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TOKAT GAZİOSMANPAŞA UNIVERSITY

HEALTH SCIENCES INSTITUTE

INVESTIGATING THE ROLE OF WASF3 IN THE DEVELOPMENT OF PTERYGIUM

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With this document, I declare that all the information in this thesis has been collected and presented in accordance with academic rules and ethical principles, and as a requirement of these rules and principles, I have cited all data, thoughts and results that do not belong to me.

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The Student Who Prepared the Thesis:

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Signature

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ÖZET

Pterjium Gelişiminde WASF3'ün Rolünün Araştırılması

Aso Ibrahim Khaleel

Yüksek Lisans, Tibbi Bioloji Ana Bilim Dalı

Tez Danışmanı: Üye:Prof.Dr.Hacı Ömer ATEŞ

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Pterjium displastik Üçgen şekilli ve etli fibro vasküler bir yapıdır. Genel olarak pterjium rengi gözlerde inci beyazından pembeye kadar görünür. Pterjium, dejeneratif bulbar konjonktival dokunun alt epitelyal büyümesi olarak limbus boyunca korneaya doğru büyür. Pterjium patolojisinin risk faktörleri olarak bilinen çeşitli nedenlerle ilişkili olduğu daha önce yapılan çalışmalarda belirtilmişti. Bu faktörler çevresel, genetik, immünolojik, coğrafi, viral ve görünüşe ait enflamasyon faktörlerinden oluşmaktadır. WASF3, hücre morfolojisi, aktin polimerizasyonu, hücre iskeletinin yeniden şekillenmesi, hücre hareketliliği ve istilasının düzenlenmesinde önemli roller oynayan Wiskot-Aldrich sendromu proteinleri WASP/WAVE aktin bağlayıcı protein ailesine aittir. hücre iskeletinin yeniden şekillenmesinin yanı sıra göç ve metastaz. WASF3, çeşitli kökenlerden gelen kanserlerde de tümör ilerlemesinin ve metastazın kilit bir sürücüsü olarak sınıflandırıldı. Bu çalışmanın amacı, WASF3'ün pterjium hastalığının davranışları üzerindeki etkisini araştırmak ve klinik etkisini değerlendirmektir. Bu çerçevede, tanıdık bir aktin bağlayıcı protein olan ve tümör baskılayıcı gene benzer olan WASF3'ün, tümör benzeri davranış sergileyen pterjiyum ile ilişkisini değerlendirdik. Çalışmamızda 27 hastaya ait pterjium dokusunda WASF3 geninin ekspresyon düzeyi qRT PCR yöntemi ile belirlendi. Elde edilen verilere göre pterjiyumlu dokularda WASF3 geninin ekspresyon seviyeleri kontrol grubuna göre istatistiksel olarak anlamlı bulundu ($p<0,005$). WASF3 geninin ekspresyonundaki azalmanın pterjiyum oluşumu ve gelişimi ile ilişkili olabileceği düşünülmektedir.

Anahtar Kelimeler: Pterjiyum, Real-Time PCR, WAVE 3

Abstract

Investigation The Role Of WASF3 In The Development Of Pterygium

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Masters Thesis, Department of Medical Biology

Thesis Supervisor : Üye:Prof.Dr.Hacı Ömer ATEŞ

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Pterygium is a dysplastic Triangular-shaped and fleshy fibro vascular structure. In general pterygium color appears as pearl white to pink in the eyes. Pterygium grows up across the limbus toward cornea as sub epithelial ingrowth of degenerative bulbar conjunctival tissue. Previously, it has been mentioned by the studies that pathology of pterygium correlated with several causes which are known as risk factors. These factors consist of environmental, genetic, immunological, geographical, viral and aspectic inflammation factors. WASF3 belongs to the Wiskot-Aldrich syndrome proteins WASP/WAVE family of actin-binding protein that play essential roles in regulating cell morphology, actin polymerization, cytoskeleton remodeling, cell motility and invasion.it regulates many aspects of cancer progression, cell invasion, cell migration and metastasis, as well as remodeling of cytoskeleton. WASF3 was classified as a key driver of tumor progression and metastasis in cancers from several origins as well. The aim of this study was to investigate the effect of WASF3 on the behaviors of pterygium disease and to evaluate its clinical impact. In this framework, we assessed the association of WASF3, a familiar actin-binding protein and analogous to tumor suppressor gene with pterygium which exhibits tumor-like behavior. In our study We analyzed the expression level of WASF3 gene in pterygium tissue belonging to 27 patients was determined by qRT PCR method. According to the obtained data, the expression levels of the WASF3 gene in tissues with pterygium were statistically significant compared to the control group ($p < 0.005$). It is thought that the decrease in the expression of the WASF3 gene may be related to the formation and development of pterygium.

Key Words: Pterygium, Real-Time PCR, WAVE 3

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Abbreviations		Definition
MAPK	:	Mitogen-Activated Protein Kinase
qRT-PCR	:	Quantitative Real Time Polymerase Chain Reaction
dNTP	:	Deoxynucleotide Triphosphate
DNA	:	Deoxyribonucleic Acid
RNA	:	Ribonucleic Acid
rtPCR	:	Real Time Polymerase Chain Reaction
WHD	:	WASF homology domain
WAS	:	Wiskott-Aldrich
Arp	:	Actin-Linked Protein
ACTB	:	Actin
MMC	:	Mitomycin C
mRNA	:	Messenger Ribonucleic Acid
TNF-A	:	Tumor Necrosis Factor Alfa
E-CAD	:	E-Kaderin
HPV	:	Human papilloma Virus
DNMT3b	:	DNA Methyltransferase 3b
EC	:	Endothelial Cells
LOH	:	loss Of Heterozygosity
PGE-2	:	Prostaglandin E-2
TOPK	:	T-Lymphokine Activated killer cell-Originated Protein Kinase
EPCs	:	Endothelial Progenitor Cells
SCF	:	Stem Cell Factor
VEGF	:	Vascular Endothelial Growth Factor
VEGFR	:	Vascular Endothelial Growth Factor Receptor
CD4+	:	Cluster of Differentiation 4
CD8+	:	Cluster of Differentiation 8
VCAM-1	:	Vascular Adhesion Molecule-1
ICAM-1	:	Intercellular Adhesion
HLA-DR	:	Human Antigen Leukocytic RR
MMPs	:	Matrix Metalloproteinases
EMT	:	Epithelial Mesenchymal Transition
LSCs	:	limbal Stem Cells
UVA	:	Ultraviolet A
UVB	:	Ultraviolet B
BC	:	Breast Cancer
ECM	:	Extracellular Matrix
UVR	:	Ultra Violate Radiation

AMD	:	Age-related Macular Degeneration
NF-kB	:	Nuclear Factor Kappa Beta
TNBC	:	Triple-Negative Breast Cancer
CDNA	:	Complementary Deoxyribonucleic Acid
NF-jB	:	Nuclear Factor-Kappa-B
CM	:	Cutaneous Melanoma

1.INTRODUCTION

Pterygium, it is come from a Greek word (pterygos) which gives the meaning of wing. Pterygium is a dysplastic Triangular-shaped and fleshy fibro vascular structure. In general pterygium color appears as pearl white to pink in the eyes. Pterygium grows up across the limbus toward cornea as sub epithelial ingrowth of degenerative bulbar conjunctival tissue(Jaros and Deluise., 1988). Pterygium consists of three main divisions: head, neck and body. The sclera, which is covered by the body pterygium, surface of limbus is involved by the neck and fore cornea is occupied by the head. The primary mark of pterygium is the cap which exists prior to head of pterygium(Anguria et al., 2014).

Previously, it has been mentioned by the studies that pathology of pterygium correlated with several causes which are known as risk factors. These factors consist of environmental, genetic, immunological, geographical, viral and aspectic inflammation factors. Environmental factors undertake wind, dust, trauma, dryness of eyes and smok. there are some factors else which have role in pterygium disease as well. For instance, sex (male>female), age (adult > young), race and sun exposure(Claudia et al., 2019).

Pterygium zone is an area which is located in equatorial regions, it is also most frequently discovered with a larger level of intensity in this region. The estimation of pterygium incidence rate within this area is recorded to be above 22% and < 2% apart from there. It is conveying the pterygium pathogenicity is likely linked to (UVR). Histopathologically pterygium characteristics assist the theory of (UVR) which are analogous to ruined skin by (UVR). According to epidemic proofs, UVR pathogenicity factor of pterygium stays settled. environmentally the pterygium sunlight UVR is defined as the main cause in pterygium growth(Jaros and Deluise., 1988).

Pterygium pathogenicity which marks by pterygium family incident. In spite of this understanding, pterygium etiological stays the same. Clinical symptoms of pterygium arise distinctly as the result of crossing genetic and environmental factors in several forms (Liu et al., 2013). The symptoms are generated in eyes by pterygium are various. Such as tearing, eye soreness, redness, itching, irritation, degradation of tissue, hypertrophy, strange body sensation and irregularities of cornea(Anguria et al., 2014).

In case of pupil is engulfed by a triangular molded development. It turns to a critical visually impaired issue. Pterygium may lead to sight problems (disrupting the tear film) or vision impairments as well. It’s expected to be aesthetically inadmissible. Pterygium is more common at the nasal limbus and happens bilaterally as well. Visual axis is rarely intersected by pterygium, but when it does, it causes visual impairment(Islam et al., 2001).

Any moderate treatment is mostly indicative and impermanent for the beginning phases of the illness. Surgical excision is the fundamental form of treating pterygium. To

afford warmth and relief from the feeling of strange body. Traditional therapy is preferred which consists of utilization of lubricant ointment or artificial tears for instance corticosteroids and vasoconstrictors (Isyaku., 2011).

Due to pterygium pathogenesis is unclear, after surgery, reoccurrence pterygium might be more violent than the essential development. In spite of difficulties associated with pterygium, anticipation of its incidence and reoccurrence following surgery has not succeeded(Anguria et al., 2014).There is no agreement on the precise pathophysiological mechanism not withstanding numerous progressed and traditional theories of pathogenesis which have been published.

The pterygium is made of fibro-vascular tissue with elastosis in the collagen fibers, according to microscopy findings. Except for the top, the pterygium is completely covered by conjunctival epithelium. At pterygium peak microscopic extensions of fibrous connective tissue which fills the cavities is visible and cornea is invaded by pterygium`s head which is caused by fragmentation and encroachment of Browman`s membrane.

Mastocytes, plasma cells and infiltrating lymphocytes (T-lymphocytes) are histopathological characteristics of pterygia constant inflaming. In combination with angiogenesis and fibrosis, the chronic inflammation is significantly contributed by the mast cells (mastocytes). Therefore, angiogenesis is thought to play a possible role in pterygium. There are some hallmarks else affirm the chronic inflammation existence of pterygium. They are existence of anormal elastic fibers, rising of degenerately altered collagen fibers, amount of fibroblast and recently made blood vessels(Petrovic et al., 2010).

Extracellular group of molecules like extra cellular matrix (ECM) are discharged by supportive cells which assist the cells around them structurally and biochemically(Claudia et al., 2019). Natural pointers of pathological and physiological status are collagen, fibrin and elastin. They are the main component parts of ECM as well, elastin provides resistance and regeneration, leading to various actions in the cell, such as cell proliferation vs cell migration(Perez-Rico et al., 2014).

Since it’s extremely exposed vascular and ECM growth as characters of fibro vascular tissue, it is considered that pterygium proliferative development connected instantly with infiltration of inflammatory cells, ECM abnormal manifestation, elastic fibrils and collagen transformation proteins. EMT (epithelial to mesenchymal transitional) plays a key role in cancer metastasis, which involves cancer cells obtaining aggressive phenotype through the transition from their specialized state to further nonspecialized state(Sossey-Alaoui et al., 2009). EMT in base epithelial cells is thought a crucial role in pterygium pathogenesis(Kato et al., 2007).

“WASF3 belongs to the Wiskot-Aldrich syndrome proteins WASP/WAVE family of actin-binding protein that play essential roles in regulating cell morphology, actin polymerization, cytoskeleton remodeling, cell motility and invasion (Molly., 2013).”it

regulates many aspects of cancer progression, cell invasion, cell migration and metastasis, as well as remodeling of cytoskeleton.

WASF3 is one of the main drivers of metastasis in various origins of cancer among various WSAP and WAVE proteins. the phenotype and invasive characteristic of cancer cells related with metastatic potentiality and controlled by the mechanism that regulates the expression WASF3 in cancer cells(Sossey-Alaoui et al., 2009).

it`s already pointed out that WASF3 facilitates metastatic and invasive morphology acquirements by some human cancers like (BC). The transformation of growth factors in metastatic BC cells which promotes the WASF3 expression particularly and vigorously (Molly., 2013). WASF3 expression inhibition causes a transition in cell morphology similar to mesenchymal to epithelial reversal, suggesting that WASF3 is involved in this cellular mechanism. furthermore it`s been documented that WASF3 over expression leads to mobility, invasion potentiality and migration cancerous cells, which for metastasis and cancer invasion altogether are demanded(Sossey-Alaoui et al., 2009).

the fact that the metastasis of cancer is a multi-stage mechanism involving a complicated protein interaction is obvious. While WASF3 proteins are well known in terms of cell mobility in the form of some cancer cell lines, investigating WASF3 function can give understanding into the participation of these proteins to the other functions of cell(Ping Weeks et al., 2016).

One of the extremely argumentative eye disorders is pterygium, whom pathology is not completely understood yet. however, it is described as a harmless wound, epithelial and fibrovascular excessive growth on the eye surface functions analogous to tumor development because of its *overgrowth* (*proliferative*), aggressive (invasive) and largely vascularized microscopic formations. WASF3 is an actin-binding protein that play essential roles in regulating cell morphology, actin polymerization, cytoskeleton remodeling, cell motility, and invasion.

WASF3 was classified as a key driver of tumor progression and metastasis in cancers from several origins as well. The aim of this study was to investigate the effect of WASF3 on the behaviors of pterygium disease and to evaluate its clinical impact. In this framework, we assessed the association of WASF3, a familiar actin-binding protein and analogous to tumor suppressor gene with pterygium which exhibits tumor-like behavior. We analyzed the expression level of WASF3 gene in our study.

2. Literature review

2.1 Pterygium and it's Prevalence

Duke Elder defines pterygium as a triangular proliferative and degenerative disease. Pterygium is an abnormal wing-shaped growth of fibrovascular and epithelial tissue(Houa et al,. 2013). It occurs in the center and lateral of the eyelid opening, and the bulbar conjunctiva invades the cornea (Figure 2.1) (Maske and Richard., 1989).Environmental conditions may be related to determining the eye surface conditions that allow primary pterygium development and exposure to ultraviolet light, and other conditions that cause chronic irritation of eye have been proposed. In a search for biological explanations, the researchers investigated potential changes in the proliferative aspects of wing-shaped fragments as well as some of the inflammatory actions and the matrix proteins regulation(Wong et al., 2014).

Aesthetic problems, poor vision and uneven astigmatism are significant problems linked herewith disease. The most major risk factor for pterygium is Prolonged exposure to the sun's UV rays. Nevertheless, other variables are other probable risk factors, such as poor income, residence area (urban and rural), sex (male>female), smoking, age (adult > young) (Claudia,et al., 2019), latitude, farming, and environmental factors (Wind, dust, shock, dryness and smokes are environmental variables). pterygium is more than 20% of few populations and generally affects the younger inhabitants and can possibly lead to blindness (Yue and Gao,. 2019).

There have been several prior reports on spread of the pterygium disease and risk factors for populations, occurrence of pterygium differs extensively depending on the age, geography, and gender of diverse examples, and the figures Is still imperfect and restricted(Liu et al., 2015). The frequency of pterygium in research carried out in different regions of the world ranged from 1.3 percent in the Tehran Eye Study to 53percent in Taiwan due to differences in age groups examined and studies (population-based versus clinical studies).

