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DETERMINANTS OF PTERYGIUM OCCURRENCE AND RECURRENCE IN A RURAL AFRICAN POPULATION

Peter Anguria

A Thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree

of

Doctor of Philosophy

Declaration

I, Peter Anguria, declare that this thesis is my own work. It is being submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted previously for any degree or examination at this or any other University.

.....

Peter Anguria

12 th day of May 2015

Dedication

This thesis is dedicated to my family

PRESENTATIONS

The following presentations at international and national meetings arose from the database of this study.

1. Anguria P, Ntuli S, Carmichael T. Epidemiology of pterygium occurrence in a rural African Population: preliminary report. Ophthalmological Society of Southern Africa Congress, Sun City 2010, February 11.
2. Anguria P, Ntuli S, Stegmann F, Carmichael T. Association of pterygium extent and recurrence time with pterygium recurrence in African patients. Ophthalmological Society of Southern Africa Congress, Port Elizabeth 2011, March 25.
3. Anguria P, Ntuli S, Interewicz B, Carmichael T. Mode of inheritance in pterygium occurrence. Poster, Research day, University of the Witwatersrand 2012, September 19.
4. Anguria P, Ntuli S, Kitinya J, Carmichael T. Inflammation in pterygium occurrence. Ophthalmological Society of Eastern Africa Congress, Kampala 2012, August 23.

PUBLICATIONS

This is a list of publications arising from the database of the present study.

1. Anguria P, Ntuli S, Carmichael T. Relationships of heredity and dry eye with pterygia in black African patients. South African Medical Journal 2011; 101 (2): 110.
2. Anguria P, Ntuli S, Interewicz B, Carmichael T. Traditional eye medication and pterygium occurrence in Limpopo Province. South African Medical Journal 2012; 102 (8): 687-90.
3. Anguria P, Ntuli S, Carmichael T, Kitinya J. Chronic inflammatory cells and damaged limbal cells in pterygium. African Health Sciences 2013; 13(3): 725-30.
4. Anguria P, Ntuli S, Carmichael T. Young patient's age determines pterygium recurrence after surgery. African Health Sciences 2014; 14(1): 72-6.
5. Anguria P, Kitinya J, Ntuli S, Carmichael T. The role of heredity in pterygium development. International Journal of Ophthalmology 2014; 7(3): 563-73.

Abstract

Pterygium, a wing-shaped fibrovascular growth of the conjunctiva onto the cornea, can impair vision and be cosmetically unacceptable. Its frequency varies in Africa and post-surgical recurrence in blacks may be high. Determinants of pterygium occurrence and recurrence in rural Africans are not known. This study aimed to establish the determinants of pterygium occurrence and recurrence in rural blacks.

The case controlled study comprised 230 patients and 157 controls. Interviews and eye examination were conducted; however, data from 150 patients and 150 controls were analyzed as pre-calculated. Families of 51 cases and 50 controls were studied. Surgery was done on 200 eligible patients. Those who experienced post-surgical recurrence were sub-classified as cases and those who did not, controls. Immunohistochemistry was done on 59 pterygium sections and 7 controls.

Family history of pterygium was present in 46 cases (30.6%) of 150, and 15 controls (10%) of 150; Odds ratio (OR) =3.93; p <0.01. Traditional eye medication was used by 79 cases (52.6%) of 150, and 60 controls (40%) of 150; OR =2.03; p <0.01. The tear film was unstable in 10 cases (6.6%) of 150, and 26 controls (17.3%) of 150; OR =0.30; p <0.01. Groups of 3-5 individuals per household were pterygium-affected in 36 pterygium families (70.5%) of 51 vs. 1 control (2%) of 50. After surgery, only 190 patients completed follow-up for a minimum duration of 6 months, and 52 (27.4%) experienced post-surgical recurrence. Of the 52 cases, 21 (40%) had grade 2 pterygia v. 8 post-surgical controls having grade 2 pterygia (6%) of 138; OR =9.1; p <0.01. The limbal basal epithelium expressed p53 in 11 pterygia (18.6%) of 59 v. 5 controls (71.4%) of 7; p <0.01. It expressed matrixmetalloproteinase-1 (MMP1) in 14 pterygia (23.7%) of 59 v. 5 controls

(71.4%) of 7; p =0.02. MMP2 and MMP3 were detected in 16 cases (27.1%) of 59 v. 5 controls (71.4%) of 7; p =0.03.

