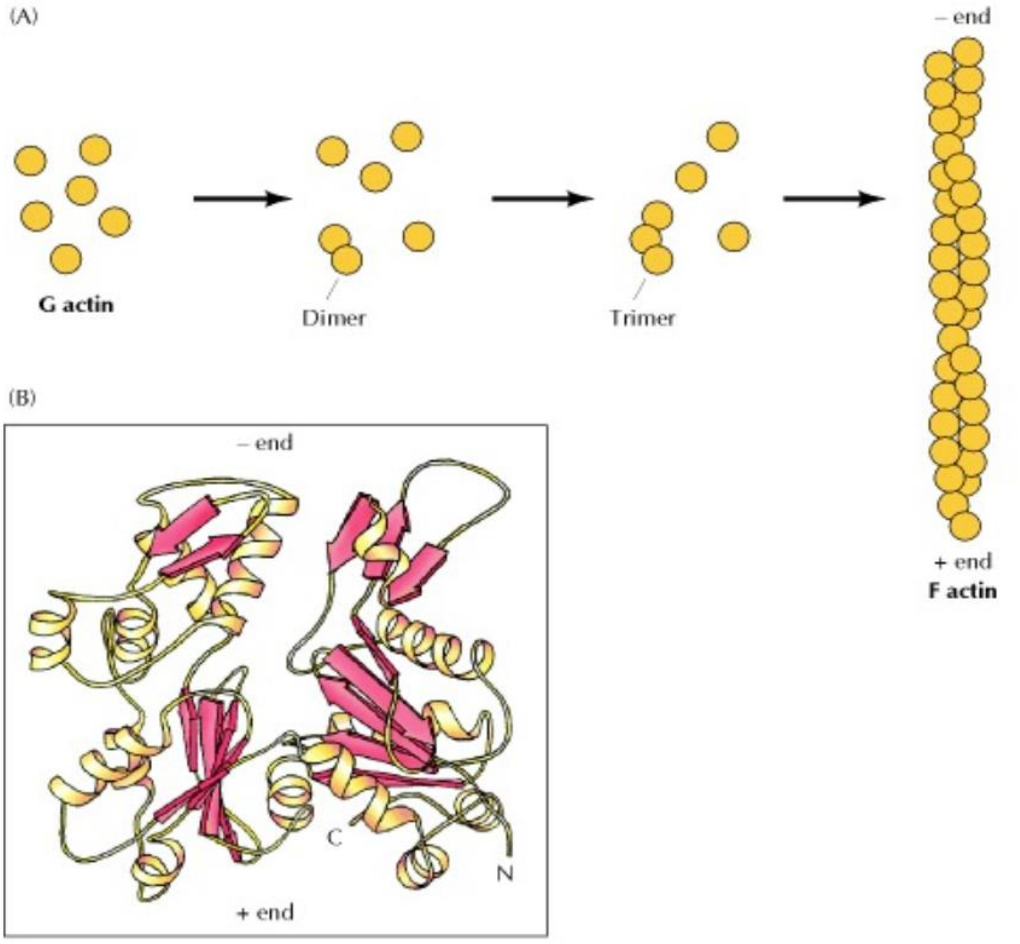


Figure 2.6: Assembly and structure of actin filaments

(A) Actin monomers (G actin) polymerize to form actin filaments (F actin). The first step is the formation of dimers and trimers, which then grow by the addition of monomers to both ends. (B) Structure of an actin monomer. (C) Space-filling model of F actin. Nine actin monomers are represented in different colors. (C, courtesy of Dan Richardson.)

2.6.2 Activation mechanisms of the WASP Family (family proteins regulate actin dynamics)

The actin cytoskeleton reorganizes quickly in response to morphological characteristics and cell mobility, being signal-regulated molecular switches that trigger actin polymerization, WASP Family have gotten a lot of interest. The first member, WASP, was discovered to be the result of a gene that causes Wiskott-Aldrich syndrome, a congenital human disease, when it malfunctions. WASP, WASL (N-WASP), WAVE/Scarl, 2, and 3 are the five members of this protein family at the moment.

Cdc42, a small Rho family GTPase that interacts in the philosophy, has functional and physical connections with WASP and WASL. But on the other hand, it has been established that the WAVE/Scar proteins are linked to Rac, a Rho family protein that governs membrane turbulence. At the C-terminus of all WASP proteins is a VCA domain

that activates the Arp2/3 complex, allowing actin polymerization to germinate. Unexpected activities of WASL and WASP proteins in multicellular animals have just recently been discovered through analysis of model organisms (Miki et al., 2003).

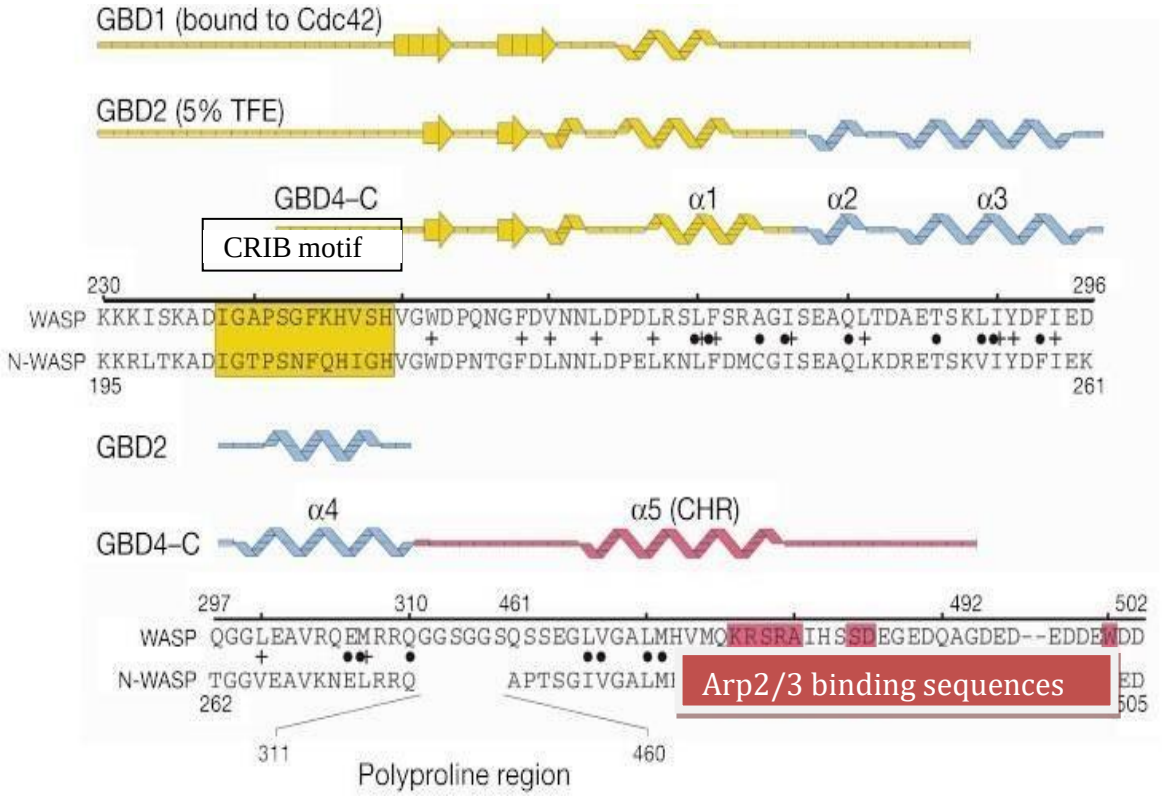


Figure 2.7: Amino-acid sequences of human WASP and , along with the GBD constructions of WASP's consensus secondary structure, are presented above (Kim et al., 2000).

WAVEs are all members of the WASP superfamily, as are the junction-mediating and regulatory protein (JMY). WASP, WASL and WAVE cause fast polymerization of actin under the biological membrane, which is essential for a range of pathological and physiological activities such as immunological response, synaptogenesis and pathogen, tissue morphogenesis, cancer invasion and metastasis. WASP, WASL and WAVE appear to be critical in the progression of cancer, according to a growing body of research (Kurisu et al., 2010; Machesky et al., 1999).

2.6.2 Wasl and relation to Cancer

Actin polymerization is accelerated by WASL activating the ARP2/3 complex, which transmits signals from cell surface receptors to the actin cytoskeleton (Uenishi et al., 2013). Additionally, studies confirmed that WASL mRNA expression was significantly increased in Uveal Melanoma cells. Downregulation of WASL had the same result as overexpression of miR-142-3p, which inhibits UM cell proliferation and/or migration. Previous researchers found that WASL overexpression acted as an oncogene in cervical cancer by enhancing the migration and invasion of cervical cancer cells in vitro (Hou et al., 2017). Unfortunately, the effect and associated processes of WASL on the prognosis of cervical cancer patients remain unknown. Recent research indicated that WASL expression was substantially correlated with the pathological stage of cervical cancer patients and that lower WASL expression was associated with improved cervical cancer patient survival. Several human tumors have been linked to the biological activities of WASL. Human breast cancer cells were found to be considerably less invasive when WASL was knocked down (Schwickert et al., 2015). According to Peng et al., melanoma cell migration was inhibited when WASL was downregulated (Peng et al., 2019). Li et al. found that the down-regulation of WASL by MicroRNA-214-5p prevented the invasion and migration of liver cancer cells (Li H. et al., 2018). Cervical cancer cells' growth may be aided by WASL, according to these researches. Knowing the mechanisms underpinning invasive progression, the first phase in metastasis, is crucial to understanding cancer-related death because cancer mortality is predominantly caused by progress to be invasive and metastatic conditions. Actin polymerization just at the point of the edge of migrating cells provides a force that push the plasma membrane forward when tumor cells become invasive. Its own main role is to maintain intercellular mechanical adhesion (cell to cell adhesion) and to govern the form of the cells, and actin polymerization at the point of the edge of migrating cells.