“Despite numerous researches to establish the kind and quantity of effects of each causative elements or factors, there is a difference of estimations amongst the studies and sometimes the results are contrary. The intention of the current analysis of studies is to make available a precise estimation of the incidence of pterygium and to detect its determining factors by means of a grouping of all population-related and clinical studies throughout the world (Rezvan et al., 2018)”. Even though pterygium has historically been connected to ophthalmoheliosis, there are advancements in a group of disorders induced by the toxic and harmful effects of sunlight on the eye, in recent decades molecular genetics have allowed additional and potential possible pathways to be discovered.

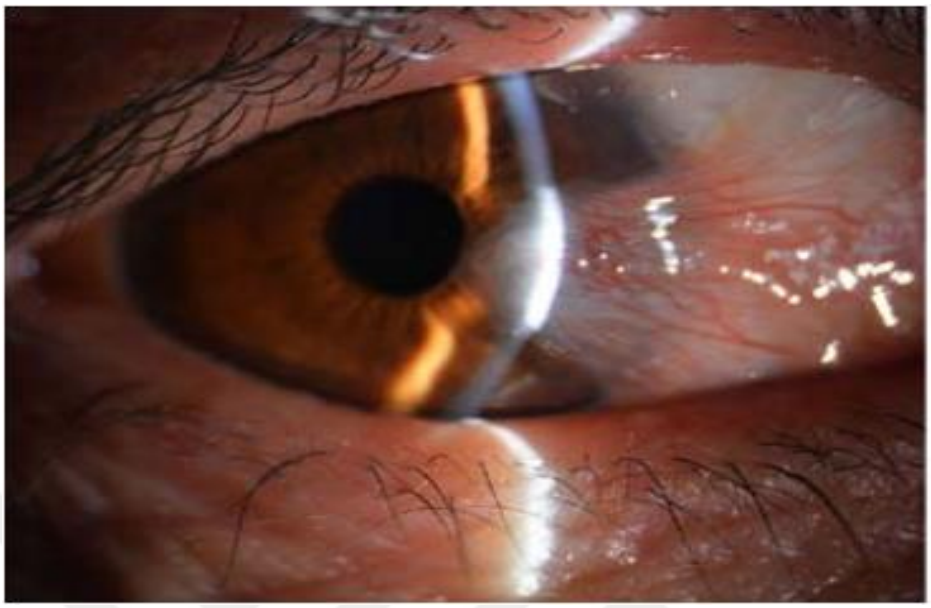

Figure 2. 1 the morphology of pterygium on the cornea of a 52-year-old female patient.

Case management, Cross-sectional and cohorts are designed as observational epidemiologic studies, either clinic-related or population-related. It provides a prevalence and risk (odds ratio) translucent report to obtain prevalence and odds ratio combined indices. Furthermost epidemiological research finds that long-term exposure to sunlight is a major cause-effect factor due to climate change, notably ozone-layer changes, increasing life expectancy and contemporary lifestyles. Studies, on the other hand, have revealed varying degrees of effectiveness.

The findings of a meta-analysis indicated that continuous and extended exposure to sunlight increased the incidence of pterygium by up to 24 percent (OR = 1.24). (In a couple of different studies) the range of first research odd ratio was 1.10 and 3.45 for the second research.in cooperation both of studies observed evidence correlated to sunlight exposure and released UV in their systematic examination. According to a Research, which shows pterygium is starting with alterations in fibroblast and stem cells, followed by activation of pre-inflammatory cytokines, growth factors and process accelerating matrix metalloproteinases(Liang et al., 2010).

In addition, its discovered that the evolving pterygium odds in people with outdoor jobs, such as farmers, hunters vs military personnel, had 1.46 times greater risks of getting pterygium than those who worked indoors. Indeed, outdoor work and employment alone are not a self-determining risk factors, but are more important for factors, such as gender and age, to be adapted as UV radiation and to persistent sunshine

exposure. “It has been shown that rural inhabitants have a higher risk of developing a pterygium than city dwellers. The study of a disease's epidemiological features permitted Insights into their pathophysiology can indicate the importance of numerous risk factors and Finally, a remark concerning the efficacy of the suggested prophylactic. The purpose of the research was this (Rezvan et al., 2018)”.

2.1.1 Laterality of Pterygium

the unilateral pterygium comes off a little more often than the bilateral before In a Investigation by Lu et al. was at 60% pterygium patients localization unilateral and 40% bilateral. According to most of the eye studies, the majority of people have been diagnosed with unilateral pterygia rather than bilateral (Droutsas et al., 2010).

2.1.2 Localization on the eye

the typical place of manifestation of the pterygium is the nasal limbus. In the temporal limbal area comes a real one Pterygium less common and in the superior or inferior limbus practically never in front. One possible explanation for this observation is that while eyebrows, nose and cheeks make one Protection against laterally incident UV radiation grant the temporal Cornea is relatively exposed what after Coroner's theory of the making of a nasal pterygium favored. Exophthalmometry of patients with pterygium showed greater protrusion of the affected versus healthy Partner eye (Demirok et al., 2008).

2.1.3 Pterygium and blindness

In a large epidemiological study of the causes of blindness. observed under 2076 Study participants 230 eyes blinded by pterygium, but only 2 people with bilateral blindness (perception of light). A pterygium-related, Bilateral, severe visual impairment was not recorded. Gazzard et al. could among 1210 people of an Indonesian population did not observe a single case of bilateral blindness. A pterygium-related, monocular reduction in visual acuity on 0.1 was registered in 3 patients. The visual acuity of eyes with pterygium was statistically significantly lower overall than from healthy (Gazzard et al., 2002). Shown in the Barbados Eye Studies A pterygium-related visual acuity reduction occurred in one patient (0.2%) within a period of 9 years. Also, in Singapore no pterygium-induced blindness was recorded among 1,717 people (Demirok et al., 2008).

2.1.4 Comorbidity

The Blue Mountains Eye Study showed one Association of the pterygium with the posterior shell opacity of the crystalline lens. The 5-year results of the Blue Mountains Eye Study, eyes with pterygium have a double risk of mild AMD (soft drusen, central RPE irregularities) and a 3- multiple risk of a late form (exudative AMD or geographic atrophy) to suffer from AMD. Simultaneous occurrence with a pinguecula was observed in 4% (Demirok et al., 2008).

2.1.5 Conclusion for practice the traditional concept of a central role of chronic UV exposure

(be it from the sun and / or artificial UV light) in the pathogenesis of the pterygium is confirmed by most epidemiological studies. In addition to a geographical location close to the equator, long-term Outdoor activity, increasing age, and male gender represent the major risk factors, being too these possibly only secondary phenomena of the chronic UV exposure. The irritation of the surface of the eye by chemical or other active substances is discussed. Regular wearing of ad- adequate sun protection has a proven protective effect and will Recommended as inexpensive and uncomplicated prophylaxis, as well as public education, especially for groups of people who practice their profession outdoors (Cinal et al., 2008).

2.2 Potential Risk Factor

2.2.1 UV exposure

The literature provides very strong evidence for a causal relationship between chronic exposure to sunlight and the pterygium formation (MT, 1993). Coroneo explains the origin of the pterygium due to the property of the corneal periphery, laterally incident light so too breaks that rays transcameral focused on the posterior side of the distal limbus area with 20 times the radiation intensity. He assumes that chronic UV exposure to one focal alteration of the limbal stem cells, which creates a disruption of the stem cell barrier and thus the prerequisites for conjunctivalization the cornea sets.

The individual UV exposure is shown in the studies both directly, on the indication of the time daily spent outdoors was and whether regular sun protection in the form of sunglasses and a hat, or indirectly through the collection of parameters such as geographical Localization of the place of life, occupation and Gender, estimated. The mean annual solar radiation of a place increases with increasing Proximity to the equator, where the angle of incidence of the sun's rays is the steepest. The highest prevalence rates in the general population are between latitudes, according to Cameron 40 ° N (north) or 40 ° S (south) of the Equator, to be found in the so-called pterygium belt (Cameron., 1965).

In a study. 278 pterygium patients were compared with a healthy eye Control group. There was an increased risk of pterygium in those who lived in the first 30 years of life on one Latitude of <30 ° resided, on Worked on the beach or spent more than 50% of their time outdoors by the age of 5 (Mackenzie et al., 1992). Not just the direct, but also out UV radiation reflected from the environment, the so-called diffuse radiation seems to favor the development of a pterygium. The effect of diffuse radiation is reflected in the results of various Studies against: People who work in in water basins have significantly higher rate of pterygium compared to the people who work in dry conditions (Mathur et al., 2005).

Norn observed a prevalence in an Eskimo population in Greenland. This population is a strong diffuse radiation due to the snow and ice reflection exposed (Norn, 1979). Mountain dwellers are also exposed to higher levels of radiation because the sun's rays have a shorter path through it take the filter of the earth's atmosphere. Accordingly, in this population higher prevalence rates observed. Another hint for the central role of UV rays - and not des Sunlight in general - is the higher Prevalence among welders (Demirok et al., 2008). Pterygium is a frequent eye surface disorder related with exposure to prolonged UV Proliferation, inflammatory infiltrates, angiogenesis, and extracellular matrix (ECM) disintegration define pterygium (Chui et al., 2008). Pterygium fibroblasts grow significantly quicker in a medium with a low percentage of serum and can grow in a semisolid agar than normal fibroblasts, showing that these cells are tumor-like altered cells (Chen et al., 1994).

In the occurrence of pterygium, cataracts and skin cancers, Ultraviolet radiation in the sun rays (UVA and UVB) plays an essential role. The early study on the UV-induced illness process was concentrated on Ultraviolet B, but Ultraviolet A's role was not respected Ultraviolet A has only been demonstrated to induce damage in many cell biomolecules in recent years, which can contribute to skin malignancy and melanoma formation (Rigel, 2008).

While UVB radiation directly impair and harm cellular DNA, the UVA radiation produces significant oxidative stress on the cells, oxidizing cell biomolecules such as DNA and lipids; initiates ECM degradation and gene transmutation; activating proteins and phosphatases; modulating transcription factors; and producing inflammation. All of these factors above are able to produce skin cancers (Chao et al., 2013).The phenomena of peripheral light focusing, in which accidental light travels through the anterior chamber and is concentrated at the distal (nasal) limbus, where limbal stem cells (LSCs) exist, may explain the limbal preference as well (Figure 2.2) (Chui et al., 2011).

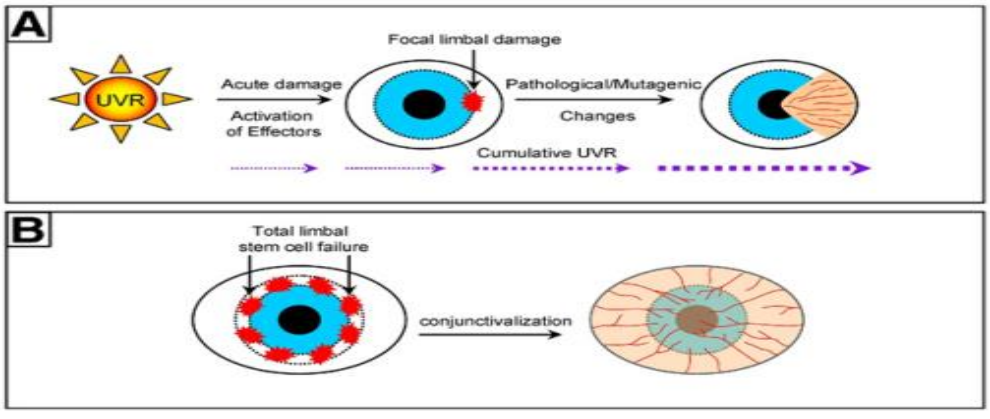

Figure 2. 2 The role of cumulative UV radiation exposure in pterygium development. **A:** Model for the pathogenesis of pterygium: focal limbal damage from UV radiation triggers migration of altered LSCs toward the central cornea. **B:** In total LSC deficiency, damage to the limbal niche or depletion of stem cell reserves results in conjunctivalization of the cornea from all directions.

2.2.2 Outdoor activity

Years of outdoor activity increase the cumulative UV exposure of the Eyes. The effect of UV radiation was demonstrated in the study by Gazzard et al. clear occupied: people who 10 years previously longer worked outdoors for 5 hours a day, had a pterygium rate twice as high as the other participants. Several studies show a high prevalence valence in occupations that involve an activity are connected outdoors, such as farmers, fishermen or foresters (figure2.3) (Luthra et al., 2001) .

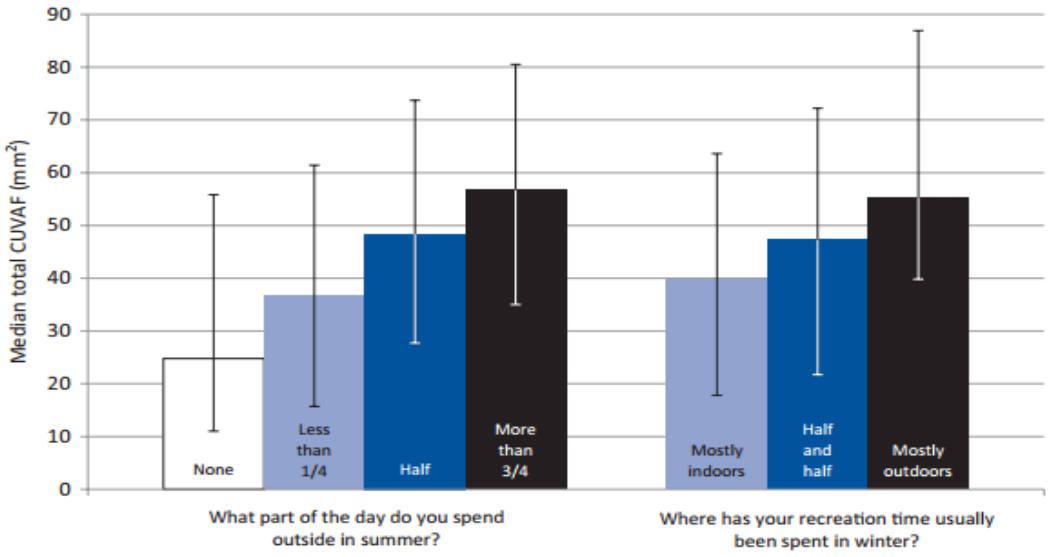

Figure 2. 3 Conjunctival ultraviolet autofluorescence (CUVAF) and participant recall of outdoor time measured by questionnaire.

2.2.3 Chronic irritation of the Surface of the eye

Not just the UV light, but also exposure to mechanical or chemical irritants in the air seems to play a role in pterygium formation. During the examination of petroleum industry workers in Nigeria A pterygium was statistically highly significantly more common in technicians who in contact with chemical irritants than found in non-technicians (Taylor et al., 2004). Compared with people who worked in closed rooms, the group of Mackenzie et al. 11 times the risk of illness for participants living in a sandy environment or a 4-fold risk for those who are in were employed in a grassy environment (Mackenzie et al., 1992).

2.2.4 Age

“According to epidemiological research, the prevalence of ocular disorders and eye problems rises with age. According to some findings, the occurrence of pterygium rose with age, with a total frequency of 19.5 percent among people over the age of 80. This is consistent with the majority of population research that demonstrate that the occurrence of pterygium increases as a result of the age (Rezvan et al., 2018)”. In a study from Indonesia, 50-year-olds had 7 times the risk of developing the disease compared to younger ages between 21 - 29. (figure 2.4) (Demirok et al., 2008)

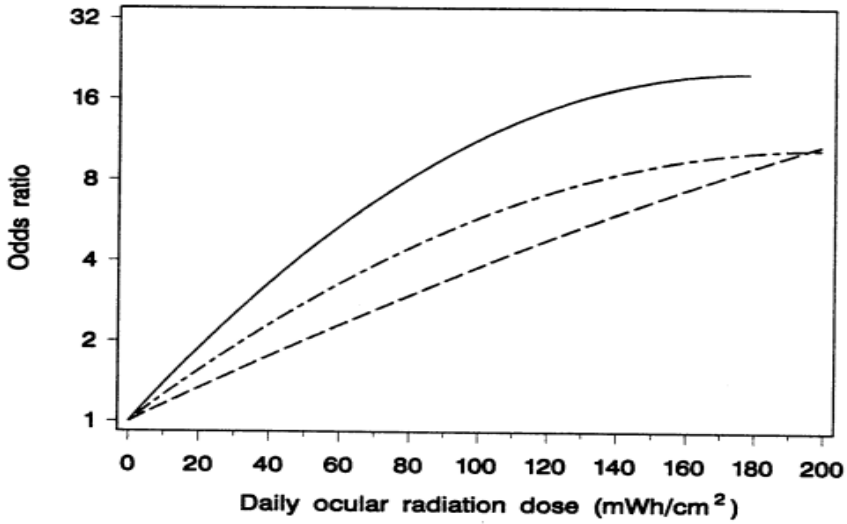

Figure 2. 4 Pterygium odds ratio and estimated average daily ocular radiation dose from the continuous relationship in logistic regression models incorporating a quadratic term, for three periods: whole life (solid line), age 0 to 9 years (dotted and dashed line), and age 10 to 19 years (dashed line).