Pterygium occurred in families and was associated with traditional eye medication. Pterygium occurrence was not associated with unstable tear film, p53, and MMPs. Post-surgical recurrence was connected to grade 2 pterygia.

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This work would have been impossible to accomplish without certain authorities or persons. I thank The Limpopo Provincial Department of Health and Social Development for permission to conduct this research in Limpopo and to publish results. I thank Mr Sam Ntuli for performing statistical analysis. I thank Mr DB Mashishi for preparing the specimens for histological analysis and Prof James Kitinya for guidance in reading the slides. Dr B Interewicz guided me in calculating the likely mode of inheritance. Dr F Stegmann and Dr S Msutwana performed the surgeries, and Dr S Thompson and Dr A Bvumbi confirmed the outcome of surgery. The antibodies and reagents used were provided by the Oral Pathology laboratory, with permission from Dr G Kaleebi. To all the participants in this study, your acceptance to participate is a great gift to humanity. I thank Prof Yoswa Dambisya for showing a keen interest in my work. And to my supervisors, Prof Trevor Carmichael, and Prof James Kitinya your guidance is tremendously valuable. Special thanks to Prof Trevor Carmichael for your patience with me; you are like a father to me.

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Nomenclature

UV	Ultraviolet
CAT	Free conjunctival autotransplant
LCAT	Limbal conjunctival autotransplant
ROS	Reactive oxygen species
MMP	Matrixmetalloproteinase
TOPK	T-lymphokine activated killer cell-originated protein kinase
COX-2	Cyclooxygenase-2
IL-8	Interleukin-8
PGE2	Prostaglandin E2
bFGF	Basic fibroblast growth factor
PDGF	Platelet derived growth factor
VEGF	Vascular endothelial growth factor
TGFβ	Transforming growth factor-beta
sma	Small
mad	Mothers against decapentaplegic
smad	Small and mothers against decapentaplegic
smurf	Smad ubiquitin regulatory factor
CTGF	Connective tissue growth factor
Dlg5	Discs large factor-5
TBUT	Tear film breakup time
Small pterygium	≤grade 2
Large pterygium	≥grade 3
Extra-large pterygia	Grade 4 or 5

LCA	Leucocyte common antigen
TRS	Target retrieval solution
TBS	Tris buffered saline
HRP	Horseradish peroxidase
DAB	3,3'diamino-benzidine-tetrahydrochloride
OR	Odds ratio
Low inflammatory cell count	≤200
High inflammatory cell count	>200
CI	Confidence interval
SD	Standard deviation
Short recurrence time	≤3 months after surgery
Long recurrence time	>3 months after surgery
Young age	Less than 50 years old
Old age	50 years old or more
Determinant gene	Inactive gene
HB-EGF	Heparin binding epidermal growth factor-like epidermal growth factor

Preface

From the beginning of written history human beings have had a tendency to blame outsiders rather than self. For example, Eve blamed the serpent for her desire to become wise and Adam blamed his wife for his desire to please her (The bible. Genesis chapter 3, verses 1-24. e-sword. KJV. Copyright 2011. Rick Meyers). Such a tendency may be the reason why it is easier to believe falsehood rather than the truth.

My ethnic group of people believed that 'if a child ate ox liver that child would defecate in the dwelling house rather than the designated area'. Of course, even good things if taken in quantities larger than what the systems can handle may be hazardous. However, problems may occur after a modest or minimal intake of a good thing, which would suggest fault in the individual taking the good thing. It is like driving at 50 km/hr along a highway in a car having a grossly faulty breaking system. Nevertheless, liver tastes great and it is a rich source of vitamin A. Moreover, by itself it doesn't cause such unsocial behaviour. Our traits follow us wherever we go and often manifest unless vigilantly self-controlled.