3. MATERIAL AND METHOD

3.1 Materials (equipment)

3.1.1. Sampling

The participants in this study were twenty-seven people who had been diagnosed with pterygium and had applied to the Department of Eye Diseases at Tokat Gaziosmanpaşa University Medical Faculty in Turkey. During the study's group formation process, pterygium tissues harvested during the operation were designated as the patient group, and healthy conjunctival tissues from the same eye as the patient group were designated as the control group. The necessary permission for the study was obtained by the Clinical Research at Tokat Gaziosmanpaşa University's Faculty of Medicine by project number 2019/110. Tokat Gaziosmanpaşa University Scientific Research funded this research with project number 19.KAEK-024. The research was carried out in Medical Biology and Genetics laboratories.

3.1.2 Storing Tissues

With the permission and knowledge of the participants involved in the research, tissue samples taken during the surgery were quickly cut into desired pieces for further study. The residual (remaining) tissues were frozen at -80 degrees Celsius for RNA isolation, cDNA extraction, and gene expression analysis.

3.1.3 Devices Used in the Study

- Refrigerator (Arcelik, 570 465 MB, Turkey) Shaker (Ika, Germany)
- -20 ° C freezer (Arcelik, 2052DY, Turkey)
- -80 ° C Deep Freezer (Nuaire, NU-9483E, USA)
- Distilled Water Device (Elga-option Q7, UK)
- Oven (Memmert, Germany)
- Power Supply (Biorad, 1645070, USA)
- Precision Balance (Kern ABT, WB0750631, Germany)
- Homogenizer (Next Advance Storme 24, BBY24M-CE, USA)
- Magnetic Stirrer (Velp Scientifica, F20520162, Italy)
- Microwave (Arcelik, MD554, Turkey)

- Autoclave (HMC, HV25, Germany)
- Automatic pipettes (Gilson, France)
- pH Meter (Hanna Instruments, HI2020W, USA)
- Plate (Applied Biosystems, 4346907, England)
- Plate adhesive (Applied Biosystems, 201405092, UK)
- Qubit 2.0 Fluorometer (Invitrogen by life technologies, Australia)
- Real-Time PCR (Applied Biosystems, UK)
- Centrifuge (Hettich, D78532, Germany)
- Refrigerated centrifuge (Hettich, Germany)
- Vortex (Velp Scientifica, F20220176, Italy).

3.1.4 Chemical Materials Used, Kits and Kit Contents

- cDNA synthesis kit (GeneAll, Hyperscript, First strand synthesis kit Catalog # 601-005, Korea)
- Reverse Transcriptase Enzyme 10X RTase Reaction Solution Mix 10 mM dNTP
- RNase Inhibitor
- Oligo (dT) 20
- Random hexamer (primary)
- Nuclease-free water
- Ethanol (Sigma-Aldrich Catalog No: E7023, USA)
- Qubit ssDNA Assay Kit (Invitrogen Catalog No: Q10212, USA)
- Qubit RNA HS-Assay Kit (Invitrogen Catalog No: Q32852, USA)
- GeneAll RiboEx^{TM}LS RNA Isolation kit (GeneAll Biotechnology, Catalog No:302-002, Korea)
- Lysis Solution
- Washing Solution 1
- Washing Solution 2
- RNase-free Water
- RNaseZap (Thermo Catalog No: AM9780, USA)
- Running Solution (ThermoFisher, NP0002, USA)

3.2 Method

The tissues that were removed from the patient during surgery were stored at -80°C. The tissue samples were utilized to extract RNA and perform gene expression analysis on the extracted RNA samples.

3.2.1 RNA Isolation from Tissue

RNA isolation from tissues were done with GeneAll RiboEx^{TM}LS kit, working protocol was depend on kits manual instruction.

1- Using a homogenizer, homogenize pterygium specimens in 0.75 ml RiboEx^{TM}LS (50–100 mg of tissue).

2- Pipette or vortex 0.25 ml of the specimen into 0.75 ml RiboEx^{TM} LS.

3- Incubate the mixture at room temperature for 5 minutes.

4- Transfer the supernatant to a new tube after centrifuging at 12,000 x g for 10 minutes at 4°C.

5- Mixture was mixed with 0.2 ml chloroform (0.2 ml per 0.75 ml RiboEx^{TM} LS). Mix thoroughly for 15 seconds, then set aside for 2 minutes at room temperature.

6- Centrifuge for 15 minutes at 4°C at 12,000 x g, then transferred the aqueous phase to a new tube.

7- Incubate specimens for 10 min at room temperature.

8- Centrifuge at 12,000 x g for 10 minutes at 4°C then remove the supernatant.

9- Wash the RNA pellet with 1 mL of 75 percent ethanol. The RNA precipitated can be kept in a 75 percent ethanol solution at 4°C.

10- Centrifuge for 5 minutes at 7,500 x g at 4°C. Thoroughly remove the supernatant, ethanol, after that air-dry the RNA pellet for 5 minutes.

11- Incubate for 10 to 15 minutes at 56°C after dissolving RNA in DEPC-treated water or a 0.5 percent SDS solution.

3.2.2 Measurement of RNA Amount

The ABP iQuantTM RNA HS Assay kit was used for RNA measurements. DNA measurements could be made because all of the necessary steps had been done. There must be an introduction of the standards before a measurement may be standardized and produced appropriately. Before using anything, we made sure it was all at room temperature.

First, 1µl of iQuant Reagent and 199 µl of iQuant buffer were mixed and vortexed per sample. This solution was prepared in both standards and sample measurement. For standards, 190 µl of solution was taken into 0.5 ml tubes. 10 µl Standard 1 and Standard 2 were prepared in separate tubes and standard readings were made on the device. For RNA kit 57, Standard 1: 0 ng / µl, Standard2: 10ng / µl. The device is read as if these are samples, and control is provided. For sample measurement; According to the amount we have, samples in the range of 1µl-10µl were taken into tubes and completed to 200 µl with Working solution. The sample was mixed thoroughly with a finger tap. Vortex is not preferred. Thus, the samples whose measurements were completed were ready for the next experimental steps.

3.2.3 cDNA Synthesis

The reverse transcriptase enzyme converts RNA to cDNA in the opposite direction of the transcription event. The A.B.T.TM cDNA Synthesis Kit with RNase Inh was used, which is a cDNA synthesis kit that contains all of the components needed to acquire and synthesize a cDNA (Table 3.1). Furthermore, the RNase Inhibitor included in the kit adequately preserves RNA templates from destruction.

TABLE 3.1. Reverse transcriptase reaction recommended volume

For total 20 µl Reverse Transcriptase Reaction Volume	
10X Reaction Buffer	2 microliters
dNTP mixture (2.5 mM each)	1 microliter
Randomized hexamer (50 µM)	2 microliters
Reverse Transcriptase (200 U/µl)	1 microliter
RNase Inhibitors	0.5 microliter
RNase free Water	3.5 microliters
RNA Template	10 microliters

TABLE 3.2. PCR program applied for cDNA synthesis

RT Steps	Temp (°C)	Time	Cycle
Step 1	25	10 minutes	1
Step 2	37	120 minutes	1
Step 3	85	5 minutes	1
Step 4	4	∞	1

3.2.3 Calculation of cDNA Concentration

The amount of cDNA was measured using the Qubit 2.0 device and the ssDNA Assay Kit from Qubit (Invitrogen, Q10212). The amount of cDNA to add to each assay was calculated to be 60 ng, and also the amount of cDNA to add to each sample was calculated independently.

3.2.4 qRT-PCR step

Real-time PCR, which enables simultaneous monitoring of nucleic acid amplification, is a technique based on the detection of an increasing fluorescent signal proportional to the amount of DNA. The level of amplification is evaluated using fluorescent dyes (such as SYTO9, SYBR Green) or probe sequences that generate signals as a result of DNA degradation (Tsai et al., 2009).

TABLE.3.3. PCR reaction components (20 µl) The reaction cycle was set up on the Applied Biosystems Real Time PCR instrument as follow

Components	Volume
A.B.T.™ 2X qPCR SYBRGreen Master Mix (with ROX)	10 microliters
1X ROX Dye (50X)	0.4 microliter
Forward Primer	1 microliter
Reverse Primer	1 microliter
Template cDNA	3 microliters
Rnase-free Water	4.6 microliter

3.2.4 qRT-PCR program implemented

50°C	**2 Min**		
95°C	**10 Min**		
95°C	**15 Sec**	**40 Cycles**	
55°C	**1 Min**		
Melt Curve	**95°C for 30 Sec.**	**60°C for 15 Sec.**	

TABLE: 3.4. The condition for the cycling reaction.

4. RESULT

Result of working group

In this study, 27 pterygium patients (18 men and 9 women) were referred to the Tokat Gaziosmanpaşa University Hospital's Ophthalmology Department. The study's patients were 33 percent female and 67 percent male, with ages ranging from 43 to 78 years and the average age was 58. The laboratories in the Tokat Gaziosmanpaşa University Faculty of Medicine were used to complete the entire investigation, as shown in **Figure 4.1**, which shows the gender distribution of the studied patients.