2.2.5 Gender

"Some studies show that the risk of developing the disease for men is 2 to 5 times higher than that of women. This is applied to the work-related increased UV exposure of the Men returned. In in other publications no significant difference between the sexes was observed (Demirok et al., 2008). The major cause for the variation in risk between men and women seems to be differences in lifestyle such as spending more hours outside (Rezvan et al., 2018)".

2.2.6 Body pigmentation

The results regarding a possible protective effect of strong body pigmentation are controversial. As part of A prospective, controlled study of an Australian population showed a protective effect of body pigmentation on pterygium formation: People with medium and light skin color had a 1.5- Up to 1.7 times the risk of illness compared to dark-skinned people. Study participants who have a bad or reported slow browning, had 3 times the risk compared to people with rapid tanning (Mackenzie et al, 1992).

2.2.7 Place of residence (city vs. country)

"Life in the country is in comparison associated with higher sun exposure to city life. The prevalence is correspondingly higher in this population (Demirok et al., 2008). It has been discovered that rural inhabitants have a higher risk of developing a pterygium than city dwellers. Pterygia is more common in those who live in rural areas (Rezvan,et al., 2018)".

2.2.8 origin

existing genetic components could include through the collection of epidemiological data from different ethnic groups. In practice, however, this comparison is hardly possible since the cumulative UV exposure fluctuates both between and within the populations examined. There are reports of a prevalence among Caucasians between 1% (Copenhageners) and 23% (residents of the Croatian island of Rab). This difference was attributed to increased exposure to the sun by the Aborigines (Moran and Hollows, 1984).

2.2.9 Alcohol consumption

Another risk factor in the pterygium development found in a research was alcohol intake, which is associated to living styles. The risk of pterygium was 10 percent greater in alcoholics than in non-consumers, according to this research results. No considerable connection among this exposure and the prevalence of pterygium was identified in most epidemiological investigations done in various parts of the world. Some researches have discovered that the probabilities of pterygium were 70% greater for alcohol drinkers than those not consumed, after adjustments for confounding variables such as age, gender, smoking and education (Marmamula et al., 2013).

2.2.10 smoking

“In the development of several malignancies, cardiovascular illnesses and even some eye problems including refractive error, macular degeneration and blindness connected to age, the harmful consequences of smoking are demonstrated. One study found that the risk to smokers of pterygium in comparison with non-smokers was 0.80. That demonstrates how smoking has a protective role on pterygium formation. The most potential reasons for the protection effect of smoking against pterygium include removed production of inflammatory factors, changes in antibody production, and eye surface protection from irritants, as well as changes to tear composition produced by smoking (Rezvan et al., 2018)”.

2.3 Molecular factors

The degenerative lesions, as illustrated by Bovman's layer degeneration and elastose, were regarded historically pterygias. Pterygia, meanwhile, is currently defined as a proliferative disease similar to an abnormal wound cure (Chui et al., 2011).Visual impairments are caused by the lesion, and there are presently no medicinal therapies available except from surgical excision. The process that causes pterygium to develop is unknown. Proofs from epidemiological and histopathological research emphasizes the fact of the invading proteolytic activity of ulcers in pterygium as fibroblast cells are dysfunctional, proliferating and release aberrant matrix and matrix metalloproteinases(Houa et al,. 2013).

Hyperplastic and centralized development of modified limbal epithelial cells with lay dissolving of Bowman is Histopathologically characteristics of pterygium. The pathogenesis of this disorder continues to be unknown, however ultraviolet (UV) radiation is usually thought to be involved in the pathogenesis, and research which have shown a clear correlation between both the closeness to the tropics and a higher pterygium occurrence have demonstrated this.

In addition, a variety of molecular components have been generally assumed, including Epigenetic abnormalities, mesenchymal epithelia transition, inflammatory activated fibroblastic stroma, neovascularity, matrix remodelling, basal epithelial cell proliferation, adjacent corneal epithelium invasion, mechanisms of anti-apoptotic and immunologic, VEGF inhibitors and lymphangiogenic stimulation. Antiangiogenic agents. Deregulating of modulators of extracellular matrix, of cell progenitor and BONE marrow-derived progenitor (Ca´rdenas-Cantu´ et al., 2014). mediated by cytokines, growth factors, and matrix metalloproteinases working together (figure 2.5) (Chui et al., 2011).

The majority of these variables, though, are more connected to the disease's growth and improvement than to its etiology. Some of these variables have been linked to UV light exposure, either direct or indirect. The first thing that affects the course of this illness is the existence of gray defined dots close to the limbus cornea with tensed conjunctive simultaneously that emerge opposed to the region of the corneal affection and the plica displacement.

A wing-shaped expansion of the bulbar conjunctiva's fleshy development onto the cornea occurs later. A full-grown pterygium has a well-formed ‘‘peak" (the apex region of the cornea), ‘‘body" (the scleral section spreading between the limbo also the canthus), and "neck" (limbal portion) (Ca´rdenas-Cantu´ et al., 2014). There is no unanimity on the actual pathophysiological processes despite the various traditional and innovative hypotheses of pterygium etiology that have been described (Song et al., 2014).

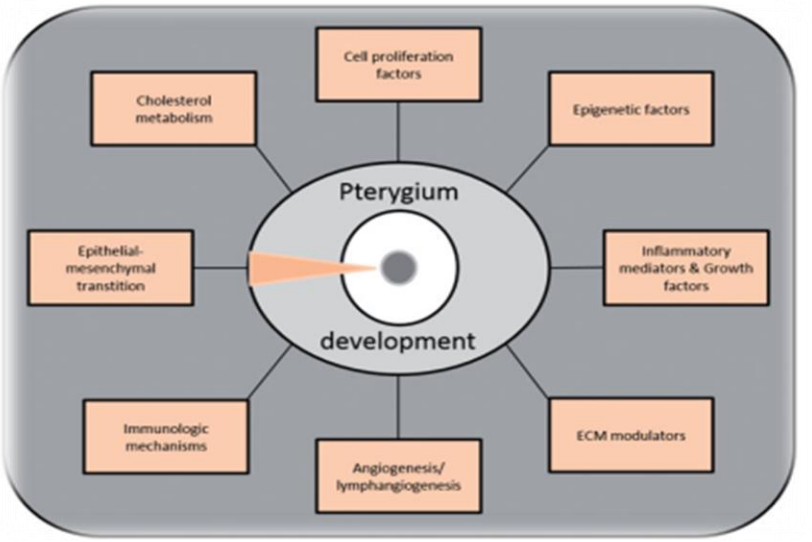

Figure 2. 5 Molecular factors related to pterygium development.

2.3.1 Heredity

Even though Heredity is essential for the development of pterygium and it is stated that it does not play a part in pterygium. The inheritance is dominating with poor penetration, but it seems that the real wound is not transferred, but rather that the eye tends to interact to stimuli for the surroundings (Ricahrd et al., 1989). Family history involving genetics, regardless of the customary usage of eye medicine and the imbalanced tear film, is linked to pterygium incidence.

Relatives with pterygium have been diagnosed, indicating that genetics is related to the appearance of pterygium and establishing the familial appearance of pterygium. The same solar exposure existed in patients with pterygium with the unaffected people, that implies the family occasions were attributable not to the environment but to genetics. (Exposure to the sun is only a pterygium trigger) (Anguria et al., 2014).

2.3.2 Apoptosis and cell proliferation

Modified developmental features: Pterygium fibroblasts demonstrate altered cell characteristics, which include heterozygosity loss and the instability of the microsatellite. In addition, an enhanced fibrovascular layer proliferation in recurrent pterygium has been identified in one study, but others have documented no such changes (Girolamoa et al., 2004).

2.3.3 epithelial mesenchymal transition EMT

As the origins of pterygia fibroblasts, one recent study investigated the performance of epithelial mesenchymal transition, a process in which epithelial cells involve taking on features of mesenchymal cells. MMPs, such as MMP-1 (collagenase), MMP-2 (gelatinase A), MMP-3 (stromelysin 1), MMP-7 (matrilysin), and MMP-9 (gelatinase B), are increased in pterygia, especially in the developing heads. MMP-8 (neutrophil collagenase) and membrane-type MMP (MMP-14 and MMP-15) expression

has also been detected (Naib-Majani W, 2004). Endogenous inhibitors of MMP activity, such as a2-macroglobulin and tissue inhibitors of metalloproteinases, regulate MMP activity (TIMPs). Pterygia has been linked to a deregulation of MMP versus TIMP activity. The production of matrix metalloproteinase has a role in pterygia migration and growth but is unlikely to be a condition for development. MMP production variations may clinically describe variability in observed development of pterygia (Bradley et al., 2009).

2.3.4 immunologic

Although the notion is not completely understood, in its pathophysiology or as a result of its generation it is likely that the immune system is connected to development of pterygium directly. There has been an elevated number of immunological biomarkers, include molecules-1 of the cellular vascular adhesion (VCAM-1), molecule-1 of the intercellular adhesion (ICAM-1) as well as human antigen leukocytic RR (HLA-DR). Up-regulating of E-cadherin14 and b-catenin16 have also been observed. There has already been reported a rise in the number of pterygium mast cells, lymphocytes, plasma cells, cells of dendritic and CD4+ and CD8+ T cells (Beden et al, 2003).

The most of immunological intensity masses of immune system cells found in pterygium conjunctive tissue have been described as having T-lymphocytes. In pterygium tissue, macrophage types have been distributed unequally because the severity of the inflammatory process changes according to the amounts of antigen (Ca´rdenas-Cantu´ et al., 2014).

2.3.5 deregulation of extracellular matrix modulators

Pterygium appears to have abnormal extracellular matrix (ECM) remodeling, as demonstrated by a new research that found ECM genes such as versican, collagen III, and fibronectin were elevated in pterygium. Pterygia has an increased level of matrix metalloproteinases (MMPs), a class of structurally similar zinc-dependent and ECM-degrading proteinases.

MMPs have been linked to a range of normal and pathologic cellular processes, including embryogenesis, angiogenesis, wound healing, and cancer, and are able to damage approximately all components of the ECM (Nagase H, 1999). In pterygium, the majority of MMP family members have been shown to be upregulated. The aggressive character of the pterygial wound is considered to incorporate modified MMP activity, since numerous reports have now demonstrated a strong link between MMPs and tumor development/ raid (Seet et al., 2012).

2.3.6 Growth Factors

Growth factors, a class of compounds (molecules)to encourage cell cycle progression, are capable of stimulating mitosis and cell cycle proliferation. Pterygium has been found to contain a variety of growth factors, including growth factor transforming (TGF-β) factor, vascular endothelial growth factor (VEGF), insulin-like growth factor

(IGF), basic fibroblast development factor (bFGF), and nerve growth factor (NGF) (Girolamo et al., 2004).

For instance, amongst the most significant growth molecules in eye disorders is VEGF. In ocular diseases, the VEGF family regulates pathological angiogenesis and increases capillary permeability. Pterygium had increasing amounts of VEGF and vascular endothelial growth factor receptor (VEGFR) than normal conjunctiva (Fukuhara et al., 2013). Elevated VEGF production promotes neovascularization (angiogenesis)and lymphangiogenesis, which may significantly affect conjunctiva cell metabolism and encourage their transformation into pterygium cells (Feng et al., 2017).

2.3.7 Endothelial progenitor cells

The role of endothelial progenitor cells (EPCs) in the pathology of pterygium is being explored, as well as the mechanism behind the selective activation of EPCs throughout this mechanism (Lee1 et al., 2007). Pterygium immunohistochemical analyzes have shown significant immunodeficiencies of progenitor cell markers. New research nevertheless indicate that bone marrow-derived circulating endothelial and epithelial progenitor cells (EPCs) can also help postnatum neovascularization (angiogenesis). The hypothesis is, pterygium comes from the UV-modified limbal stem cells, and more recently the suggested contribution to their pathogenesis has been bone-marrow progenitor cells. This idea is supported by the separation of cells with leukocyte markers (CD45, CD11b, CD11c, CD14 and HLA-DR) (JEANIE CHUI, 2008). Several modulators, including vascular endothelial growth factor (VEGF), stem cell factor (SCF (c-kit ligand)), and substance-P, are recognized to activate EPCs (epithelial) (SP) (Rameshwar et al., 1993).

Furthermore, it is recognized earlier that pterygial tissue contains bone marrow-derived stem and progenitor cells (Song YS, 2005) These observations are, however, restricted to local investigations of pterygium effects and the physiological process behind the limited manifestations of such stem cell modulatory factors are not well characterized. In recurring pterygium, the production of these markers was likewise higher (Lee et al., 2007).

2.3.8 modifications in cholesterol metabolism.

In a study which is confirmed by Peiretti et al, they discovered a connection between the growth of pterygia and changes in metabolism of cholesterol. The scientists discovered reduced density lipoprotein receptor and elevated amounts of hydroxyl-methylglutaryl-coenzyme A-reductase, which they believe may be engaged in fibroblast proliferation promotion (Peiretti , 2006). In reality, the pterygia fibroblasts proliferation was reduced by some lipid metabolism modifying medications. The coming decades treatment approach seems to be to inhibit the formation and proliferation of pterygia with this relationship (Bradley, 2010).

2.3.9 Angiogenesis

In pterygium pathogenesis, the overabundance of factors of pterygium angiogenesis have been shown to be irritating to limbal basal cells and to generate ingrowing vessel to encourage pterygium development. Furthermore, the absolute amount of different pro-angiogenic and anti-angiogenic substances which (regulate angiogenic processes and activities) controls angiogenesis (Pepper, 1997).

The major "on" switching are also known as angiogenesis growth factors while angiogenesis inhibitors are the main "off" switching." This approach can be referred to as the switching of angiogenesis or "angiogenic switch." When angiogenic growth factors surpass(outweigh) angiogenesis inhibitors, the balance moves toward blood vessel expansion, but angiogenesis is inhibited when inhibitor levels increase angiogenic stimulator levels. Several angiogenesis promoters were already found (Fang, 2013).

2.3.10 Inflammation

Inflammation is a self-protective mechanism in the human body to eliminate irritant. It occurs in both ordinary physiological mechanism such as disease pathologies such as malignancy and wound healing (Fang, 2013). In pterygia exposed to the same dose of UV radiation, the degree of production of inflammation has changed, indicating that sunlight exposure might not be significant to the severity of the inflammation.

There may be genetically controlled inflammation intensity. Some people may be insufficient in the T-lymphokine activated killer cell-originated protein kinase (TOPK) and the weakness appears to enhance inflammation by sunlight (Zhu et al., 2011). lipids of cell membrane are phosphorylated by species of Reactive oxygen, leading to a rise in lipid metabolic products. Prostaglandin E-2 (PGE-2) is one of these lipid molecules that has been linked to pterygia (Koshima et al., 2007). every pterygium specimen contained inflammatory cells, demonstrating inflammation.

Although inflammation was considered to be the penultimate step in the development of pterygia, that’s why the leukocytes were mostly found in the epithelium, it was assumed to be a sort of hypersensitivity(Anguria,et al., 2014). inflamed pterygia (inflammation) cells were observed to activate the transforming. Pterygium is characterized by collagen degradation and ECM modification, angiogenesis and fibroblast proliferation are activated by MMPs in pterygium. Tissue fibrosis is characterized as cell proliferation and increased extracellular matrix production as a result of serious inflammation (Fang, 2013).