I have realised from this study that: to follow original tradition is better than to follow fashion; inappropriate additions should be cut-off; if dislocated from the natural environment it is beneficial to carry along sincere uniqueness; and if the new environment gets irreparably damaged one should relocate to the natural environment. (Paul in the bible: Romans chapter 12, verse 2. e-sword. KJV. Copyright 2011. Rick Meyers).

Chapter 1: Introduction

The word pterygium derives from the Greek word pterygos, which means wing.[1] Therefore, pterygium is a wing-shaped[1] fibrovascular growth of the conjunctiva across the limbus onto the cornea.[1,2,3] Pterygium is divided into the body that overlies the sclera, the neck that includes the superficial limbus, and the head that invades the anterior cornea.[4] The cap is the grey area that extends centrally from the apex of the head and it is deep to the corneal epithelium.[2,5]

Pterygium consists of conjunctival epithelium on the surface below which is the conjunctival stroma.[2] At the apex transition from conjunctival to corneal epithelium is sharp.[5] Across the limbus the conjunctival stroma is at the plane of Bowman's membrane that it replaces.[2] Fibroblasts that are in front of the advancing conjunctival stroma are superficial to Bowman's membrane and when the latter is fragmented the fibroblasts are in the underlying corneal stroma also.[5] Numerous blood vessels are present in the pterygium stroma[2,3,6] and chronic inflammatory cells in varying degrees of infiltration are also found.[2,6,7,8]

1.1 The problem

Pterygium can impair vision by obstructing the visual axis, and inducing corneal astigmatism, and by the glare due to the corneal haze caused by the cap.[9-12] It may be cosmetically unacceptable.[9,12,13] Pterygium is frequently encountered by Eye Health Care providers in Africa,[14] however, its frequency may vary in different countries[9,14,15,16] or even within the same country.[9,17] Pterygium frequently recurs after

surgery and the recurrent growth may be more aggressive than the primary growth.[5,14,18]

Determinants of pterygium occurrence or recurrence after excision in a rural African population have not been reported and the pathogenesis of pterygium is not clear. Therefore, the research questions were what determine pterygium occurrence and recurrence in a rural African population? And what is the pathogenesis of pterygium? The hypothesis of the present study was that pterygium occurrence and recurrence after excision are determined by multiple modifiable factors, which interact to develop pterygium. This research was aimed to establish the determinants of pterygium occurrence and recurrence after excision in a rural African population. The objectives were to:

- Explore epidemiological factors in pterygium occurrence and recurrence after excision.
- Determine whether the tendency to pterygium occurrence were hereditary or acquired.
- Determine whether pterygium occurrence and recurrence was associated with damaged or deficient limbal stem cells.
- Confirm and assess chronic inflammatory cells in pterygia.
- Describe the pathogenesis of pterygium.

1.2 Rationale

Multiple factors such as sunlight,[19] dust,[20] dry eyes,[21] and hereditary predisposition[22] have been associated with pterygium occurrence. And sunlight,[23] young age,[24] pterygium fleshiness[25] and large extent[26] have been associated with pterygium recurrence after surgery. Multiple pathological changes such as collagen degeneration,[5,27] chronic inflammation,[7] growth factor production,[8] and limbal stem cell damage[28] have been reported in pterygium samples. Genetic factors have also been reported.[29] Inspite of those reports the determinants of pterygium occurrence and recurrence after excision in a rural African population are not known. The pathogenesis of pterygium is not clear.

Knowledge of the determinants of pterygium occurrence and its recurrence after excision, and of the pathogenesis of pterygium will facilitate prevention of its occurrence and recurrence after surgery. This will reduce the occurrence of visual impairment or disfigurement due to pterygium.

1.3 Preview of the organization of the thesis

The background to this study and the literature review are discussed in chapter 2. The materials and methods are explained in chapter 3. The results are presented in chapter 4 and they are discussed in chapter 5. A model of pterygium development is proposed in chapter 6. Chapter 7 presents the conclusions.