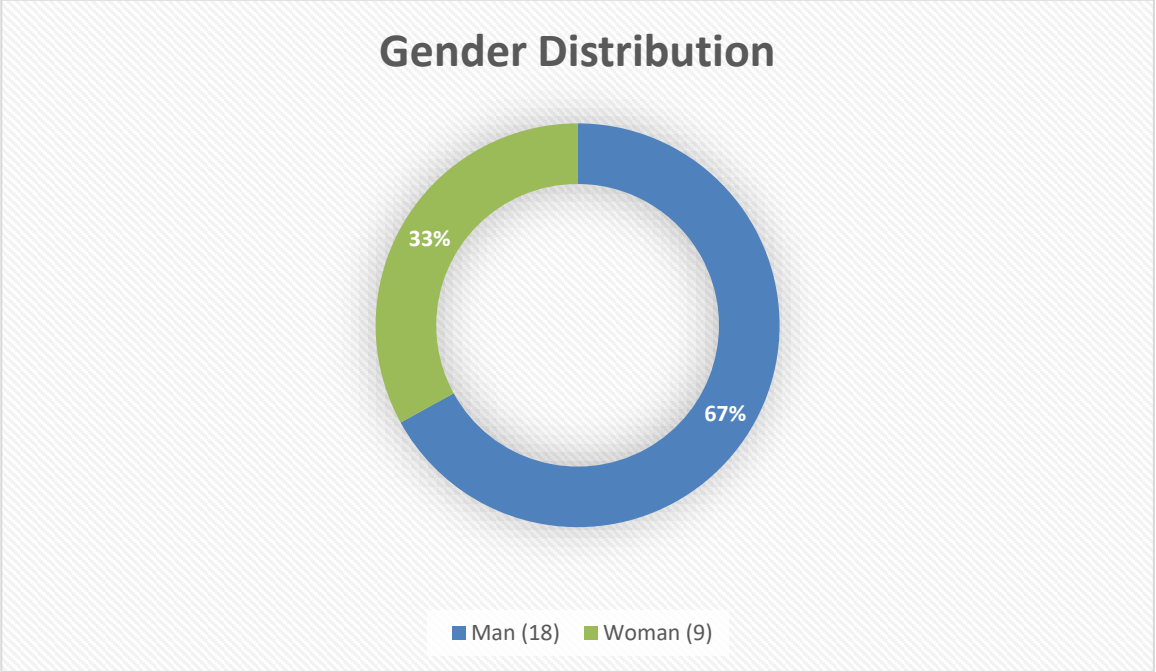


Figure 4.1: The Gender Distribution of the studied Patients

Results of quantitative real-time PCR (qRT-PCR) analysis

4.1.1. qRT-PCR image and evaluation of the WASL gene

The quantitative Real-Time PCR method based on SYBR Green was used to evaluate the gene expression with WASL mRNA, for WASL and the gene preferred as a reference (Actin: ACTB), samples of cDNA (1/10: 1/100: 1/1000 and 1/10000) at different concentrations were prepared and serial dilution was made.

In the figures below, the amplification curve and melting peak of ACTB and WASL genes observed as a result of qRT-PCR are given, respectively (Figure 4.2, Figure 4.3, Figure 4.4,Figure 4.5)

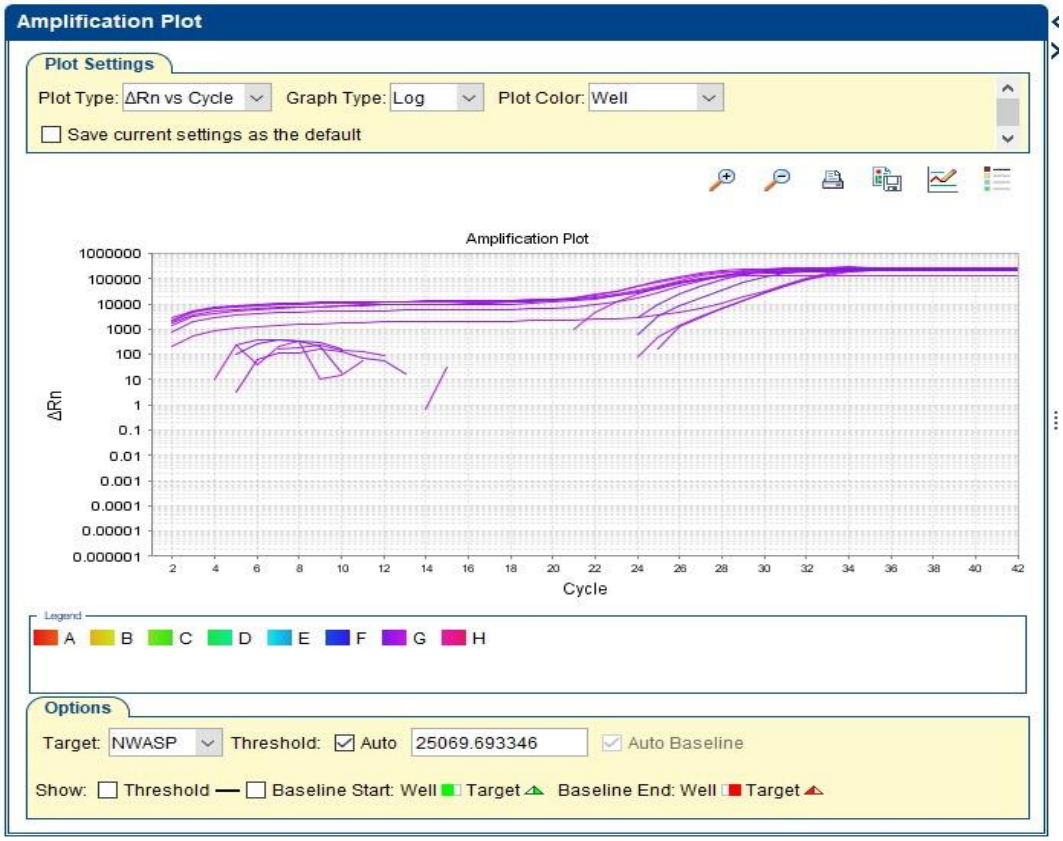


Figure 4.2: WASL(nWASP) gene amplification plot.

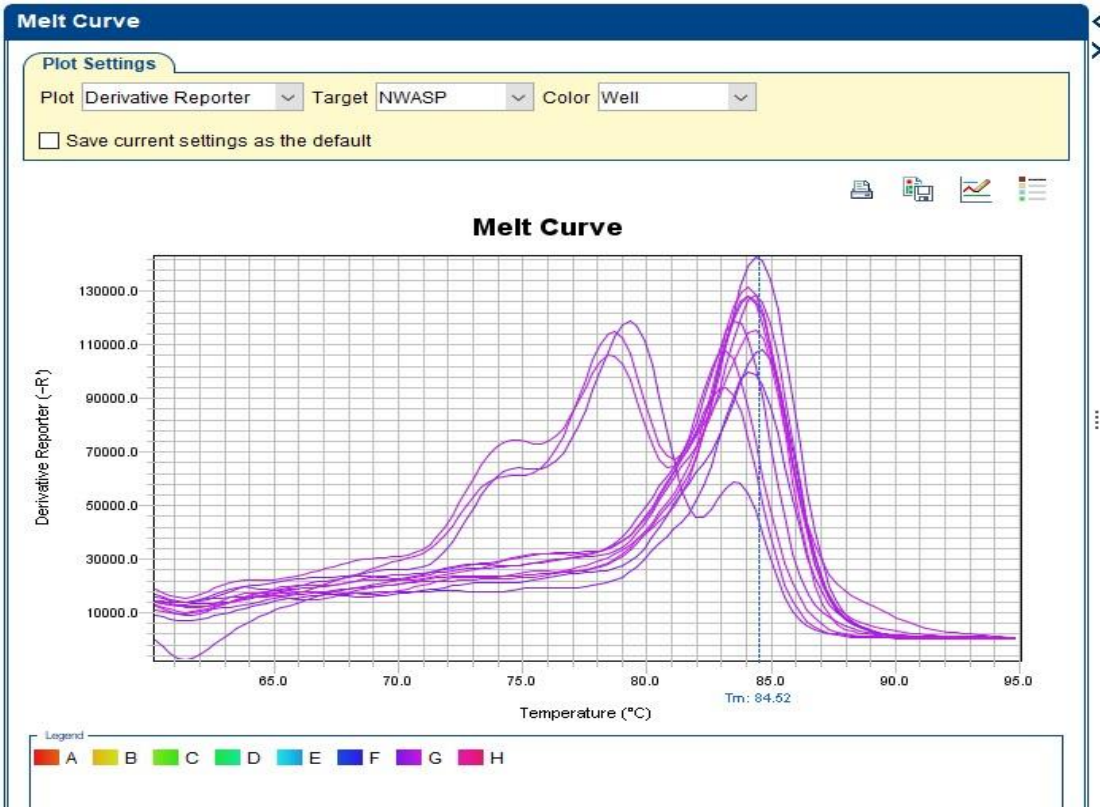


Figure 4.3: WASL(nWASP) gene melt curve.