2.3.11 pterygium pathogenetic co-factors and viral infections Theories

Pterygium was once thought to be a degenerative disorder. Furthermore, the discovery of significant molecular genetic changes in pterygium in recent years has cast doubt on this hypothesis (Aikatereni et al., 2013). pterygium co-factors include genetic polymorphism of hOGG1, loss of heterozygosity (LOH) of microsatellite DNA, and elevated P53 expression altered (mutated)versions (Fang, 2013). therefore, for the

pathogenesis of pterygium a multi-step pathogenetic process has been postulated with the inclusion of inheritance of genetic, UV exposure and, over all else, oncogenic viral infections.

DNA damage results from UV exposure and epigenetic changes with gene mutation. "according to this 'two-hit' hypothesis, inherited genetic alterations or exposure to environmental factors such as UV could predispose individuals to this benign neoplastic disease ('first hit'). Oncogenic viruses, or additional UVR exposure, adding further damage to an already susceptible genetic material, may be the stimulus for the outset of development or recurrence of pterygium ('second hit')." the identification of HPV strains believed to be of high risk for cancer development has lately backed up this theoretical paradigm. Most studies concur that HPV is found in at least one pterygia subgroup that both etiology and (including recurrence) clinical behaviors of these wound be influenced by HPV infection (Aikatereni et al., 2013).

2.3.12 Proliferation and angiogenesis

Angiogenesis is an ordinary development method for new blood vessels from pre-existing vessels in response to tissue demand such as growth and differentiation, ovulation, injury cure, and serious diseases, such as neoplasms and eye diseases complicated by severe vision interference and angiogenesis deregulation (Carmeliet, 2003). Angiogenesis-dependent conditions are caused by the increased or inadequate growth of new blood vessels. Angiogenesis is triggered by the diffusion of angiogenic growth agents in the preexisting vascular because of the spread of angiogenic growth factors. Endothelial cells (EC) are activated when growth factors connect to their receptors and convey signals from the cell's surface to the nucleus. This causes extracellular matrix breakdown, cell proliferation, and migration, which leads to the formation of new blood vessels (Risau, 1997). The localized invasive pterygium may range from moderate dysplasia to cancer in site with varying amounts of aberrations.

According to recent research, Pterygium illness has been an active method of cell proliferation, continuous renovation of connective tissue, angiogenesis, and inflammation. Pterygium is a disorder characterized by epithelial hyperplasia and fibro-vascular proliferation that arises at the corneo-conjunctival junction. Altered limbic stem cells move from this location and pass through the cornea. It leads light sensitivity(photophobia)lachrymation, and pain. Recurrences are prevalent following surgical treatment. Altogether, pterygium is a proliferative invasive illness accompanied by localized sclero-corneal limbus inadequacy and linked with increased UV light exposure. Chronic inflammation and angiogenesis define this condition, which eventually leads to connective tissue modification (Coroneo MT, 1999).

This etiopathogenic concept is in conflict with the classical one, in which pterygium is seen as a typical degenerative and normal process. The investigation indicated a significantly higher than in normal conjunctiva vascularization in the pterygium. These vasculatures were also most noticeable in the subepithelial connective

tissue. The shape of these vasculature is unique to (tumor)neoformation: they are tiny in diameter, twisted and branching, and the lumen is seldom visible (Livezeanu et al., 2011).

2.3.13 epigenetic aberrations

Pterygium has cancer resemblances, such as cell growth, invasion and post excision recurrence. The pterygium epithelial cellular layer was over-expressed in comparison to healthy conjunctivitis with proteins associated with enhanced proliferation activity (Ki-67, nucleic antigen [PCNA] and erythropoietin), low levels of cellular apoptosis (Bcl-2 and p 53), and malignant disease (Hsp90) (Kim et al., 2013). These proteins were discovered to be elevated in many forms of cancer (Lester et al, 2005) and utilized for an evaluation of the proliferative activities of a tumor.

Significant alterations in the pattern of DNA methylation are prevalent in several cancer forms, and some researches have been discussed on patterns of pterygium methylation due to the parallels between cancer and pterygium. Pterygium invasion is linked to altered DNA methylation of genes that are involved in cell adhesion (TGM-2 and CD24 hypermethylation) and matrix remodelling (MMP-2 hypomethylation) (Riau et al., 2011). Moreover, overexpression of the DNA methyltransferase 3b (DNMT3b) in pterygium has been associated to hypermethylation of the tumor suppressor gene p16. the alternatives of DNA mutations are These epigenetic changes, which may lead to unregulated expression or repression of significant genes linked to pterygium pathogenesis. These anomalous methylation patterns may be attributed to oxidative damage induced by UV light, but so far, no study was ever carried out (Ca´rdenas-Cantu´ et al., 2014).

2.4 Relations between lymphangiogenesis and the size of pterygium (lymphangiogenesis and pterygium)

The lymphatic system as well as plays an important function in immunological response to infectious pathogens in addition to maintaining homeostasis of tissue fluids. Whilst the vessels offer entrance pathways for cells of the immune effector (e.g., alloreactive T lymphocytes, memory T lymphocytes), the lymph vessels corticoafferent to the lymph nodes and lymph bodies transition from APC to the local lymph nodes and the lymph bodies (Bradley et al., 2010).

Human pterygia have recently been investigated by Cimpean et al using immunochemistry and an LMD elevation in pterygium has been detected compared to normal conjunctiva. They observed furthermore that D2-40- positive lymph endothelial cells proliferate vigorously, corresponding with the immunostaining finding of Ki-67, but not in normal conjunctive activity. The findings simply show that lymphangiogenesis is active in this diseased state and the existence of active lymphatic vessels in human pterygium is reported. The occurrence of enhanced LMD in primary pterygium shows a correlation between immunologic mechanism and pterygium size and lymphangiogenicity (Liu et al., 2012).

2.5 Pterygium Treatment and protection

2.5.1 Pterygium Treatment

Until recently, surgical excision is the main therapy for pterygium. However, following surgery, it reverted back aggressively, and extensive removal, radiotherapy and antimitotic chemotherapy are further therapeutic modalities (Yue and Gao., 2019). If the pterygium is small and does not have an unpleasant appearance and does not cause redness and irritation of the eyes, it does not need special treatment, but artificial tear drops. In the case of larger pterygiums, which are important in terms of appearance and beauty or have caused blurred vision by causing astigmatism, a course of treatment with steroid drops or other anti-inflammatory drops can be performed or the pterygium can be removed surgically.

People with inflamed pterygium are found in half of the recurrences. In these people, in addition to removing the pterygium, it is better to use complementary methods such as conjunctival transplantation or the use of special drugs (such as mitomycin) during surgery. In very large pterygiums that involve the middle of the cornea, it may be necessary to use a layered corneal transplant to correct the shape of the cornea in addition to the above procedures (Noor., 2021). After pterygium operation, recurrence is a difficult and chronic issue. To address this, many sorts of additional medicines were employed. Mitomycin C was commonly used to decrease recurrence, as a traditional medicine. Post-surgery inflammation can cause pterygium reappearance again, as shown in earlier investigations. in the beginning of postoperative phase, therefore, it is particularly crucial to manage inflammation.

In the research artificial tears, a safe and frequently used medication (hydroxypropyl methylcellulose), were tested as a supplement following surgery. When compared to the other group that did not get this medication (control group), the group that received topical ointments had a massively reduced recurrence rate (Kampitak et al., 2016). In a research, the conjunctival Autograft Incisional pterygium techniques and the conjunctival autograft rotating with mitomycin C were compared. The findings further showed that CRA with mitomycin C and CA technique with mitomycin C both are able evenly to help decrease recurrence in pterygium surgery as well. Based on the findings of this investigation (Bamdad et al., 2017).

2.5.2 protection /Prophylactic measures

Exposed to toxic influence of exposure to sun in the pterygium development, the need for broad border caps and recommended sunglasses, especially of the elderly and middle-aged people, must be trained in individuals whose work description also makes them at risk of exposed to sunlight. It furthermore demonstrates a 53 percent reduction in the risk of pterygium for those who frequently use sunglasses compared with individuals who are not using it in most studies (figure 2.6) (Yam and Kwok., 2014). The relevance of sunglasses in particular for persons with higher risk is demonstrated by this degree of protection achieved via the study. The chance of pterygium in rural inhabitants was shown

to be superior to urban. “The effect of lengthy exposure to the sun in rural residents is also demonstrated. Furthermore, it is advised that risk groups learn about the necessity for UV protection using appropriate caps or laterally protected sunglasses (Rezvan,et al., 2018)”.

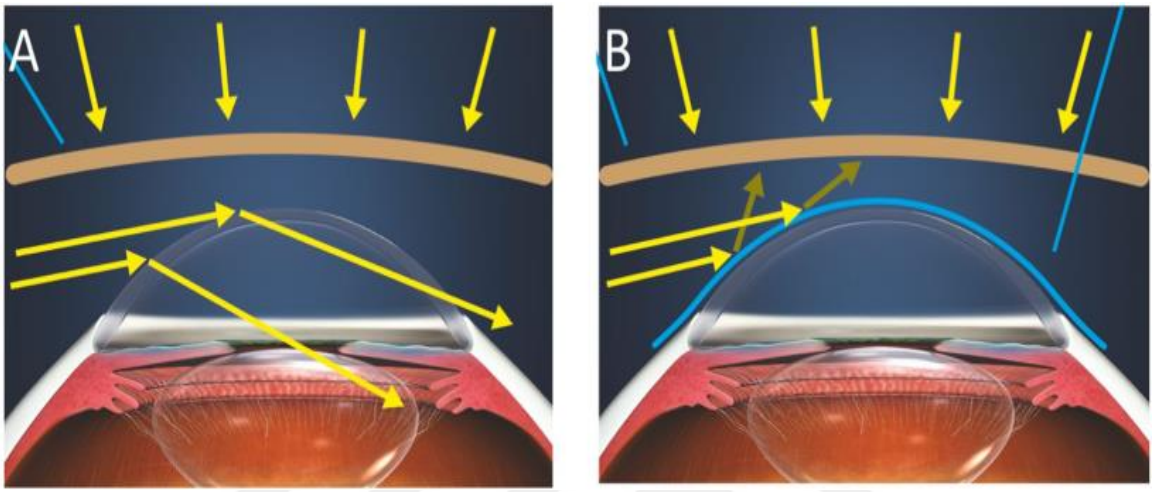

Figure 2. 6 UVB blocking contact lenses effectively block UVA and UVB light. (A) Protective UV-absorbing eyewear, such as sunglasses, alone are not able to protect the ocular surface from UV rays coming from the side direction (depicted in yellow arrows) (B). UV blocking contact lenses offer total protection of the cornea and the limbus and when combined with UV protective eye ware the entire ocular surface is protected.

2.6 WASF3 gene

2.6.1 WASF3 gene identification

An essential function in cell invasion and metastatic activation is played by a family of proteins – the Wiskott-Aldrich syndrome protein (WASP) family. The family comprises of 5 individuals, separated by their resemblance into two subfamilies. The subfamily WASP (WAVE) includes WASF1, WASF2, and WASF3. In the case of all members, the excessive phosphoactivation (e.g., cytokines or growth factor) causes their motifs of C-terminus to be exposed and subsequently interact with numerous factors, including the (Arp) 2/3 complex actin-linked protein (Arp) (Teng, 2021).

The WAS gene sends signals on the production of the WASP protein. All the blood cells have this protein. WASP is engaged in the transmission to the actinic cytoskeleton of a net of fibers forming the structural frame of the cells, of signal from the

surface of the blood cells. WASP signals cause the cell to migrate and connect with neighboring cells and tissues (adhesion). This communication enables the actinic cytoskeleton to create interactions between cells and foreign invaders in white blood cells to defend the body against infection (immune synapse) (Pike and Bethesda., 2020).

Actin cytoskeleton is an important part of a range of cellular activities, including changes in cell form, cytokinesis, mobility, cellular proliferation and membranes trafficking. Fundamental to these activities is the exact regulation of the production, dynamics and structure of actin filaments forming the center of the actinic cytoskeleton. Nevertheless, irregularities in normal Actin regulation, like cancer metastasis and Wiskott-Aldrich syndrome may lead to human illness, to mention just a few. Numerous genes, including those encoding the protein family WASP/WAVE, were shown to be participated in regulating actin-cytoskeleton arrangement (Sossey-Alaoui, 2013).

" WASF3 gene Locates on 13q12.13 And has 14 Exons (figure 2.7). Gene type of wasf3 is protein coding gene which encodes a member of the Wiskott-Aldrich syndrome protein family. The gene product is a protein that forms a multiprotein complex that links receptor kinases and actin. Binding to actin occurs through a C-terminal verprolin homology domain in all family members. The multiprotein complex serves to transduce signals that involve changes in cell shape, motility or function. Broad expression in brain (RPKM 34.0), fat (RPKM 12.7) and 15 other tissues. (Teng, 2021)."

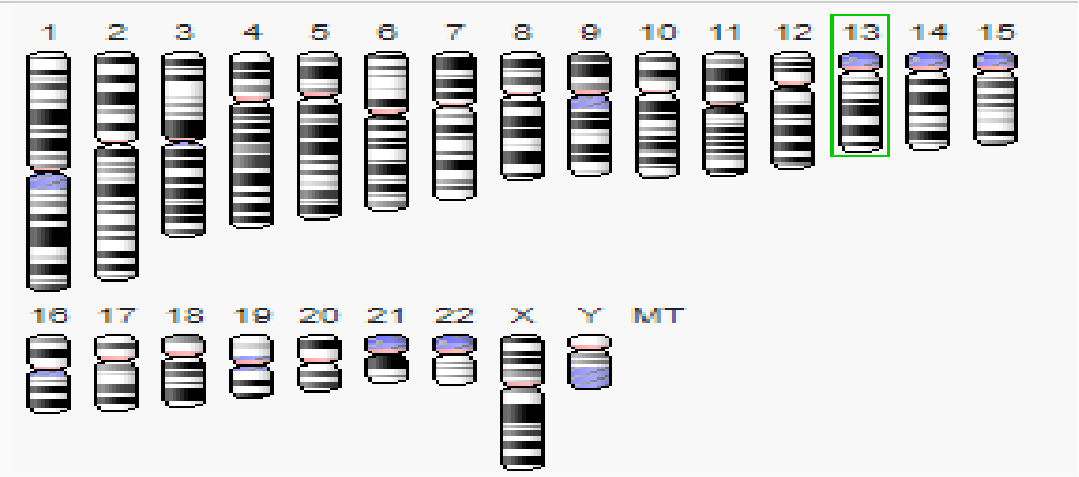

Figure 2. 7

There have been over 350 genetic changes (mutation) in the WAS gene causing Wiskott-Aldrich syndrome, is defined by aberrant (immune deficiency) occupation of the immune system, eczema (an inflammatory skin illness that characterizes the anomalous red, itchy skin patch) and decreased blood clotting capability. The major implications of this disease are men (figure 2.8) (Blunde et al., 2010).

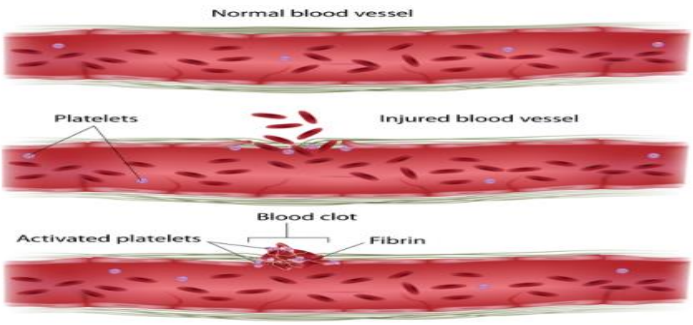

Figure 2. 8 Blood clot formation

Individuals with Wiskott-Aldrich syndrome have microthrombocytopenia, which is a decrease in the number and size of blood cells involved in clotting (platelets). This platelet abnormality, which is typically present from birth, can lead to easy bruising, bloody diarrhea, or episodes of prolonged bleeding following nose bleeds or minor trauma. Microthrombocytopenia can also lead to small areas of bleeding just under the surface of the skin, resulting in purplish spots called purpura, or variably sized rashes made up of tiny red spots called petechiae.