Chapter 2

2.1 Background

I practiced privately after graduating as an Ophthalmologist and the commonest surgical disease I treated was pterygium (2 cases out of 5 in a two weekly theater list). Even after commencing employment with the Limpopo Provincial Department of Health, pterygium cases were seen frequently – about 8% of the out-patients per year according to the 2006 eye clinic attendance register. This condition is fascinating. Many ophthalmologists consider pterygium to be a trivial condition and yet the growth frequently presents a problem by way of its recurrence after excision.

Pterygium is ancient and as reported by Jaros and DeLuise[1] as well as Raju,[30] Susruta, who is believed to have lived around 1000B.C was the first practitioner to write about pterygium. It has been reported that the writer described the different forms of pterygium as separate diseases[30] but there is no report of whether Susruta described the aetiology of pterygium occurrence. According to Raju, Susruta reported that pterygium recurs due to improper surgery.[30]

Looking after patients is enjoyable but, now, the desire to discover is more appealing, which led to this research. Professor Yoswa Dambisya who is a pharmacologist and friend suggested choosing supervisors who are conversant with the topic to be researched on. In 1994 Dushku and Reid suggested that pterygia may arise because of damage to limbal basal cells, which spread in all directions.[31] This raised the possibility that wide excision would prevent pterygium recurrence. Completeness of

excision of damaged limbal epithelial cells has not been demonstrated histologically. It looked as if studying the relationship between these cells and pterygium recurrence after surgery could be conducted at doctoral level. Professor James Kitinya who is a Pathologist advised that enrolling with a University that has a reputation for research would be helpful.

Therefore; I consulted Professor Trevor Carmichael, who is the Head of the Division of Ophthalmology at the University of the Witwatersrand Johannesburg. Professor Carmichael recommended broadening of the investigation to encompass determinants of pterygium occurrence and recurrence. While writing the proposal the magnitude of the task ahead was appreciable as pterygium is enigmatic but, the inspiration was the fact that it is not clear why pterygium occurs. Moreover, the overall prevalence rate of pterygium occurrence in South Africa including Limpopo Province is not known;[32] the approach of first understanding why pterygium occurs was tempting to believe that this would facilitate understanding why it recurs after surgery.

2.2 Literature review

The main categories of factors that have been reported to be associated with pterygium occurrence are environmental and hereditary. Environmental factors seem to be related to histological factors. For example, sunlight may degrade collagen,[5,33] damage limbal stem cells,[34] induce chronic inflammatory cell infiltration[35] as well as growth factors.[8,36] Environmental factors also seem to be related to some clinical factors. For example, a dry atmosphere may encourage the tear film to evaporate thereby

contributing to the occurrence of dry eyes.[37] However; acquired clinical conditions of the ocular surface are environmental factors. Hereditary factors have been underemphasized,[32] and so, it is not clear whether heredity contributes to pterygium formation.

2.2.1 Environmental factors

This includes ultraviolet (UV) radiation, dust, smoke, and smoking. Excessive exposure to sunlight radiation, which is widely believed to be the main reason for the formation of pterygium[19,38,39,40,41] is more likely to occur in semiskilled or unskilled individuals[41,42] as these are more likely than the skilled to be occupied outdoors.[19,41,42,43,44] Due to outdoor occupations males are more frequently affected by pterygium[41,42] however, males and females may be equally affected as these may be equally likely to work outdoors.[44] As spectacles or sunglasses may protect eyes from UV radiation[45] these were reported to prevent pterygium occurrence in a study dominated by blacks in the Barbados.[19] However, the influence of spectacles or sunglasses in rural Africans is not known. Increasing age is associated with pterygium occurrence[39,41,44,46] possibly as individuals with increasing age are the ones who have a large accumulation of sunlight exposure.[39,40] Nevertheless, a high accumulation was not associated with pterygium occurrence independent of rural residence or lower fasting blood sugar.[47] Moreover, the peak age of pterygium occurrence was less than 50 years old in Africans in one study.[48] Hence, some degree of exposure is necessary and it seems there might be another factor besides exposure to sunlight, which might be also important for pterygium to develop.