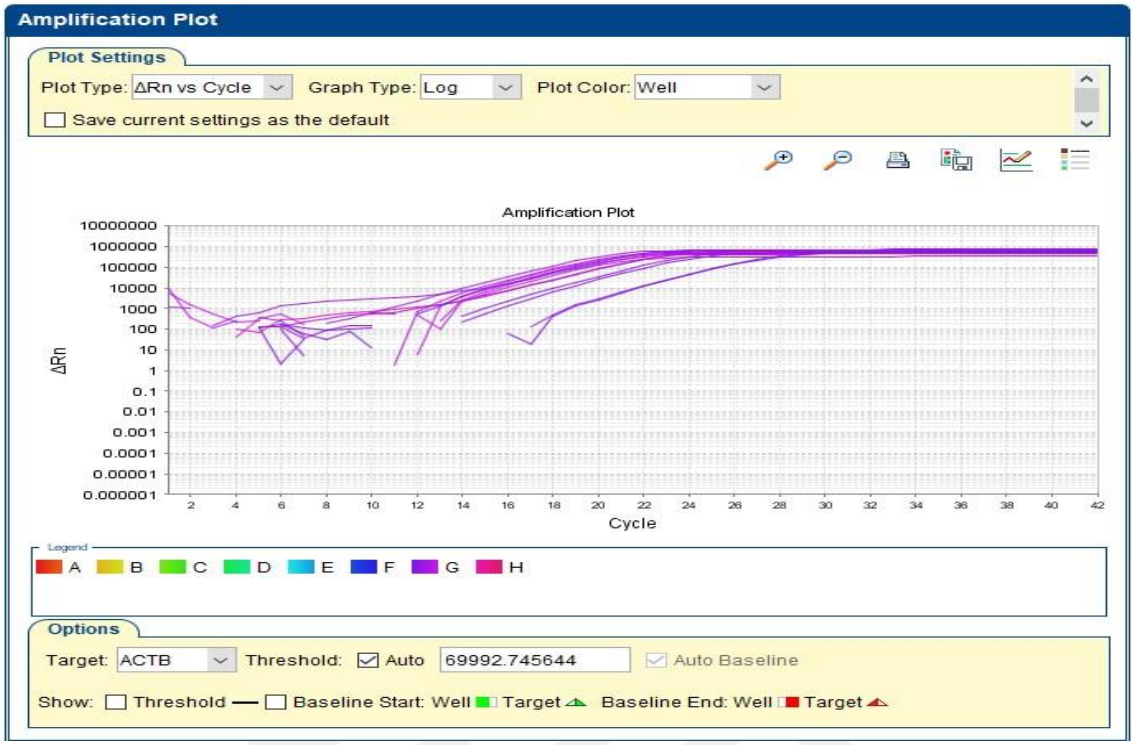


Figure 4.4: ACTB amplification plot.

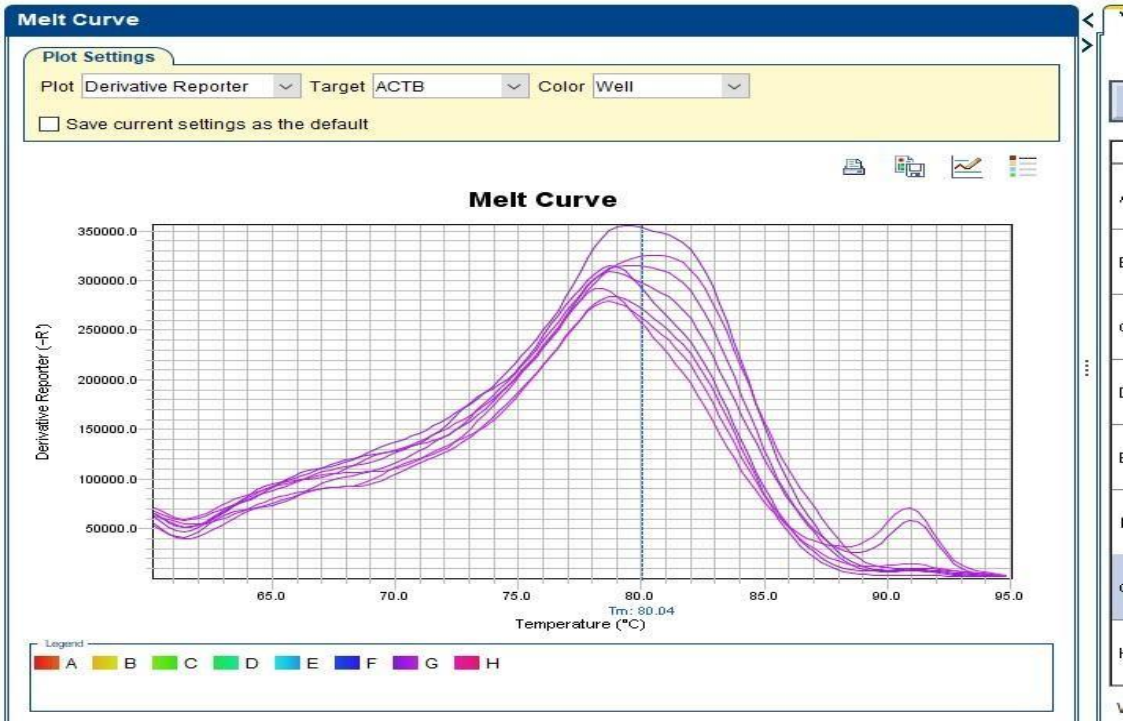


Figure 4.5: ACTB melt curve.

4.2. Statistical analysis of qRT-PCR findings of the WASL(nWASP) mRNA gene

qRT-PCR analysis was performed for mRNA level expression of WASL gene found in pterygium tissues and control conjunctiva tissues. The qRT-PCR results of WASL and ACTB genes were quantified with step one plus software v2.3. The data belonging to the WASL gene was normalized with the ACTB gene and the $2^{-\Delta\Delta Ct}$ formula was used to calculate the fold change. Statistical analysis was performed with the data obtained from this process. All data was given as mean ± SEM. Analysis of the results was done according to the range of 0.9 – 1.1. At values less than 0.9, the expression level of the relevant gene is decreased in pterygium tissue compared to normal conjunctival tissue. If it is in the range of 0.9–1.1, it does not change compared to normal conjunctival tissue, and at values higher than 1.1, there is an increase in the expression of the gene compared to normal conjunctival tissue. (Schmittgen and Livak., 2008). Statistical analysis of expression data was performed using SPSS 16.0 software. The T test was used to examine the data's significance levels. If $p \leq 0.05$ is significant, the statistical significance threshold was accepted as 0.05; if $p > 0.05$, the evaluation that there is no difference.

4.2.1. T-Test

Analysis results

TABLE4.1: Statistical analysis of $2^{-\Delta\Delta Ct}$ values of WAS gene and conjunctival tissues

Gene	Tissue	
	Pterygium tissue (N=27)	Normal Conjunctiva (N=27)
WASL gene (AVG±SEM)	1.320±0,254	
P	0,001	

According to the qRT-PCR analysis results When the expression level of the WASL gene was compared with pterygium tissues, it was found that it increased WASL gene expression in the pterygium tissue (1.320±0,254). Statistically significant in terms of WASL gene mRNA expression level (p=0,001). When the expression level of the WASL gene was compared with the pterygium and normal conjunctival tissues, it was determined that the pterygium tissue increased (1.320±0,254-Fold change) compared to the normal conjunctival tissue (Figure 4.6). In terms of mRNA expression level, the gene was found to have a statistically significant ($p \leq 0.05$). That means there is a significant difference was found between expression levels of WASP gene in pterygia disorder tissues and healthy conjunctival tissue ($p \leq 0.001$).

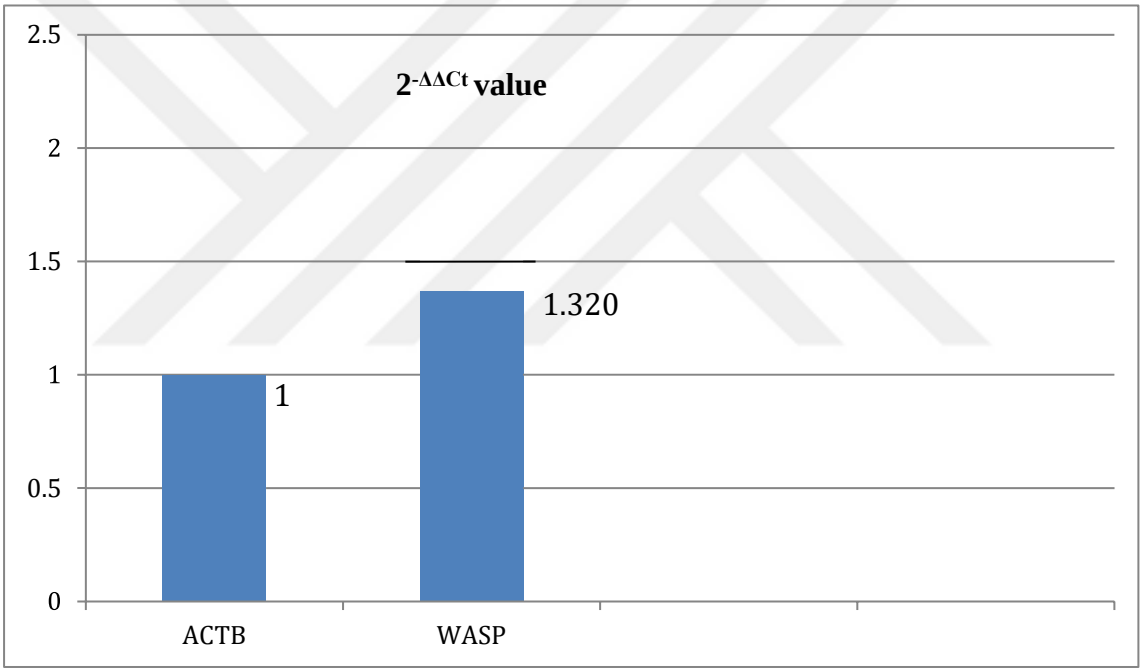


Figure: 4. 6. Average of $2^{-\Delta\Delta Ct}$ values of WASL and ACTB genes.