In some cases, particularly if a bleeding episode occurs within the brain, prolonged bleeding can be life-threatening. ("Inactive and active WASF domain confirmations. At resting state, WASFs exist in an autoinhibited closed conformational state due to the interactions between members of the *N*-terminally located WASF homology domain (WHD) and the *C*-terminus. Members of the protein complex at WHD include HSPC300, ABI, NCKAP1 (NAP1), CYFIP1 (SRA1), and WASF1/2/3. Upon activation by Rac, WHD interactions are disrupted and WASFs adopts an open conformation where their verprolin-cofilin-acidic (VCA) region can interact with the actin-related protein (Arp) 2/3 complex and monomeric actin (G-actin).Phosphatidylinositol (Frugtniet et al.2015) (3,4,5)-trisphosphate (PIP3) may also recruit the WASF complex to the membrane by binding to the basic domain (B) of WASFs.

The Arp2/3 complex then initiates actin polymerization and branched filament network formation by generating a new nucleation core and binding to pre-existing filaments. From this branched nucleation process, actin projections at the leading edge of the cell (lamellipodia) and actin matrix-degrading structures (invadopodia) can be subsequently formed and used to facilitate cell motility and matrix remodeling. Under normal conditions, the formation of these structures is strictly governed to safeguard

tissues against disruption and damage. Under pathological conditions, however, cancer cells exploit this process to locally invade and metastasize throughout the body (figure 2.9) (Teng, 2021)." WASF3 Carcinoma Protein needs important proteins like HSP70 and HSP90 for Activation and Stability. Inactivating HSP90 and HSP70 leads to a decline of invasion of a range of tumor cell lines, likely because the molecular chaperone cells that impact the phenotypic have been destabilized by, and yet characterized. The WASF3 gene was already demonstrated to be overexpressed in cancers of top condition and to lose invasion and metastasis by its lower expression (Teng, 2012).

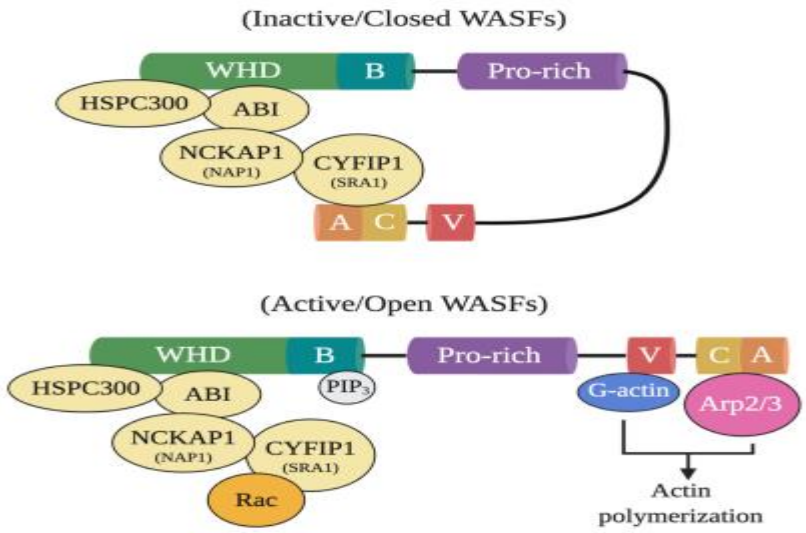

Figure 2. 9Inactive and active WASF domain confirmations.

2.6.2 WASF3 interaction Is Essential for the Survival and Invasion of Cancer Cells

The new actin-regulating proteins (WASP, N-WASP, and WAVE 1, 2, and 3) in the Wiskott-Aldrich syndrome protein WASP family might have a critical and regulatory function in cell shape, cellular membranes actin polymerization, and control, encouraging cell motility, invasions, and metastases. What is confusing is that a few types of the WASP family can serve whether as tumor malignant promoters or silencers according to the kind of tumor and the stage of cancer (Hao et al., 2012).

The earlier investigations have revealed that WASP inadequacies could contribute in anomalous restoration of morphological characteristics as well as cell motility (Miki et al., 1998). Verprolin Homologous Protein 3 (WASF3), which is rich in proline protein and has a strong impact on cell motility, cell shape and actin polymerization, WASF3 is

the key WASP Wiskott-Aldrich Syndrome component. WASF3's function in tumor cell invasion and motility has been established in a number of investigations. Davuluri et al. have shown that WASF3 has been linked to several signal transduction pathways and also that the NF-kB signaling has been implicated in cell migration control. Interaction of WASF3-NFκB is fundamental to invasion, metastasis and cancer (Bing et al., 2020).

WASF3 investigation has shown its over-expression in each kind of cancer investigated, the majority of research has shown that WASF3 is over-expressed in specific kinds of cancer, includes bladder cancer, pancreatic cancer (Huang et al., 2018) , esophageal cancer (Bing et al., 2020), ovarian cancer (Lu et al., 2017), breast cancer (Shibuya et al, 2020), prostate cancer (Herman et al., 2010), hepatocellular carcinoma (Ji a et al., 2015), gastric cancer (Nie et al., 2019), colorectal cancer(Hao et al., 2012), and Intrahepatic Cholangiocarcinoma (Zheng et al., 2016).

WASF3 has been linked to cell cytokinesis, cell migration, and proliferating, and it has also been revealed that WASF3 stimulates the epithelial-mesenchymal transition (EMT) method, which can increase the tumor progression of some cancers (Taylor et al., 2013). A few researches have looked at the methods by which WASF3 impacts the biologic characteristics of specific cancers. Matrix metalloproteinases (MMPs), mitogen-activated protein kinase (MAPK), and Snail, for example, have all been reported to be influenced by WASF3. Thus, WASF3 improves the proliferation, motility and invading capacity of cells in specific cancer types, and the precise mechanisms have parallels and variances (Huang et al., 2018).

2.6.3 WASF3 and MMPs

For many normal physiological processes, e.g., development, cell migration, growth and healing of wounds, destruction of the Extracellular Matrix (ECM) is necessary through MMP activities. on the other side, enhanced MMP activity and production are also linked to tumorigenesis, metastases and angiogenic. Most MMPs usually have modest expression in tissues and are activated only when the extracellular matrix has to be renovated (Reunanen et al., 2002).

It was discovered that WASF3 is required for the control of the production of several MMPs via the MAPK signaling pathway. The expression levels of MMP-1, -3, and -9 were significantly reduced when WASF3 was lost. When these MMPs were inhibited as a result of WASF3 elimination, cell migration and invasion were dramatically reduced in both in vitro wound repair and Matrigel tests.

For the very first moment, proof for a new involvement of WASF3 in the control of MMP activity via the p38 MAPK axis was shown in a research (Teng Y et al., 2011). The position of WASF3 as a crucial factor in cancer development and metastasis has now been established by both mechanical and medical investigations. Medical research has now established WASF3 as a biomarker for BC development and metastasis, and much

significantly, as a driving factor behind the etiology of the severest BC subgroup, TNBC (Sossey-Alaoui and Khalid., 2013).

While JAK-STAT3 signaling controls many aspects of cancer genesis and growth, it also tends to stimulate invasion and metastasis by activating important metastasis-encouraging genes including WASF3.STAT3 increases expression of WASF3, while JAK2 stimulates it separately, which is necessary for invasion. WASF3 activity is required for JAK-STAT3 signaling since its deactivation inhibits invasion in cells that express JAK-STAT3.Upregulation of WASF3 promotes NFB and ZeB1, both of which enhance invasion by regulating target genes implicated in metastasis. NFB commonly works with STAT3 to overexpress metastasis-stimulating genes such cytokines and matrix metalloproteinases, as well as decrease microRNAs that inhibit invasion (figure 2.10) (Teng et al, 2014).

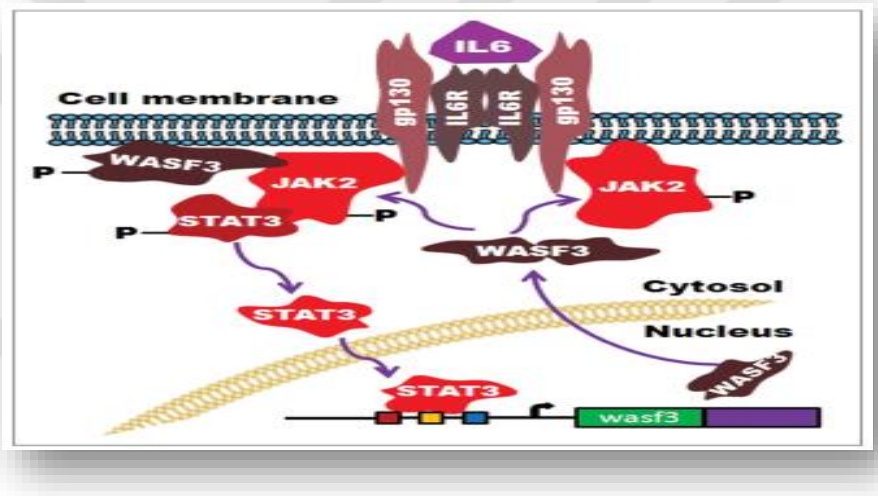

Figure 2. 10 Summary of the iL-6/JAK-STAT interaction with the wASF3 gene. Activation of STAT3 leads to increased expression of wASF3 which is then recruited to the membrane where it is activated by JAK2 to promote invasion.

3. MATERIALS AND METHODS

3.1. MATERIEL

3.1.1 Surgical Technique

"Limbal conjunctival autograft" surgical method was used in this study. Topical proparacaine hydrochloride (Alcaine, Alcon) was applied to all patients 5 minutes before surgery, 2 times at 1-minute intervals. Periocular tissue was cleaned with 10% povidone iodine and attached to blepharos by using a sterile eye cover. The ocular surface was irrigated with 5% povidone iodine and kept for 3 minutes. Later, povidone iodine was removed from the ocular surface with a balanced salt solution. 0.1 cc lidocaine HCl 20 mg / ml + epinephrine 0.0125 mg / ml (Jetokain, Adeka A.Ş.) were injected under the pterygium tissue with a 26 G injector tip.

At the limbus level, the pterygium tissue was entered under the pterygium with Westcott scissors, separated from the sclera and separated from the cornea by blunt dissection starting from the base to the apex. Pterygium residues were removed with superficial keratectomy and the sclera surface was freed from pterygium tissue until the cornea, limbus and medial rectus insertion site with the help of a scalpel. The pterygium was excised with scissors so that the caruncle could not be reached.

The graft area in the upper temporal region was marked with a sterile pen with a distance of approximately 3 mm parallel to the limbus and 5 mm in the conjunctiva parallel to the fornix. Before this procedure, 0.1 cc lidocaine HCl 20 mg / ml + epinephrine 0.0125 mg / ml was injected with a subconjunctival 26 G injector tip. The graft was sutured with single 8/0 vicryl sutures to the conjunctival opening formed where the pterygium tissue was removed, paying attention to the localization of the limbus. The graft area was left for secondary wound healing.

3.1.2. Establishing working groups

Tokat Gaziosmanpaşa University Hospital Eye Diseases 27 patients (9 females, 18 males) with a diagnosis of pterygium who applied to the outpatient clinic were included. The pterygium tissues taken during the operation are placed in the same eye of the patient group and each patient. using the healthy conjunctival tissue as a control group. groups were created.

The necessary permission for the study was obtained by the Tokat Gaziosmanpaşa University Clinical Research Ethics Committee with the project number 19. KAEK-024. The entire study was carried out in Tokat Gaziosmanpaşa University Faculty of Medicine Medical Biology and Genetics laboratories. This thesis work was supported by the Scientific Research Projects Directorate of Tokat Gaziosmanpaşa University with the project number 2019/110.

3.1.3. Storage of tissues

During surgery, with the permission and knowledge of the participants involved in the study Tissue samples taken are quickly divided into desired pieces and frozen in liquid nitrogen and then stored at -80 ° C for work. One of the fragmented tissues part of it was sent to the pathology laboratory for histopathological examination. Belong to each patient the pterygium tissues were divided into two parts as head and trunk. These tissue samples, which were divided into smaller pieces, were pooled into one group. This group is RNA isolation followed by cDNA extraction and expression analysis. Likewise, a pool system was created for the control group and the tissues were divided into a group for RNA analysis and removed to -80 ° C to be studied.

3.1.4 Histopathological Examination of Tissue

Pterygium excisional biopsy materials were dissected into two equal parts appropriately, and one half was fixed in 10% buffered formalin solution for 24 hours, and then tissue follow-up was performed. Routine tissue follow-up was created from the stages of detection, dehydration, transparency and infiltration. These stages were carried out on an automatic tissue tracking device.

Tissue samples, whose tissue follow-up procedure was completed, were turned into paraffin blocks and sections cut in a microtome at a thickness of 4 micrometers were taken and stained with hematoxylin-eosin dye. The diagnosis of pterygium was histopathologically confirmed by observing areas of capillary vascular proliferation consisting of endothelial cell proliferation in mixed areas within fibroblastic stromal areas covered with conjunctival epithelium in preparations ready for microscopic examination. In the histopathological image of the tissue with pterygium given in Figure 3.1 above, numerous congested ectasic capillary vascular areas were detected in the stromal areas under the conjunctival epithelium.

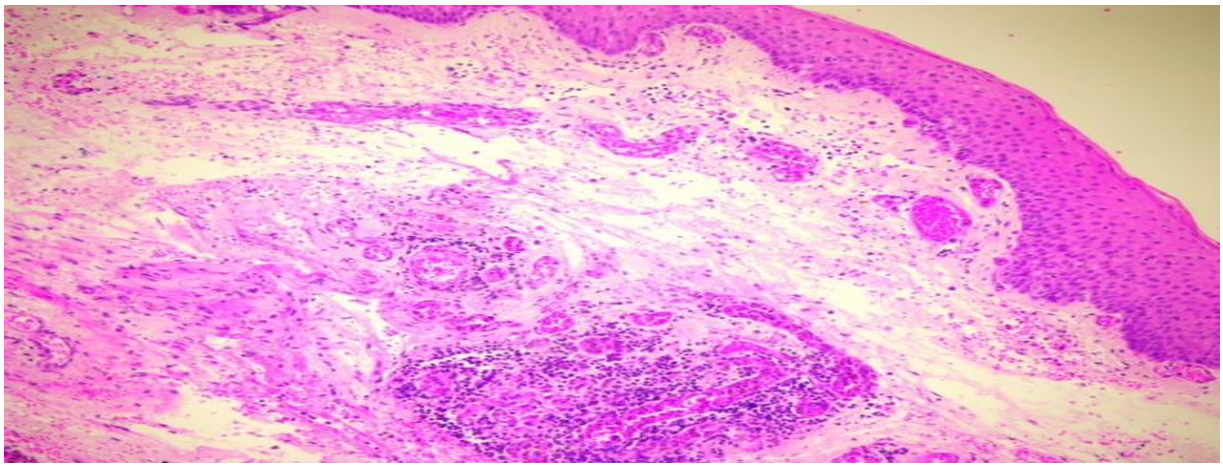

Figure 3. 1 Histopathological Image of Pterygium Tissue (HE, X100).