5. DISCUSSION

The conjunctiva expands like winged along of the limbus and toward the cornea in pterygium, an invading fibrovascular development. UV radiation from sun light is a significant environmental component in pterygium growth. Despite the fact that pterygium is considered a degenerative lesion with elastic degeneration. According to recent findings, because it has numerous tumor-like characteristics, such like invasion of healthy tissue, higher frequency recurrence percentages post excision, & subsequent premalignant lesions, pterygium is considered as a pre-malignant tissue (Bozkurt et al., 2020).

Pterygium pathogenesis has been linked to a mutation in the p53 gene in numerous studies. As a consequence, pterygium is more likely to be considered a tumor caused by uncontrolled cell proliferation than a growth condition like neoplasia. The reality that pterygium is a benign neoplastic lesion with limited local invasion and no metastasis, as well as the fact that its cells show tumor-like genetic features, lends credence to the notion that it is a benign neoplastic lesion. (Weinstein et al., 2002).

The generation of reactive oxygen species (ROS) is linked to the dynamic reorganization of the actin cytoskeleton, which is implicated in cancer cell movement and metastasis. As a result, reducing the production of reactive oxygen species (ROS) and actin polymerization in cancerous cells could be a promising anti-cancer treatment.

Intracellular ROS levels are greater in malignant cells than non-metastatic parent cell, and the intracellular ROS signal mediates melanoma cell prometastatic characteristics. Rac1 activation and WAVE2 expression are linked to ROS levels, cell migration, and melanoma cell invasiveness, implying that WAVE2 is play role in the migration of invasive melanoma cells via b (Park et al., 2012).

A large increase in BCL-2 expression with anti-apoptotic capabilities reveals that both cell proliferation and death involved in the pathophysiology of pterygium (Liang et al., 2011). By evaluating removed pterygium tissue and superior conjunctival tissue that was acquired by PCR, the expression of MiR-21 was determined. After pterygium surgery, human pterygium fibroblasts (HPFs) were collected and a primary culture was kept. A total of 58 patients with a unilateral pterygium issue were evaluated.

Relative to healthy conjunctival tissues, the pterygium tissue had a higher amount of miR-21. With the severity of the pterygium, the amount of miR-21 rises. By raising PTEN expression, the miR-21 inhibitor promoted death and inhibited proliferation in HPF cells, while the PTEN VO-Ohpic inhibitor trihydrate lowered p- AKT expression. (Li et al., 2018).

The human genome contains two WASP paralogues, WASP and WASL (also known as N-WASP). WASL has been discovered to be a tumor suppressor in T cell lymphoma. WASP and WASP Interacting Protein (WIP) regulate signal transmission by the T cell antigen receptor (TCR) in T cells, although their significance in lymphoma is unclear. WASP and WIP expression in anaplastic large cell lymphomas (ALCL) is often low or nonexistent, in contrast to other T-cell lymphomas. ALK's oncogenic activity influences WASP and WIP via STAT3 and C/EBP-pathway mediators. CDC42 connected to active GTP increased in the absence of WASP, and genetic deletion of the CDC42 allele was enough to affect (impair) lymphoma growth. WASL's primary known effector function is actin polymerization nucleation, which is mediated by the interaction of its C-terminal domain with the Arp2/3 complex and actin monomers (Galletta et al., 2008). Different WASL domains are necessary for WASL to interact with other proteins, which can impact WASL's stability, activation, or function. Actin as a major component of cytoskeleton gives cell support in a network like form and makes cytocol gel like material and it has important role like in driving cell locomotion, phagocytosis, and intracellular trafficking including motility of lipid vesicles, organelles and invasive pathogens. When actin is nucleated which leads to formation of new actin filament or formation of branched actin filament. Arp 2/3 complex with presence of NPFs and ATP by the help of its seven subunits which consists of two major component Arp2 and Arp3 which they resemble actin in structure 61 and have role in formation of a dimer that helps in formation of new branched actin filament. And five additional subunits ARPC1-ARPC5. Like the WAVE complex, N-WASP plays an important role at the plasma membrane. In untransformed cells, however, N-WASP appears to play only a minimal role in membrane appendages. N-WASP, on the other hand, is required for the formation of appendages in transformed cells. I

Some microarray research showed that WASL is involved in a variety of processes in UM cells, including the regulation of endocytosis, the development of actin cytoskeletal structure, and cell junction formation (Peng et al., 2019). Phagocytosis and inflammatory activities Dysregulation contribute to the onset of autoinflammatory disorders. The actin cytoskeleton is essential for the regulation of inflammatory reactions. A deficiency of WASL, which regulates the actin cytoskeleton, has been related to autophagy and inflammation, according to new studies. Bacterial clearance difficulties, greater activation inflammation, and host tumor growth are all hallmarks of WASL deficiency due to unequal septin cage-like cell formation and the generation of defective canonical autophagosomes (Lee et al., 2017). WASL is a critical regulator of actin polymerization in hematopoietic cells, with 5 well-defined domains involving signal transmission, cell motility, and immunological synapses development. Alterations in the WASL gene can result in a variety of symptoms, the most of which are, if not fully, reliant on the mutations. WASL deficiency affects all aspects of cell proliferation, phagocytosis, immune synapses, adhesion, and direct migration, resulting in cytoskeletal abnormalities. Many studies involving molecular pathogenesis were blossoming in order to develop new nonsurgical treatments for pterygium, and from these studies, noncoding RNAs were a substantial group, proposed to be potential molecular targets for treatment or therapeutic monitoring biomarkers (Engelsvold et al., 2013; Wu et al., 2014; Liu et al., 2016). For example, miR-216b inhibits apoptosis in pterygium fibroblasts, which contradicts the curative effect of hydroxycamptothecin, raising the possibility that a miR-216b inhibitor could be an effective therapeutic drug (Xu et al., 2014). Although there were numerous potential applications for miRNA-based treatment, obstacles such as inadequate cellular absorptivity, action position, or target deviation, and long-term safety in the body remained, which hampered the importance of mechanisms research (Garzon et al., 2010). WASL has also been demonstrated to play a role in the creation of lymphoid and monocytic myeloid cells, as well as expanded diagnostic options and therapeutic options, which range from symptomatic conventional therapy to curable hematopoietic stem cell transplantation and gene therapy. WASL facilitates nuclear translocation of the central factor kappaB and has been linked to the development

and function of monocytic myeloid cells, as well as diagnostic potential and extended treatment options (Ochs et al., 2006; Blundell et al., 2017).

Cancer cells spread by invading local tissue and then traveling to various organs via through bloodstream or lymphatic system (metastasis). A multi-step process that causes to metastatic progression is known as a metastatic cascade. Cancer cells, particularly those of epithelial origin, diminish cadherin-based cell adhesion and it can destroy the underlying basement membrane at first, despite the fact that some cellular activities are altered throughout these processes. The cells then become movable in the extracellular stromal matrix (ECM) and show increased motility with high proteolytic activity, culminating in a pathological state called local invasion.

Understanding the mechanisms underpinning invasive progression, the first stage in metastasis, is crucial to understanding cancer-related death because cancer mortality is predominantly caused by progression to invasive and metastatic states, the actin cytoskeleton plays a vital role when tumor cells become invasive: its major function is to sustain intercellular mechanical adhesion (cell–cell adhesion) and to govern the morphology of the cells. An epithelial-mesenchymal transition (EMT) occurs in some benign cancers.

Actin networks at intercellular junctions are actively modified to lessen cell-cell adhesion, while actin polymerization at the tip of migration cells' edges pushes the plasma membrane upward, WASL and WAVE cause fast polymerization of actin under the biological membrane, which is essential for a range of pathological and physiological activities such as immunological response, synaptogenesis and pathogens, tissue morphogenesis, and cancer invasion and metastasis, to name a few. According to an increasing amount of studies, WASL and WASP appear to be important in the progression of cancer (Kurisu et al., 2010). According to the results of the real-time PCR analysis, there was even a significant correlation between the gene and the disease in the expression analysis (p 0.001). When the pterygial and conjunctival tissues were compared, it was found that the gene expression level of the pterygium compared to the conjunctiva (1.320±0,245-Fold change, p=0,001) significantly increased ($p \leq 0.001$).

The findings imply that the WASL gene plays a role in pterygium disease, but additional investigation is needed to determine its efficacy and further understand the WASL gene's association with pterygium. More research on the WASL gene should be done in the future, using different approaches with more patients to achieve various results and better understand the WASL gene's role in pterygium.

6. REFERENCES

ALEKHINA, O., BURSTEIN, E., & BILLADEAU, D.D. (2017) Cellular functions of WASP family proteins at a glance. J Cell Sci 130:2235–2241.

ALTAY, Y., & BALTA, Ö. (2016). Intraoperative application of 5-fluorouracil and mitomycin C aschemoadjuvants in primary pterygium surgery. *Turkish Journal of Medical Sciences*, *46*(2), 321–327.

ANDO, R., KASE, S., OHASHI, T., DONG, Z., FUKUHARA, J., KANDA, A., MURATA, M., NODA, K., KITAICHI, N., & ISHIDA, S. (2011). Tissue factor expression in human pterygium. *Molecular vision*, *17*, 63–69.

ASLANKURT, M. & ASTAM, N. (2003). Pterjium etiyolojisi. *Tıp Araştırmaları Derneği*, *1*(2), 39–42.

BACON C., LAKICS V., MACHESKY L., & RUMSBY M. (2007) N-WASP regulates extension of filopodia and processes by oligodendrocyte progenitors, oligodendrocytes, and Schwann cells: implications for axon ensheathment at myelination. Glia 55:844–858.

BLANCHOIN, L., MAY, R. C. et al. (1999). Scar, a WASp-related protein, activates nucleation of actin and genotype-phenotype correlation. *Pediatric*

blood & cancer, 62(9), 1601–1608.

BRADLEY, J. C., YANG, W., BRADLEY, R. H., REID, T. W., & SCHWAB, I. R. (2010). The science of pterygia. *The British journal of ophthalmology*, *94*(7), 815–820.

CÁRDENAS-CANTÚ, E., ZAVALA, J., VALENZUELA, J., & VALDEZ-GARCÍA, J. E. (2016). Molecular Basis of Pterygium Development. *Seminars in ophthalmology*, *31*(6), 567–583.

CHEN, K. H., & HSU, W. M. (2006). Intraoperative ethanol treatment as an adjuvant therapy of pterygium excision. *International journal of biomedical science : IJBS*, *2*(4), 414–421.

CHEN, P.L., CHENG, Y.W., CHIANG, C.C., TSENG, S.H., CHAU, P.S., TSAI, Y.Y. (2006). Hypermethylation of the p16 gene promoter in pterygia and its association with the expression of DNA methyltransferase 3b. *Mol Vis.* 2006 Nov 17;12:1411-6.

CHIEN, K. H., CHEN, S. J., LIU, J. H., WOUNG, L. C., CHEN, J. T., LIANG, C. M., CHIOU, S. H., TSAI, C. Y., CHENG, C. K., HU, C. C., & PENG, C. H. (2013). Correlation of MicroRNA-145 levels and clinical severity of pterygia. *Ocular Surface*, *11*(2), 133-138.

CHUI, J., CORONEO, M. T., TAT, L. T., CROUCH, R., WAKEFIELD, D., & DI GIROLAMO, N. (2011). Ophthalmic pterygium: a stem cell disorder with premalignant features. *The American journal of pathology*, *178*(2), 817–827.

CHUI, J., DI GIROLAMO, N., WAKEFIELD, D., & CORONEO, M. T. (2008). The pathogenesis of pterygium: current concepts and their therapeutic implications. *The ocular surface*, *6*(1), 24–43.

CIMPEAN, A. M., SAVA, M. P., & RAICA, M. (2013). DNA damage in human pterygium: one-shot multiple targets. *Molecular vision*, *19*, 348–356.

CLEARFIELD, E., MUTHAPPAN, V., WANG, X., & KUO, I. C. (2016). Conjunctival autograft for pterygium. *The Cochrane database of systematic reviews*, *2*, CD011349.

CORONEO, M. T. (1993). Pterygium as an early indicator of ultraviolet insolation: a hypothesis. *The British journal of ophthalmology*, *77*(11), 734–739.

DETORAKIS, E. T., & SPANDIDOS, D. A. (2009). Pathogenetic mechanisms and treatment options for ophthalmic pterygium: trends and perspectives (Review). *International journal of molecular medicine*, *23*(4), 439–447.

DETORAKIS, E.T., SOURVINOS, G., TSAMPARLAKIS, J., SPANDIDOS, D.A. (1998). Evaluation of loss of heterozygosity and microsatellite instability in human pterygium: clinical correlations. Br J Ophthalmol; 82:1324–1328.

DI GIROLAMO, N., CHUI, J., CORONEO, M.T., WAKEFIELD, D. (2004). Pathogenesis of pterygia: role of cytokines, growth factors, and matrix metalloproteinases. *Prog Retin Eye Res.* Mar;23(2):195-228.

DI GIROLAMO, N., WAKEFIELD, D., & CORONEO, M. T. (2006). UVB-mediated induction of cytokines and growth factors in pterygium epithelial cells involves cell surface receptors and intracellular signaling. *Investigative ophthalmology & visual science*, *47*(6), 2430–2437.

DUSHKU, N., JOHN, M. K., SCHULTZ, G. S., & REID, T. W. (2001). Pterygia pathogenesis: corneal invasion by matrix metalloproteinase expressing altered limbal epithelial basal cells. *archives of ophthalmology (chicago, Ill. : 1960)*, *119*(5), 695–706.

DŽUNIĆ, B., JOVANOVIĆ, P., VESELINOVIĆ, D., PETROVIĆ, A., STEFANOVIĆ, I., & KOVAČEVIĆ, I. (2010). Analysis of pathohistological characteristics of pterygium. Bosnian Journal of Basic Medical Sciences, 10(4), 307–303.

ENGELSVOLD, D. H., UTHEIM, T. P., OLSTAD, O. K., GONZALEZ, P., EIDET, J. R., LYBERG, T., TRØSEID, A. M., DARTT, D. A., & RAEDER, S. (2013). miRNA and mRNA expression profiling identifies members of the miR-200 family as potential regulators of epithelial-mesenchymal transition in pterygium. *Experimental eye research*, *115*, 189–198.

EZE, B. I., MADUKA-OKAFOR, F. C., OKOYE, O. I., & CHUKA-OKOSA, C. M. (2011). Pterygium: A review of clinical features and surgical treatment. *Nigerian journal of medicine : journal of the National Association of Resident Doctors of Nigeria*, *20*(1), 7–14.

FANG, C. (2013). Elucidating the regulatory mechanisms of pterygium. Scientific Thesis; School of Biological Sciences, University of East Anglia, Norwich, UK.

FENG, Q. Y., HU, Z. X., SONG, X. L., & PAN, H. W. (2017). Aberrant expression of genes and proteins in pterygium and their implications in the pathogenesis. *International journal of ophthalmology*, *10*(6), 973–981.

FREZZA, C., & MARTINS, C. P. (2012). From tumor prevention to therapy: empowering p53 to fight back. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy*, *15*(5-6), 258–267.

GARZON, R., MARCUCCI, G., & CROCE, C. M. (2010). Targeting microRNAs in cancer: rationale, strategies and challenges. *Nature reviews. Drug discovery*, *9*(10), 775–789.

GINGER-EKE, H. A., OGBONNAYA, C. E. & EZISI, C. N. (2018). Pterygium: recent trends and perspectives –A review of pathogenesis and current management options. Journal of ophthalmology; 26(2): 89-98.

GOODSELL, D. S. (2001). The molecular perspective: Ultraviolet light and pyrimidine dimers. The Oncologist, 6(3), 298–299.

GREENBLATT, M. S., BENNETT, W. P., HOLLSTEIN, M., & HARRIS, C. C. (1994). Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer research*, *54*(18), 4855–4878.

GUM, S. I., KIM, Y. H., JUNG, J. C., KIM, I. G., LEE, J. S., LEE, K. W., & PARK, Y. J. (2017). Cyclosporine A inhibits TGF-β2-induced myofibroblasts of primary cultured human pterygium fibroblasts. *Biochemical and biophysical research communications*, *482*(4), 1148–1153.

HAN, S., CHEN, Y., GAO, Y., SUN, B., & KONG, Y. (2019). MicroRNA-218-5p inhibit the migration and proliferation of pterygium epithelial cells by targeting EGFR via PI3K/Akt/mTOR signaling pathway. *Experimental eye research*, *178*, 37–45.

HAY, E. D. (2005). The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. *Developmental dynamics :*

an official publication of the American Association of Anatomists, *233*(3), 706–720.

HAYES, C. N., & CHAYAMA, K. (2016). MicroRNAs as Biomarkers for Liver Disease and Hepatocellular Carcinoma. *International journal of molecular sciences*, *17*(3), 280.

HE, S., SUN, H., HUANG, Y., DONG, S., QIAO, C., ZHANG, S., WANG, C., ZHENG, F., YAN, M., & YANG, G. (2019). Identification and Interaction Analysis of Significant Genes and MicroRNAs in Pterygium. *BioMed research international*, *2019*, 2767512.

HILGERS J. H. (1960). Pterygium: its incidence, heredity and etiology. *American journal of ophthalmology*, *50*, 635–644.

HILL, J., & MASKE, R. (1989). Pathogenesis of Pterygium. *Eye* 3(Pt 2)(2), 218-226.

HIRST L. W. (2003). The treatment of pterygium. *Survey of ophthalmology*, *48*(2), 145–180.

HIRST, L. W., AXELSEN, R. A., & SCHWAB, I. (2009). Pterygium and associated ocular surface squamous neoplasia. *Archives of ophthalmology (Chicago, Ill. : 1960)*, *127*(1), 31–32.

HOSSAIN, P. (2011). Pterygium Surgery. Royal College of Ophthalmologists.

HOU, J., YANG, H., HUANG, X., LENG, X., ZHOU, F., XIE, C., ET AL. (2017). N-WASP promotes invasion and migration of cervical cancer cells through regulating p38

HUDSON, L. G., CHOI, C., NEWKIRK, K. M., PARKHANI, J., COOPER, K. L., LU, P., & KUSEWITT, D. F. (2007). Ultraviolet radiation stimulates expression of Snail family transcription factors in keratinocytes. *Molecular carcinogenesis*, *46*(4), 257–268.

IGARASHI, N., HONJO, M., FUJISHIRO, T., TOYONO, T., ONO, T., MORI, Y., MIYATA, K., OBINATA, H., & AIHARA, M. (2019). Activation of the Sphingosine 1 Phosphate-Rho Pathway in Pterygium and in Ultraviolet-Irradiated Normal Conjunctiva. *International journal of molecular sciences*, *20*(19), 4670.

IWANO, M., PLIETH, D., DANOFF, T. M., XUE, C., OKADA, H., & NEILSON, E. G. (2002). Evidence that fibroblasts derive from epithelium during tissue fibrosis. *The Journal of clinical investigation*, *110*(3), 341–350.