3.1.5. Devices used in the study

- homogenizer (next advance storme 24, BBY24M-CF, ABD)
- Real Time PCR (Applied Biosystems, UK)
- PCR (Thermal Cycle Device, Techne, UK)
- PCR (Thermal Cycle Device, Biometra)
- Iblot Gel Transfer System (Invitrogen, IB301001, Australia)
- Vortex (Velp, Scientifica, F20220176, Italy)
- Centrifuge (Hettich, Germany)
- Refrigerated Centrifuge (Hettich, Germany)
- Qubit 2.0 Fluormeter (Invitrogen, Life Technology, Australia)
- Qubit 3.0 Fluormeter (Invitrogen, Life Technology, Australia)
- Plate (Applied Biosystems, 4346907, UK)
- Plate Adhesive (Applied Biosystems, 201405092, UK)
- Ph Meter (Hanna Instruments, HI2020W, USA)
- Automatic Straws (Gilson, France)
- Automatic Pipettes (ABT, Turkey)
- Autoclave (HMC, HV25, Germany)
- Microwave Oven (Arcelik, MD554, Turkey)
- Magnetic Stirrer (Velp Scientifica, F20520162, Italy)
- Precision Balance (Kern ABT, Wb0750631, Germany)
- Power Supply (Biorad, 1645070, USA)
- Oven (Memmert, Germany)
- Distilled Water Device (Elgaoption Q7, UK)
- Vertical Electrophoresis Set (XCell SureLock, Invitrogen, Australia)
- Horizontal Electrophoresis Set (SUB-GEL Midi, USA)
- Heated Shaker (Thermoshaker, UK)
- Refrigerator (Arcelik, 570 465, Turkey)
- -20 ° C freezer (Arcelik, 2052dy, Turkey)

- -80 ° C Deep Freezer (Nuaire, Nu9483e, USA)

3.1.6. Consumables and chemicals used in the study

-RNA Isolation Kit

- GeneAll brand RiboEx (301-001)
- Hybrid-R (305-101)

-**For cDNA synthesis**, A.B.T. brand cDNA Synthesis Kit with RNase Inh. (High Capacity) (C03-01-05)

- 10X Reaction Buffer
- dNTP Mix (2.5 mM)
- Random hexamer (50 μM)
- Reverse Transcriptase (200 U / μl)
- RNase Inhibitor
- RNase free Water
- Rnase Template

- **PCR reaction for real time PCR44**

- A.B.T. ™ 2X qPCR SYBRGreen MasterMix (with ROX)
- 1X ROX Dye (50X)
- Forward Primer
- Reverse Primer
- Template cDNA
- RNase-free Water

3.2. METHOD

The tissues taken during the surgery were separated into two parts and kept at -80 ° C. A group of tissue pieces for RNA extraction and gene expression analysis was used.

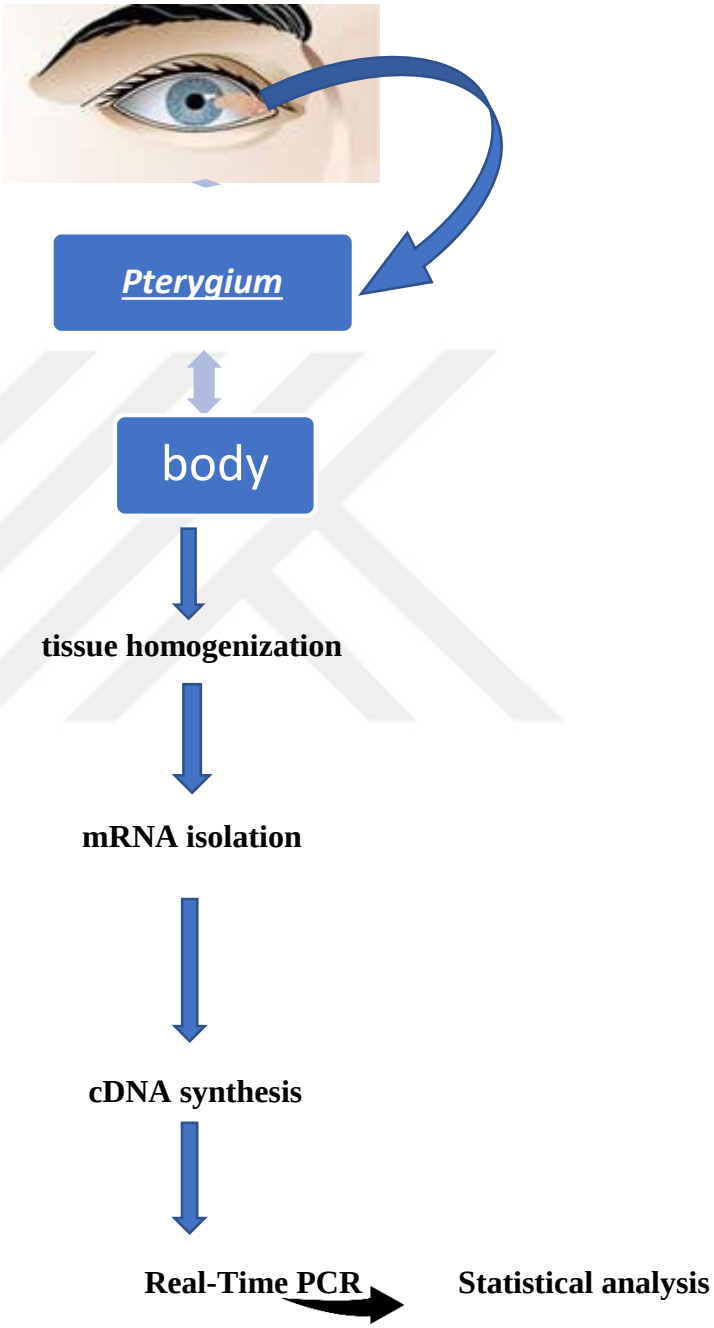

Figure 3. 2 Thesis work general flow chart

3.2.1. mRNA isolation from tissue

GeneAll brand RiboEx (301-001) and Hybrid-R (305-101) kits for mRNA isolation It was used and the isolation steps were done in accordance with the kit protocol as given below has been carried out.

mRNA isolation protocol:

1. Tissue samples were divided into small pieces with a scalpel and homogenized.

1 mL of RiboEx was added to them and vortexed.

2. Then the sample mixtures at room temperature for 5 minutes. incubated.

3. Mixture 11.000rpm, 10 min. It was centrifuged at 4 ° C and the supernatant was transferred to another tube has been transferred.

4. 200 µL of chloroform was added on the mixture and mixed for 2 min. incubated.

5. It was centrifuged at + 4 ° C at 12,000 × g for 15 minutes. The supernatant was transferred to a clean tube.

6. RB1 Buffer was added as much as the volume of the supernatant. It was mixed by pipetting.

7. 700 µL of the mixture was transferred to the column.

8. 30 sec. At 10,000 × g. centrifuge was done. The collection tube was replaced with a new one.

9. 500 µL of SW1 Buffer was added on the column.

10. 30 sec at 10,000 × g. centrifuge was done. The collection tube was replaced with a new one.

11. 500 µL of RNW Buffer was added onto the column.

12.30 sec at 10,000 × g. centrifuge was done. The collection tube was replaced with a new one.

13. 1 min at 10,000 × g to remove wash solutions remaining in the column.

centrifuge was done. The column was then moved into a clean tube.

14. 50 µL of RNase-free water was added onto the column and 1 min. kept at room temperature.

15. Then at 10,000 × g for 1 min. mRNA samples obtained by centrifugation frozen at -80 ° C

for storage.

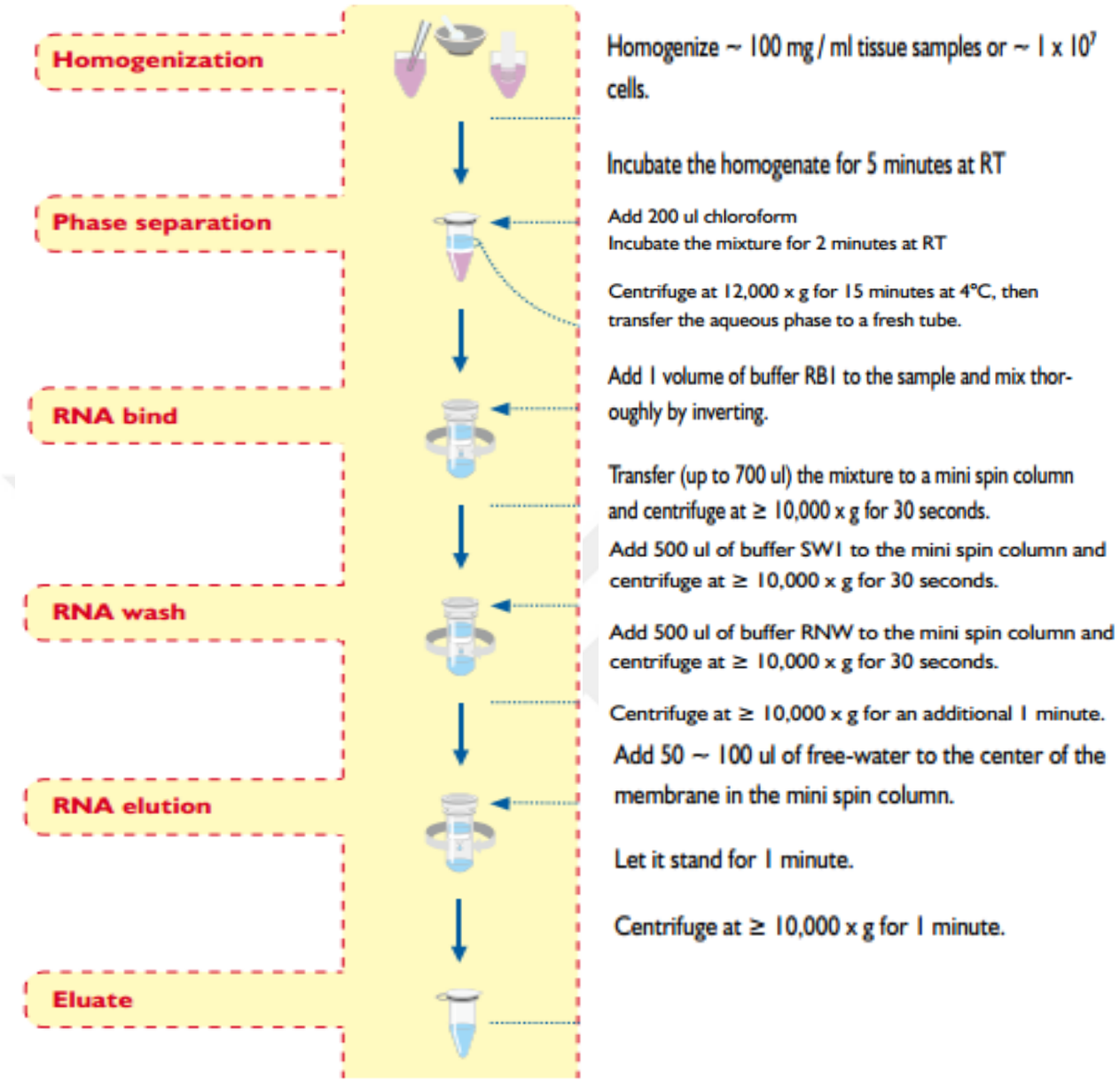

Figure 3. 3 Schematization of RNA isolation protocol summary

mRNA concentration measurement with Qubit HS RNA

RNA measurements were performed using the ABP iQuant ™ RNA HS Assay kit. The necessary procedures for DNA measurement were performed. Standardization of measurement and Standards must be introduced to the device in order to do it correctly. All components are out of use first brought to room temperature. Primarily 1µl iQuant Reagent and 199 µl per sample iQuant buffer was mixed and vortexed. This solution is both standard and sample prepared in the measurement.

For standards, 190 µl of the solution was taken into 0.5ml tubes. 10 µl Standard1 and Standard2 was prepared in separate tubes and standard readings were made on the device. RNA kit57 for Standard 1: 0 ng / µl, Standard2: 10ng / µl. Reading to the device as if these are examples control is provided by making it. For sample measurement; sample tubes in the range of 1µl-10µl according to the quantity available. It was completed to 200 µl with Working solution. With a finger tap the sample was mixed well. Vortex is not preferred. Thus, the samples whose measurements are completed are ready for the next experimental steps has arrived.

3.2.2. cDNA synthesis

After mRNA isolation, cDNA synthesis phase was started. for cDNA synthesis A.B.T. brand cDNA Synthesis Kit with RNase Inh. (High Capacity) (C03-01-05) kit The manufacturer's kit protocol was applied for the synthesis process.

CDNA synthesis was done in two steps:

1. Quantity information of the kit used for cDNA synthesis is given in the table below.

Table 3.1 Reverse transcriptase reaction volume (20 µl)

Reverse Transcriptase Reaction.	Volume
10X Reaction Buffer	2 µl
dNTP Mix (2.5 mM)	1 µl
Random hexamer	2 µl
Reverse Transcriptase (200U/µl)	1 µl
RNase Inhibitor	0.5 µl
RNase free Water	3.5 µl
RNA example	10 µl

2. After the cDNA mastermix was prepared on ice, the following conditions were followed.

Table 3. 2 PCR program applied for cDNA synthesis RT Steps Temperature (° C) Time Cycle

RT Steps	**Temperature (°C)**	**time**	**Cycle**
Step 1	25	10 min	1
Step 2	37	120 min	1
Step 3	85	5 min	1
Step 4	4	∞	1

The cDNA samples obtained were frozen at -80 ° C until the Real-Time PCR step.

cDNA concentration measurement with Qubit HS dbDNA

CDNA measurements were performed using the ABP iQuant ™ dbDNA HS Assay kit. The necessary procedures for DNA measurement were performed. Standardization of measurement and Standards must be introduced to the device in order to do it correctly. All components are out of use first brought to room temperature. Primarily 1µl iQuant Reagent and 199 µl per sample iQuant buffer was mixed and vortexed. This solution is both standard and sample should be prepared in the measurement.

For standards, 190 µl of the solution was taken into 0.5ml tubes. 10 µl Standard1 and Standard 2 It was prepared in separate tubes and standard readings were made on the device. for cDNA kit Standard 1: 0 ng / µl, Standard2: 10ng / µl. Reading to the device as if these are examples control is provided by making it. For sample measurement, according to the amount we have, samples in the range of 1µl-10µl were taken into tubes. over 200 µl with Working solution. With a finger tap for example It was mixed well. Vortex is not preferred. Thus, the samples whose measurements are completed are ready for the next experimental steps. has arrived.

3.2.3. qRT-PCR step

Real time for expression analysis after cDNA extraction from isolated RNAs The PCR phase has started. At this stage, control and pterygium tissues for the target WAVE-3 gene. Beta-actin was used as an internal control with groups. As the reaction mixture A.B.T. ™ 2X qPCR SYBR-Green MasterMix (with ROX) (Cat no: Q03-02-01) used. The reaction mixture was prepared as follows.

Table 3. 3 PCR reaction components (20 µl)

A.B.T.™ 2X q PCR SYBR Green MasterMix (with ROX)	10 µl
1X ROX Dye (50X)	0.4 µl
Forward Primer	1 µl
Reverse Primer	1 µl
cDNA template	3 µl
Rnase-free Water	4.6 µl

The reaction cycle was set up on the Applied Biosystems Real Time PCR instrument as follows: The qRT-PCR program applied

50°C 20 minute

95°C 10 minute

95°C 15 second ┐ 40 cycle

55°C 1 minute ┘

Melt Curve: 95°C 30 second, 60°C 15 second

4.RESULTS

4.1. FINDINGS ABOUT THE WORKING GROUPS

Twenty-seven patients who were diagnosed with pterygium at Tokat Gaziosmanpaşa University Hospital Ophthalmology Clinic were included in this study. The entire study was conducted in Tokat Gaziosmanpaşa University, Faculty of medicine, Medical Biology and Genetics laboratories.

The patients included in the study, 9 (33%) were female and 18 (66%) were male, and their mean age was found to be 58 ± 8.43, although the age range varies between 43-78. Demographic data of patients with pterygium included in the study Table 4.1. Figure 4.1 and Figure 4.2. 'Is also given.