JİANG, H., LEUNG, C., TAHAN, S., & WANG, D. (2019). Entry by multiple picornaviruses is dependent on a pathway that includes TNK2, WASL, and NCK1. *eLife*, *8*, e50276.

JIN, F., DONG, B., GEORGIOU, J., JIANG, Q., ZHANG, J., BHARIOKE, A., QIU, F., LOMMEL, S., FELTRI, M.L., WRABETZ, L., RODER, J.C., EYER, J., CHEN, X., PETERSON, A.C., & SIMINOVITCH, K.A. (2011) N-WASp is required for Schwann cell cytoskeletal dynamics, normal myelin gene expression and peripheral nerve myelination. Development 138:1329–1337.

KASE, S., OSAKI, M., SATO, I., TAKAHASHI, S., NAKANISHI, K., YOSHIDA, K., ITO, H., & OHNO, S. (2007). Immunolocalisation of E-cadherin and beta-catenin in human pterygium. *The British journal of ophthalmology*, *91*(9), 1209–1212.

KATO, N., SHIMMURA, S., KAWAKITA, T., MIYASHITA, H., OGAWA, Y., YOSHIDA, S., HIGA, K., OKANO, H., & TSUBOTA, K. (2007). Beta-catenin activation and epithelial-mesenchymal transition in the pathogenesis of pterygium. *Investigative ophthalmology & visual science*, *48*(4), 1511–1517.

KIM, A. S., KAKALIS, L. T., ABDUL-MANAN, N., LIU, G. A., & ROSEN, M. K. (2000). Autoinhibition and activation mechanisms of the Wiskott-Aldrich syndrome protein. *Nature*, 404(6774), 151–158.

KIM, K. W., PARK, S. H., & KIM, J. C. (2016). Fibroblast biology in pterygia. *Experimental eye research*, *142*, 32–39.

KIM, S. W., LEE, J., LEE, B., & RHIM, T. (2014). Proteomic analysis in pterygium; upregulated protein expression of ALDH3A1, PDIA3, and PRDX2. *Molecular vision*, *20*, 1192–1202.

KIM, S. W., PARK, S., IM, C. Y., SEO, K. Y., & KIM, E. K. (2014). Prediction of mean corneal power change after pterygium excision. *Cornea*, *33*(2), 148–153.

KIM, Y.-H., JUNG, J.-C., GUM, S. I., PARK, S.-B., MA, J. Y., KIM, Y. I., LEE, K. W., & PARK, Y. J. (2017). Inhibition of Pterygium Fibroblast Migration and Outgrowth by Bevacizumab and Cyclosporine A Involves Down-Regulation of Matrix Metalloproteinases-3 and -13. *PLoS ONE*, *12*(1).

KONUK, O., AKTAŞ, Z., ERDEM, Ö., YÜKSEL KONUK, E.B. & ÜNAL, M. (2011). Expression of Cyclooxygenase-2 and Ki-67 in Primary and Recurrent Pterygias. Turkiye Klinikleri J Med Sci.; 31(1):115-21.

KONUK, O., AKTAŞ, Z., ERDEM, Ö., YÜKSEL KONUK, E.B. & ÜNAL, M. (2011). Expression of Cyclooxygenase-2 and Ki-67 in Primary and Recurrent Pterygias. Turkiye Klinikleri J Med Sci.; 31(1):115-21.

KURISU, S., & TAKENAWA, T. (2010). WASP and WAVE family proteins: friends or foes in cancer invasion?. *Cancer science*, 101(10), 2093–2104.

LEE, J. H., JUNG, S. A., KWON, Y. A., CHUNG, J. L., & KIM, U. S. (2016). Expression of microRNAs in fibroblast of pterygium. *International journal of ophthalmology*, *9*(7), 967–972.

LEWALLEN, S. (1989). A randomized trial of conjunctival autografting for pterygium in the tropics. *Ophthalmology*, *96*(11), 1612–1614.

LI, H., WANG, H., & REN, Z. (2018). MicroRNA-214-5p Inhibits the Invasion and Migration of Hepatocellular Carcinoma Cells by Targeting Wiskott-Aldrich Syndrome Like. Cell Physiol. Biochem. 46, 757–764.

LIU, J., DING, X., YUAN, L., & ZHANG, X. (2016). Identification of pterygium-related long non-coding RNAs and expression profiling by microarray analysis. *International journal of molecular medicine*, *38*(2), 529–536.

LIU, J., DING, X., YUAN, L., & ZHANG, X. (2017). Identification of pterygium-related mRNA expression profiling by microarray analysis. *Eye (London, England)*, *31*(12), 1733–1739.

LIU, K.J., QI, H.Z., YAO, H.L., LEI, S.L., LEI, Z.D., LI, T.G., & ZHAO, H. (2012). An updated meta-analysis of the p53 codon 72 polymorphism and gastric cancer risk. *Mol Biol Rep.* Aug;39(8):8265-75.

LIU, L., WU, J., GENG, J., YUAN, Z., & HUANG, D. (2013). Geographical prevalence and risk factors for pterygium: a systematic review and meta-analysis. *BMJ open*, *3*(11), e003787.

LIU, T., LIU, Y., XIE, L., HE, X., & BAI, J. (2013). Progress in the pathogenesis of pterygium. *Current Eye Research*; 38(12): 1191–1197.

LIVEZEANU, C., CRĂIŢOIU, M. M., MĂNESCU, R., MOCANU, C., & CRĂIŢOIU, S. (2011). Angiogenesis in the pathogenesis of pterygium. *Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie*, 52(3), 837–844.

MA, D. H., SEE, L. C., HWANG, Y. S., & WANG, S. F. (2005). Comparison of amniotic membrane graft alone or combined with intraoperative mitomycin C to prevent recurrence after excision of recurrent pterygia. *Cornea*, *24*(2), 141–150.

MA, K., XU, L., JIE, Y., & JONAS, J. (2007). Prevalence of and Factors Associated With Pterygium in Adult Chinese: The Beijing Eye Study. Cornea, 26(10), 1184–1186.

MACHESKY, L. M., MULLINS, R. D., HIGGS, H. N., KAISER, D. A.,

MACKENZIE, F. D., HIRST, L. W., BATTISTUTTA, D., & GREEN, A. (1992). Risk analysis in the development of pterygia. *Ophthalmology*, *99*(7), 1056–1061.

MAHDY, M. A. E. S., & BHATIA, J. (2009). Treatment of primary pterygium: Role of limbal stem cells and conjunctival autograft transplantation. Oman Journal of Ophthalmology, 2(1), 23–26.

MAHESHWARI, S. (2007). Pterygium-induced corneal refractive changes. Indian Journal of Ophthalmology, 55(5), 383–386.

MAURO, J., & FOSTER, C. S. (2009). Pterygia: pathogenesis and the role of subconjunctival bevacizumab in treatment. *Seminars in ophthalmology*, *24*(3), 130–134.

MIKI, H., & TAKENAWA, T. (2003). Regulation of actin dynamics by WASP family proteins. *Journal of biochemistry*, 134(3), 309–313.

MIKI, H., SASAKI, T., TAKAI, Y., & TAKENAWA, T. (1998). Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. *Nature*, *391*(6662), 93–96.

NAKAGAMI, T., MURAKAMI, A., OKISAKA, S., & EBIHARA, N. (1999). Mast cells in pterygium: number and phenotype. *Japanese journal of ophthalmology*, *43*(2), 75–79.

NASSAR, M.K., EL-SEBAEY, A., ABDUL-RAHMAN, M.H., EL-GHONEMY, K., & SHEBI A., (2014). Clinical, pathological, and molecular aspects of recurrent versus primary pterygium. Menoufia Med J, 27:386-94.

NEMET, A. Y., VINKER, S., SEGAL, O., MIMOUNI, M., & KAISERMAN, I. (2016). Epidemiology and Associated Morbidity of Pterygium: A Large, CommunityBased Case-Control Study. Seminars in Ophthalmology, 31(5), 446–451.

NOTARA, M., LENTZSCH, A., CORONEO, M., & CURSIEFEN, C. (2018). The Role of Limbal Epithelial Stem Cells in Regulating Corneal (Lymph)angiogenic Privilege and the Micromilieu of the Limbal Niche following UV Exposure. *Stem cells international*, *2018*, 8620172.

NOVAK, N., BAR, V., SABANAY, H., FRECHTER, S., JAEGLE, M., SNAPPER, S. B., MEIJER, D., & PELES, E. (2011) N-WASP is required for membrane wrapping and myelination by Schwann cells. J Cell Biol 192:243–250.

NUWORMEGBE, S. A., SOHN, J. H., & KIM, S. W. (2017). A PPAR-Gamma Agonist Rosiglitazone Suppresses Fibrotic Response in Human Pterygium Fibroblasts by Modulating the p38 MAPK Pathway. *Investigative ophthalmology & visual science*, *58*(12), 5217–5226.

PENG, D., DONG, J., ZHAO, Y., PENG, X., TANG, J., CHEN, X., WANG, L., HU, D. N., REİNACH, P. S., QU, J., & YAN, D. (2019). miR-142-3p suppresses uveal melanoma by targeting CDC25C, TGFβR1, GNAQ, WASL, and RAC1. *Cancer management and research*, *11*, 4729–4742.

PÉREZ-RICO, C., PASCUAL, G., SOTOMAYOR, S., ASÚNSOLO, Á., CIFUENTES, A., GARCÍA-HONDUVILLA, N., & BUJÁN, J. (2014). Elastin development-associated extracellular matrix constituents of subepithelial connective tissue in human pterygium. *Investigative ophthalmology & visual science*, *55*(10), 6309–6318.