Table 4. 1 Demographic data of patient groups with pterygium

pterygium (n=27)	
Age	58 ± 8.43
Sex	9 female / 18 male

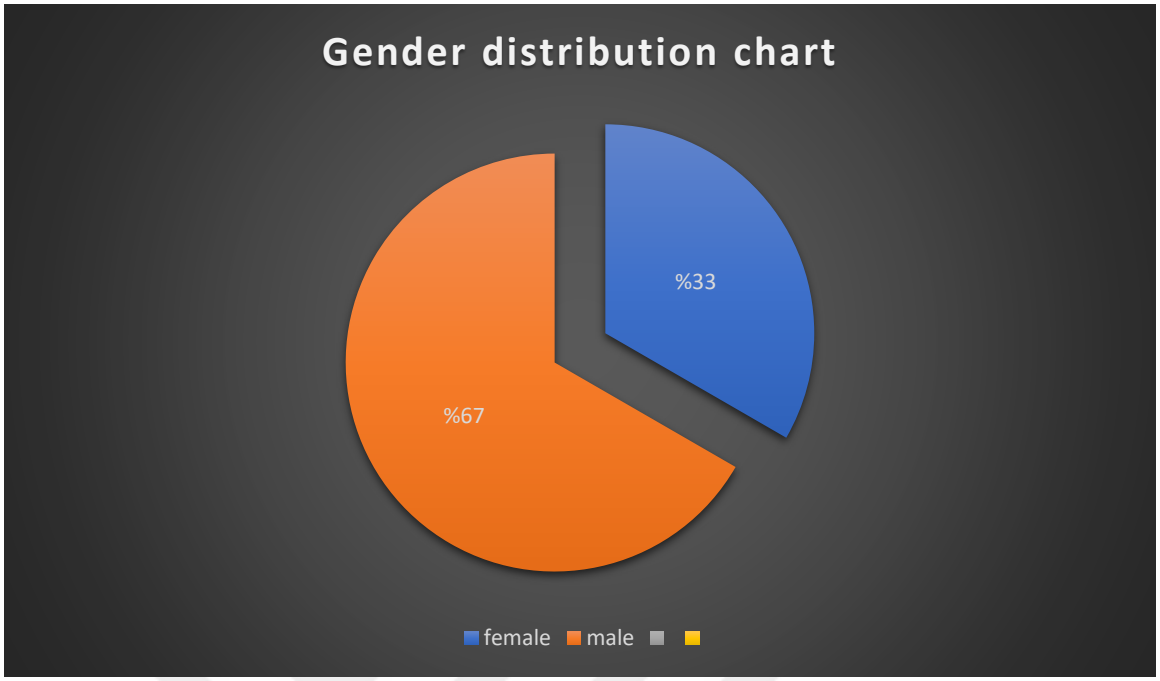

Figure 4. 1 Gender distribution chart of the patient groups with pterygium

4.2. HISTOPATHOLOGICAL EXAMINATION OF PTERJIUM TISSUES

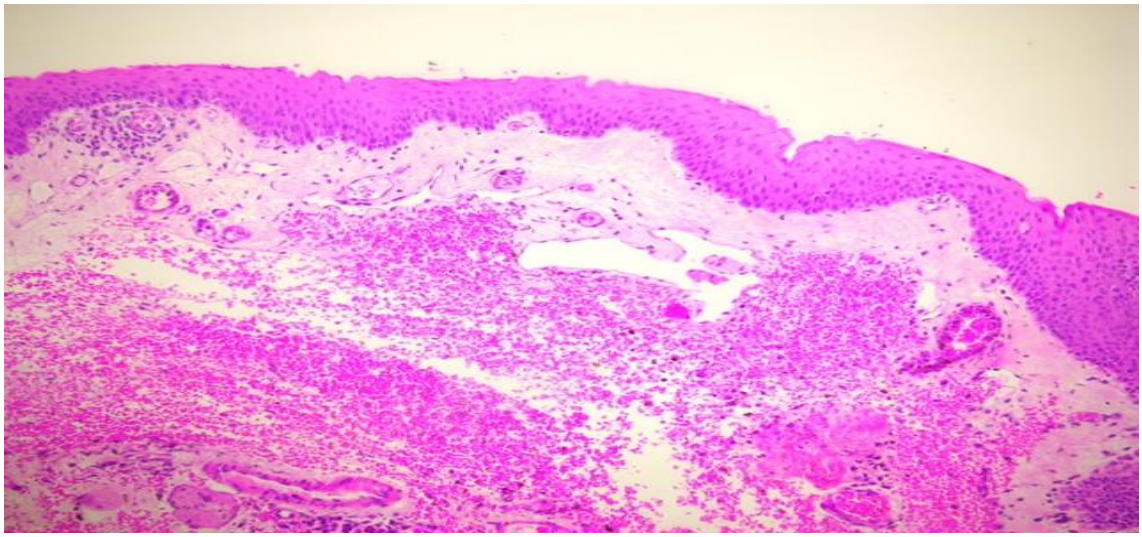

Figure 4. 2 *Histopathological view of the tissue with pterygium (H&E, X100).*

In the histopathological image of the tissue with pterygium given in Figure 4.3 above, numerous congested ectasic capillary vascular areas were detected in the stromal areas under the conjunctival epithelium.

4.3. QUANTITATIVE REAL-TIME PCR (qRT-PCR) ANALYSIS FINDINGS.

4.3.1. QRT-PCR image and evaluation of the WASF3 gene

SYBR Green based quantitative Real-Time PCR method was used to determine the expression level of WASF3 mRNA expression. For WASF3 and the gene preferred as reference (Actin: ACTB), samples of different concentrations of cDNA (1/10: 1/100: 1/1000 and 1/10000) were prepared and serial dilution was made. For each instance, Applied Biosystems Step One Plus.

Amplification efficiencies were calculated by performing 3 repeated readings on the device. After calculating the amplification efficiencies, cDNA and standard samples of pterygium and control conjunctiva tissues were studied in 3 repetitions and the mean CT (cycle threshold) value was taken. In the study, as the reference (control) gene; ACTB gene: as negative control; Real-Time mix without cDNA template and finally for positive control; An example with a good result was used before. In the figures below, the amplification curve and melting peak of ACTB and WASF3 gene observed as a result of qRT-PCR are given, respectively (Figure 4.3, Figure 4.4, Figure 4.5, Figure 4.6).

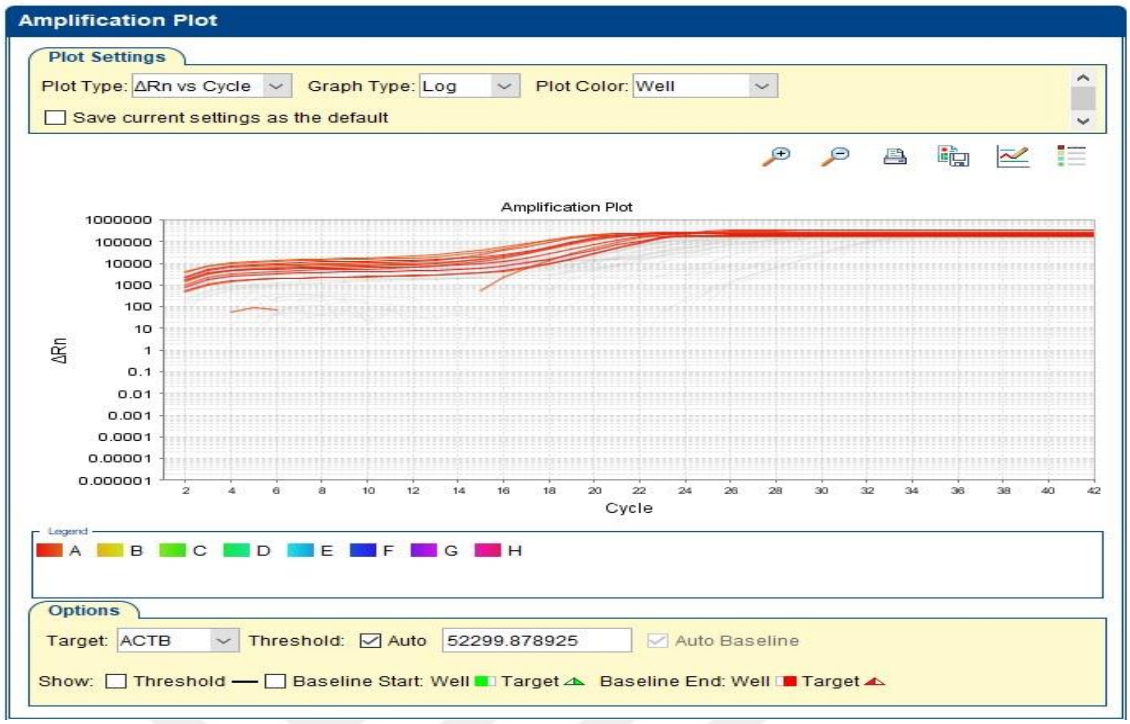

Figure 4. 3 *Belong to the ACTB gene amplification curve.*

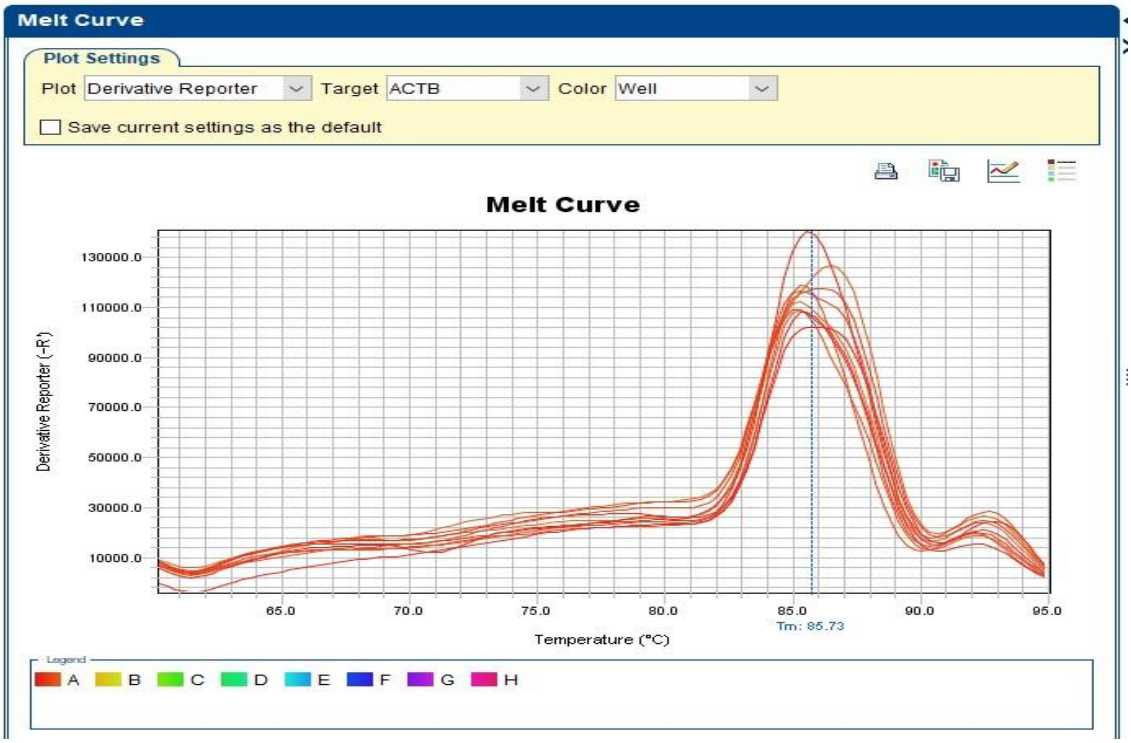

Figure 4. 4 ACTB melting peak of the gene

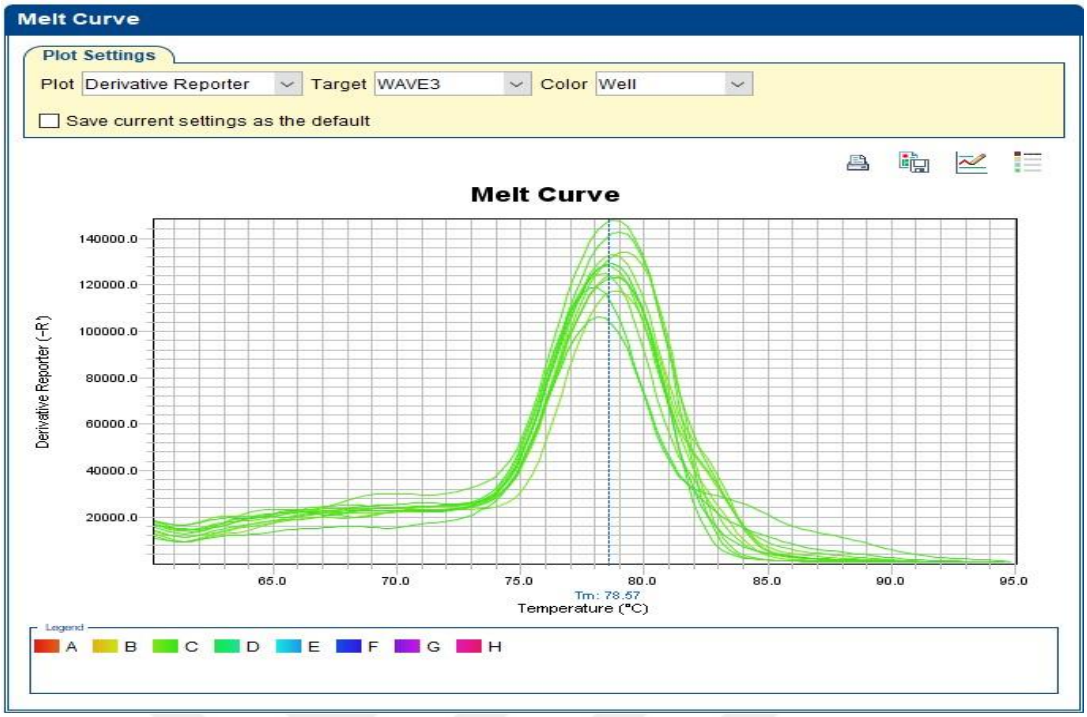

Figure 4. 5 To Wave-3 genes amplification curve

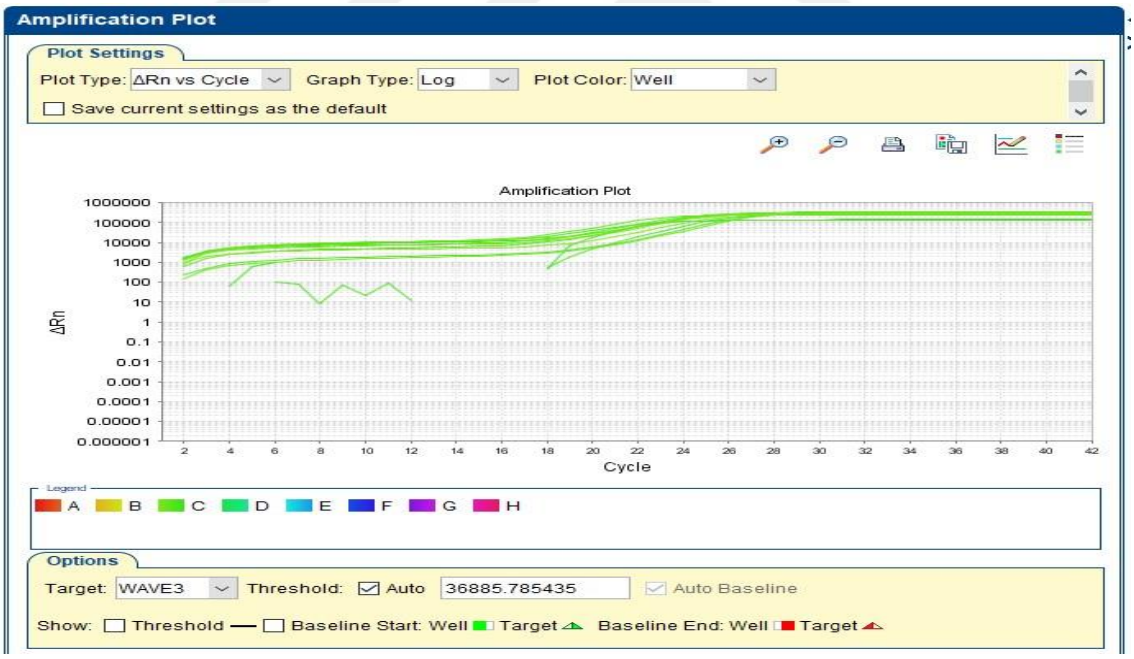

Figure 4. 6 Figure 4. 7.Melting peak of Wave-3 gene

Table 4. 2 Pterygium Tissues Ct Values

SAMPLE GROUP	2^-ΔΔCt WASF3 (Fold Change)
G1	2.11696879
G1	2.11696879
G1	2.11696879
G2	1
G2	1
G2	1
G4	1.797510253
G4	1.797510253
G4	1.797510253
G5	0.96996191
G5	0.96996191
G5	0.96996191
G6	1.442928687
G6	1.442928687
G6	1.442928687
G7	1.340712592
G7	1.340712592
G7	1.340712592
G8	0.948684315
G8	0.948684315
G8	0.948684315
G10	1.272794935
G10	1.272794935
G10	1.272794935
P1	0.923382311
P2	0.444729497
P3	0.288771446
CONTROL	1

4.3.2. Statistical analysis of qRT-PCR findings of the Wave-3 mRNA gene

QRT-PCR analysis was performed for the expression of the WASF3 gene at the mRNA level in the pterygium and control conjunctiva tissues. QRT-PCR results of WASF3 and ACTB genes were quantified with the step one plus software v2.3 program. The data belonging to the WASF3 gene was normalized with the ACTB gene and the 2-ΔΔCt formula was used to calculate the fold change. Statistical analysis was performed with the data obtained from this process. All data were given as mean ± SEM. Analysis of the results was made according to the range 0.9 - 1.1. The expression level of the relevant gene is decreased in the pterygium tissue compared to the normal conjunctival tissue in values less than 0.9, it does not change when compared to normal conjunctival tissue in the range of 0.9-1.1, and an increase in the expression of the gene compared to normal conjunctival tissue at values higher than 1.1 was accepted as). Statistical analysis of expression data was performed using SPSS 16.0 software. The significance levels of the data were analyzed with Student's t test. The statistical significance level was accepted as 0.05, if $p \leq 0.05$ was significant, and if $p > 0.05$, it was evaluated that there was no difference.