PERRA, M. T., MAXIA, C., CORBU, A., MINERBA, L., DEMURTAS, P., COLOMBARI, R., MURTAS, D., BRAVO, S., PIRAS, F., & SIRIGU, P. (2006). Oxidative stress in pterygium: relationship between p53 and 8-hydroxydeoxyguanosine. *Molecular vision*, *12*, 1136–1142.

PERRA, M. T., MAXIA, C., ZUCCA, I., PIRAS, F., & SIRIGU, P. (2002). Immunohistochemical study of human pterygium. *Histology and histopathology*, *17*(1), 139–149.

PINKERTON, O. D., HOKAMA, Y., & SHIGEMURA, L. A. (1984). Immunologic basis for the pathogenesis of pterygium. *American journal of ophthalmology*, *98*(2), 225–228.

REID, T. W., & DUSHKU, N. (2010). What a study of pterygia teaches us about the cornea? Molecular mechanisms of formation. *Eye & contact lens*, *36*(5), 290–295.

RIAU, A. K., WONG, T. T., LAN, W., FINGER, S. N., CHAURASIA, S. S., HOU, A. H., CHEN, S., YU, S. J., & TONG, L. (2011). Aberrant DNA methylation of matrix remodeling and cell adhesion related genes in pterygium. *PloS one*, *6*(2), e14687.

RIM, T. H., KANG, M. J., CHOI, M., SEO, K. Y., & KIM, S. S. (2017). The incidence and prevalence of pterygium in South Korea: A 10-year population-based Korean cohort study. *PloS one*, *12*(3), e0171954.

SALMAN, A. G., & MANSOUR, D. E. (2011). The recurrence of pterygium after different modalities of surgical treatment. *Saudi journal of ophthalmology : official journal of the Saudi Ophthalmological Society*, *25*(4), 411–415.

SCHMITTGEN, T. D., & LIVAK, K. J. (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nature protocols*, *3*(6), 1101–1108.

SCHWICKERT, A., WEGHAKE, E., BRÜGGEMANN, K., ENGBERS, A., BRINKMANN, B. F., KEMPER, B., SEGGEWIß, J., STOCK, C., EBNET,

K., KIESEL, L., RIETHMÜLLER, C., & GÖTTE, M. (2015). microRNA miR-142-3p Inhibits Breast Cancer Cell Invasiveness by Synchronous Targeting of WASL, Integrin Alpha V, and Additional Cytoskeletal Elements. *PloS one*, *10*(12), e0143993.

SEET, L. F., TONG, L., SU, R., & WONG, T. T. (2012). Involvement of SPARC and MMP-3 in the pathogenesis of human pterygium. *Investigative ophthalmology & visual science*, *53*(2), 587–595.

SERRA, H.M., SUAREZ, M.F., MACCIO, J.P., ESPOSITO, E. & URRETS-ZAVALIA, J.A., (2019). Pterygium: A Complex and Multifactorial Ocular Surface Disease. A Review on its Pathogenic Aspects. In *Rare Diseases*.

SHIMMURA, S., ISHIOKA, M., HANADA, K., SHIMAZAKI, J., & TSUBOTA, K. (2000). Telomerase activity and p53 expression in pterygia. *Invest Ophthalmol Vis Sci.* May;41(6):1364-9.

SHRESTHA, S., & SHRESTHA, S. M. (2014). Comparative study of prevalence of pterygium at high altitude and Kathmandu Valley. *Journal of Nepal Health Research Council*, *12*(28), 187–190.

SINGH, S. K. (2017). Pterygium: Epidemiology prevention and treatment. Community Eye Health, 30(99), S5–S6.

SOLOMON A. S., (2016). Pterygium. *British Journal of Ophthalmology* ;90:665-666.

SPANDIDOS, D.A., SOURVINOS, G., KIARIS, H., & TSAMPARLAKIS, J. (1997). Microsatellite instability and loss of heterozygosity in human pterygia. British Journal of Ophthalmology; 81:493–496.

STERN, G. A., & LIN, A. (1998). Effect of pterygium excision on induced corneal topographic abnormalities. Cornea, 17(1), 23–27.

SUN, Y., ZHANG, B., JIA, X., LING, S., & DENG, J. (2018). Efficacy and Safety of Bevacizumab in the Treatment of Pterygium: An Updated Meta-Analysis of Randomized Controlled Trials. *Journal of ophthalmology*, *2018*, 4598173.

TAKENAWA, T., MIKI, H. (2001) WASP and WAVE family proteins: key molecules for rapid rearrangement of cortical actin filaments and cell movement. J Cell Sci 114:1801–1809.

TAN, D. T., CHEE, S. P., DEAR, K. B., & LIM, A. S. (1997). Effect of pterygium morphology on pterygium recurrence in a controlled trial comparing conjunctival autografting with bare sclera excision. *Archives of ophthalmology (Chicago, Ill. : 1960)*, *115*(10), 1235–1240.

TI, S. E., CHEE, S. P., DEAR, K. B., & TAN, D. T. (2000). Analysis of variation in success rates in conjunctival autografting for primary and recurrent pterygium. *The British journal of ophthalmology*, *84*(4), 385–389.

TOMIDOKORO, A., MIYATA, K., SAKAGUCHI, Y., SAMEJIMA, T., TOKUNAGA, T., & OSHIKA, T. (2000). Effects of pterygium on corneal spherical power and astigmatism. Ophthalmology, 107(8), 1568–1571.

TURAN, M., & TURAN, G. (2019). Overexpression of fractalkine and its histopathological characteristics in primary pterygium. *Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*, *257*(12), 2743–2750.

UENISHI, E., SHIBASAKI, T., TAKAHASHI, H., SEKI, C., HAMAGUCHI, H., YASUDA, T., TATEBE, M., OISO, Y., TAKENAWA, T., & SEINO, S. (2013). Actin dynamics regulated by the balance of neuronal Wiskott-Aldrich syndrome protein (N-WASP) and cofilin activities determines the biphasic response of glucose-induced insulin secretion. *The Journal of biological chemistry*, *288*(36), 25851–25864.

VARTANIAN, T.K. (2006) WAVE1 is required for oligodendrocyte morphogenesis and normal CNS myelination. J Neurosci 26:5849–5859.

VISO, E., GUDE, F., & RODRÍGUEZ-ARES, M. T. (2011). Prevalence of pinguecula and pterygium in a general population in Spain. *Eye (London, England)*, *25*(3), 350–357.

WANZELER, A., BARBOSA, I., DUARTE, B., BARBOSA, E. B., BORGES, D. A., & ALVES, M. (2019). Impact of pterygium on the ocular surface and meibomian glands. *PloS one*, *14*(9), e0213956.

WILDER, R. B., BUATTI, J. M., KITTELSON, J. M., SHIMM, D. S., HARARI, P. M., ROGOFF, E. E., & CASSADY, J. R. (1992). Pterygium treated with excision and postoperative beta irradiation. *International Journal of Radiation Oncology, Biology, Physics*, *23*(3), 533–537.

WONG, T. Y., FOSTER, P. J., JOHNSON, G. J., SEAH, S. K., & TAN, D. T. (2001). The prevalence and risk factors for pterygium in an adult Chinese population in Singapore: the Tanjong Pagar survey. *American journal of ophthalmology*, *131*(2), 176–183.

WU, C. W., CHENG, Y. W., HSU, N. Y., YEH, K. T., TSAI, Y. Y., CHIANG, C. C., WANG, W. R., & TUNG, J. N. (2014). MiRNA-221 negatively regulated downstream p27Kip1 gene expression involvement in pterygium pathogenesis. *Molecular vision*, *20*, 1048–1056.

WU, X., YOO, Y., OKUHAMA, N. N., TUCKER, P. W., LIU, G., & GUAN, J. L. (2006). Regulation of RNA-polymerase-II-dependent transcription by N-WASP and its nuclear-binding partners. *Nature cell biology*, *8*(7), 756–763.

XU, X., FU, Y., TONG, J., FAN, S., XU, K., SUN, H., LIANG, Y., YAN, C., YUAN, Z., & GE, Y. (2014). MicroRNA-216b/Beclin 1 axis regulates autophagy and apoptosis in human Tenon's capsule fibroblasts upon hydroxycamptothecin exposure. *Experimental eye research*, *123*, 43–55.

XU, Y. X., ZHANG, L. Y., ZOU, D. L., LIU, Z. S., SHANG, X. M., WU, H. P., ZHOU, Y., HE, H., & LIU, Z. G. (2014). Differential expression and function of survivin during the progress of pterygium. *Investigative ophthalmology & visual science*, *55*(12), 8480–8487.

YEUNG, S. N., KIM, P., LICHTINGER, A., AMIRAN, M. D., COTE, E., TEITEL, S., & SLOMOVIC, A. R. (2011). Incidence of ocular surface squamous neoplasia in pterygium specimens: an 8-year survey. *The British journal of ophthalmology*, *95*(4), 592.

ZAVADIL, J., & BÖTTINGER, E. P. (2005). TGF-beta and epithelial-to-mesenchymal transitions. *Oncogene*, *24*(37), 5764–5774.

ZHANG, J., ZHANG, X., GUO, Y., XU, L., & PEI, D. (2009). Sorting nexin 33 induces mammalian cell micronucleated phenotype and actin polymerization by interacting with Wiskott-Aldrich syndrome protein. *The Journal of biological chemistry*, *284*(32), 21659–21669.

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