Table 4. 3 Statistical analysis of 2-ΔΔCt values of WASF3 head and body tissues

Gene	Tissue
	pterygium (n=27)
WASF3 (mean ± SEM)	(0.271 ± 0.089)
P	**0.004**

All values are given as mean ± SEM. Statistically significant data are expressed in bold.

All values are given as mean ± SEM.

According to the results of qRT-PCR analysis (Table 4.3); When the expression level of the *WASF3* gene was compared with the pterygium and normal conjunctival tissues, it was found to be decreased in pterygium tissue compared to the normal conjunctival tissue (0.271 ± 0.089 fold change, p=0.004)) Figure 4.7, Statistically significant in terms of *WASF3* gene mRNA expression level (p=0.004). and the *WASF3* gene was found to be 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 ($p \leq 0.05$).

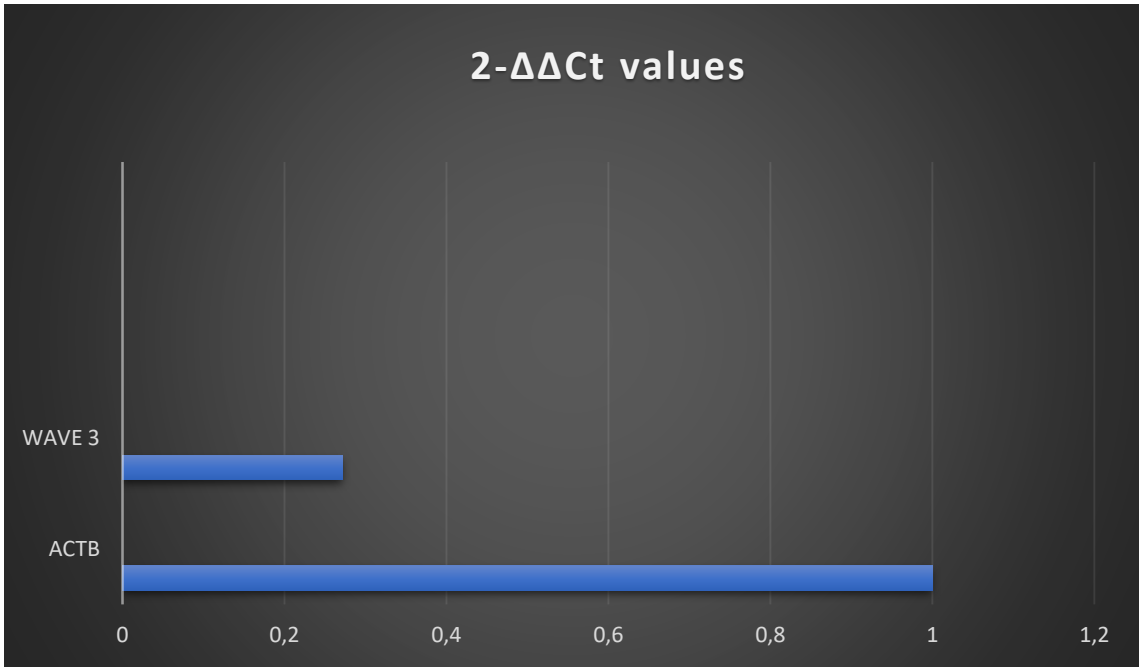

Figure 4. 7 Average of 2-ΔΔCt values of WAVE 3 (*0.271*) and ACTB (1.00) genes.

5.DISCUSSION

A vascular pink plump conjunctival development that extends into the cornea called a pterygium. There are no symptoms of most pterygias. However, the eye irritates or affects the development of the cornea occasionally, which can damage both vision and look (Ca´rdenas-Cantu´ et al., 2014).

pterygium can interfere with the view if it develops into the line of view (over the pupil's opening). Pterygiums arise mostly frequently from the inside (nasal) layer of the eye and reach to the pupil. They may also happen on the eye's side (toward the ear) (Teng et al., 2011).

The benign conjunctival alteration, winging skin, generally spreads to the horn from the inner border of the eyelid. Intensive inflammation from UV radiation/sunlight frequently stimulates the development of pterygium. Additional variables might even play a crucial part, including as genetic structure, eye damage and wind or poisoning exposure. Initially, almost no complaints are received and the alteration is more probably to be seen as visually disturbing. The initial signs show as feelings of foreign body if the cornea is also damaged, then there might be visual deficiency later (Pajic et al., 2017).

In regions closer to the equator, where lengthy exposure to radiation, especially ultraviolet (UV) radiation, pterygium is much more frequent. Others also think persistent inflammation of the eye might play an essential part. Pterygium occurs more frequently in persons, particularly in sunny conditions, who occupy their time outside. pterygium is Normal tumors (not cancerous). therefore, there is no pterygium penetration in ocular, sinus or brain. Pterygium does not expand to other bodily regions (metastasize) (Teng et al., 2011).

Pterygium has cancer resemblances, such as cell growth, invasion and post excision recurrence. The pterygium epithelial cellular layer was over-expressed in comparison to healthy conjunctivitis with proteins associated with enhanced proliferation activity (Ki-67, nucleic antigen [PCNA] and erythropoietin), low levels of cellular apoptosis (Bcl-2 and p 53), and malignant disease (Hsp90) (Kim KW .. S., 2013). These proteins were discovered to be elevated in many forms of cancer (Lester RD .. J., 2005) and utilized for an evaluation of the proliferative activities of a tumor (Ca´rdenas-Cantu´ et al., 2014)

WASF3 is one of very few biological molecular factors controlling various metastatic processes. It has been shown that WASF3 is a major regulator for previously awarded metastasis-related traits to other proteins as well as that inactivating it is enough to suppress cancer invasion and metastasis. Identifying pathways that promote tumor metastasis through the WASF3 gains and activation gives us with a greater knowledge of tumor progression biology and the adaptation necessary to enhance therapy approaches (Teng and Yong., 2021).

There have been over 350 genetic changes (mutation) in the WAS gene causing Wiskott-Aldrich syndrome, is defined by aberrant (immune deficiency) occupation of the immune system, eczema (an inflammatory skin illness that characterizes the anomalous red, itchy skin patch) and decreased blood clotting capability. The major implications of this disease are men (Blunde et al., 2010).

In a research, they explored if ophthalmic pterygium is a biomarker of greater risk of cutaneous melanoma (CM) and might thus aid in early diagnosis. The research utilized the capacity of whole population health data to evaluate if the formation of a pterygium indicates that it either malignancy or in situ CM is more likely to develop. After adjusting for gender, ages at pterygium treatments, and home postcode, a logistic regression evaluation revealed that persons who received pterygium medicine had a 24 percent higher probability of receiving a CM diagnoses.

Consequently, 23 625 individuals (64 percent men) were treated with pterygium in WA hospitals. Pterygium diagnoses and/or treatment occurred at a median age of 49 years (ranging 14–96). In the pterygium group, there were substantially more CM patients than in the control group (1083 vs 874; p0.001). After controlling other determinants, there was a 24 percent higher in the chances of developing a CM in the pterygium group compared with the control group in a logistic regression analysis (OR 1.24, 95 percent CI 1.1 to 1.4). When compared to the control group, the incidence rate ratio (IRR) of a malignancy CM diagnoses was 20% higher in persons who had a pterygium treated (IRR 1.2, 95 percent CI 1.0 to 1.4). at the end of the study, the existence of a pterygium elevates the probability of gaining CM significantly. Patients with growing pterygia should be informed about their higher risk of developing pterygia, and frequent skin monitoring should be recommended (Crewe et al., 2018).

WASF3 has been linked to cell cytokinesis, cell migration, and proliferating, and it has also been revealed that WASF3 stimulates the epithelial-mesenchymal transition (EMT) method, which can increase the tumor progression of some cancers (Molly., 2013). A few researches have looked at the methods by which WASF3 impacts the biologic characteristics of specific cancers. Matrix metalloproteinases (MMPs), mitogen-activated protein kinase (MAPK), and Snail, for example, have all been reported to be influenced by WASF3. Thus, WASF3 improves the proliferation, motility and invading capacity of cells in specific cancer types, and the precise mechanisms have parallels and variances (Huang et al., 2018).

It was discovered that WASF3 is required for the control of the production of several MMPs via the MAPK signaling pathway. The expression levels of MMP-1, -3, and -9 were significantly reduced when WASF3 was lost. When these MMPs were inhibited as a result of WASF3 elimination, cell migration and invasion were dramatically reduced in both in vitro wound repair and Matrigel tests. For the very first moment, proof for a new involvement of WASF3 in the control of MMP activity via the p38 MAPK axis was shown in a research (Teng et al., 2011). The position of WASF3 as a crucial

factor in cancer development and metastasis has now been established by both mechanical and medical investigations. Medical research has now established WASF3 as a biomarker for BC development and metastasis, and much significantly, as a driving factor behind the etiology of the severest BC subgroup, TNBC (Sossey-Alaoui., 2013).

While JAK-STAT3 signaling controls many aspects of cancer genesis and growth, it also tends to stimulate invasion and metastasis by activating important metastasis-encouraging genes including WASF3.STAT3 increases expression of WASF3, while JAK2 stimulates it separately, which is necessary for invasion. WASF3 activity is required for JAK-STAT3 signaling since its deactivation inhibits invasion in cells that express JAK-STAT3 (Teng et al., 2014).

Lack of WASF3 function in the breasts lead to loss and reduction of the metastatic potential of invasive phenotypes. By employing oligonucleotide ratings, it has been proven, with correspondingly decreased invading and lost matrix Metalloproteinases activity (MMP)-9, that the knockdown from WASF3 causes to overexpression of the KISS1 metastasis suppressor gene. The relationship between WASF3 Knockdown and KISS1 overexpression proposes one VEGF control mechanism.WASF3's downregulation showed a lower expression of VEGF in vivo, which substantially decreased the quantity and size of micro-vessels in the tumor forming from those cells. The loss of WASF3 also led to increased cytoplasmic IjBa and decreased nucleus nuclear factor-kappa-B (NF-jB) p65/50. Cell invasion was related with the tumor necrosis factor alpha (TNF-a). via promotion of the MMP-9 synthesis by KISS1 regulation of the NFjB pathway. The discovery also explains the decreased tumorigenicity of deficient WASF3 cells. The study of gene expression showed more than 35 dysfunctional genes associated in tumor invasion and metastasis implicated as a result of WASF3 down regulation (Teng et al., 2011).

The function of WASF3 in the susceptibility of stomach cancer to oxaliplatin, as well as the underlying processes, was examined in a research. They suppressed WASF3 in MGC803 cells by utilizing WASF3-siRNA. The WASF3 silencing effects on proliferation, migration, invasion and apoptosis of MGC803 cells, were then studied in a CCK-8, flow cytometry and transwell experiment. In addition, the study examined the possible mechanism in vitro to see if WASF3 causes oxaliplatin sensitivity. The use of short interfering RNA to silence WASF3 reduced the migration, proliferation, and invasiveness of gastric cancer cells. It is also discovered that knocking down WASF3 increased oxaliplatin sensitivity and accelerated cell death (apoptosis). In addition, the WASF3 knockdown-induced oxaliplatin sensitivity was dependent on Atg12-mediated autophagy suppression. Eventually, the study's findings show that targeting WASF3 is a novel possible treatment approach for stomach cancer (Nie et al., 2020).

Overexpression of miR-217 inhibited osteosarcoma cell proliferation, migration, and invasion, according to findings of a research. Knock down of miR-217 expression, on the other hand, dramatically increased cell proliferation, migration, and invasion.

WASF3 was also discovered to be a new functional pathway target of miR-217. The suppression of cell proliferation and invasion mediated by miR-217 can be partially reversed by abnormal (ectopic) expression of WASF3. Taken together, the findings show that miR-217 acts as a tumor suppressor miRNA that targets WASF3 to prevent osteosarcoma carcinogenesis (Shen et al., 2014).

The expression of WASF3, an actin cytoskeleton remodeling and metastasis promoter protein, is controlled by miR200 microRNAs, according to this study. In invasive vs non-invasive cancer cells, there was a significant inverse connection between WASF3 and miR200 microRNA expression levels. MiR200 suppresses WASF3 mRNA expression by directly targeting the 3'-untranslated regions. The loss of WASF3 expression leads in a large decrease in the invasive phenotype of cancer cells, which is unique to the loss of WASF3 expression, which is mediated by miR200. MiR200-mediated suppression of cancer cell invasion is reversed when a miR200-resistant WASF3 is re-expressed. Loss of WASF3 expression pathway of miR200 also leads in a significant shift in cell shape, which is similar to mesenchymal to epithelial transition. Finally, a unique method for the regulating of WASF3 expression has been discovered in cancer cells, which regulates cancer cells' invasive characteristics and shape, as well as their metastatic spread(Sossey-Alaoui,et al., 2009).

WASF3 is one of very few biological molecular factors controlling various metastatic processes. It has been shown that WASF3 is a major regulator for previously awarded metastasis-related traits to other proteins as well as that inactivating it is enough to suppress cancer invasion and metastasis (Teng et al, 2021). In our current study, the expression level of *WASF3* in pterygium tissue were found to be statistically significantly lower than in the normal conjunctiva ($p=0.005$). Considering all these data, our study results support the results that *WASF3* expression is decreased in pterygium tissue compared to the normal conjunctiva.

Our results may provide a molecular basis for a better understanding of the pathogenesis of pterygium. The fact that WASF3 play an essential function in cell invasion, metastatic activation and our findings as its expression is low in pterygium tissue support the neoplastic feature of pterygium, which is a tumor-like tissue, and provide a molecular basis for the development of pterygium, the pathology of which is still unclear. This decrease in WASF3 expression level may be caused by mutations in the WASF3 gene. In this context, we think that our results should be supported by further studies including WASF3 expression studies, mutation analysis, WASF3 and tissue culture that will investigate the possible relationships of pterygium with the proteins in the cellular pathways in which it is effective. Identifying pathways that promote tumor metastasis through the WASF3 gains and activation gives us with a greater knowledge of tumor progression biology and the adaptation necessary to enhance therapy approaches.

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