Some reports on pterygium occurrence and sunlight exposure from Africa seem to be contradictory. Pterygium was more frequent in the Karoo than the Transkei, yet both regions receive similar levels of UV radiation; and climatic droplet keratopathy, pinguecula, and pterygium did not occur together significantly, yet all these conditions have been reported to be associated with UV light exposure and damage;[9,17] and pterygium cases were less frequent than expected in Rwanda, an area close to the equator and high in altitude.[15] Ultraviolet radiation increases with decreasing latitude and with increasing altitude.[49]

Dust may be associated with pterygium presence,[20] however, pterygia may be infrequent in individuals who are chronically exposed to dust without protection against it.[50] It is not clear whether dust increases the risk of pterygium occurrence in rural black Africans whose employment is mainly on farms.[9] Moreover, it has not been reported whether or not smoke, which is suspended particles in air, like dust, is related to pterygium occurrence. The majority of rural Africans are exposed to smoke because of the use of fire wood for cooking and for provision of warmth in winter in conditions of poor ventilation due to poverty;[51] it has been proposed that suspended solid particles may cause micro-trauma or chronic inflammation in the ocular surface.[38]

Smoking has been associated with the occurrence of pterygia in Singapore.[41] Conversely, the same habit was reported to have no association with the existence of pterygia in Barbados.[42] And in Myanmar smoking had no relationship with pterygium presence.[44] Cigarette smoke may be cytotoxic to tissues exposed to it.[52] Whether tobacco use is related to pterygium in Africans is not known.

2.2.2 Clinical factors

Limbal stem cell deficiency might arise from damage induced by sunlight, which might be connected to pterygium formation.[53] Nonetheless, whether limbal stem cells are damaged or not only one study with a small sample has prospectively randomised patients to free conjunctival autotransplant (CAT) or limbal conjunctival autotransplant (LCAT) for the treatment of primary pterygia.[54] LCAT has been found to be superior to CAT by meta-analysis,[55] nonetheless, those results have not been compared with subsequent large randomised, controlled trials. Inconsistencies between meta-analysis and subsequent high powered randomised, controlled trials exist;[56] moreover, heterogeneity estimates in meta-analysis are doubtful.[57] It is therefore not clear whether LCAT is superior to CAT in the treatment of primary pterygium. Limbal stem cell transplantation is the treatment for limbal stem cell deficiency.[58,59]

An unstable tear film may[21,60] or may not[61] be associated with pterygium presence. It may be that the unstable tear film is unlikely to cause pterygium.[62] Nevertheless; there is no report of whether the presence or absence of unstable tear film was associated with pterygium occurrence independent of other significant factors.

Eye medication may damage the ocular surface via its pH or via reactive oxygen species (ROS) present in the medication.[63] Traditional medicine is widely used by blacks because they believe in it.[64,65] Those Africans who use traditional remedies follow certain traditions.[66] Medicinal plant extracts have been shown to have antioxidant capability.[67] and to stabilize the tearfilm in dry eye patients.[68] However; it

is not known whether or not the use of traditional eye medication is associated with pterygium occurrence. The sources and formulation of traditional eye medication used by rural Africans have not been reported.

2.2.3 Histological factors

The majority of pterygium samples may have damaged limbal stem cells, which manifest by overexpression of p53[28,31,69,70] and by the detection of matrixmetalloproteinases (MMPs) in the limbal basal epithelium.[3,71,72,73] Conversely, pterygia may have a low rate of p53 expression though previous studies cited by Tsai et al reported varying rates from 7.7% to 100%, which suggests the role of limbal stem cell damage is unclear.[74] Due to a small study sample Ateenyi-Agaba et al failed to clarify whether p53 expression rate in pterygia from black Africans is low;[34] there is no study with a large sample on p53 expression rate in Africans. MMP detection rates by immunohistochemistry in pterygia from Africans have not been described.

Ultraviolet radiation is phototoxic to the conjunctiva via ROS[75,76] thereby inducing inflammatory cells in the conjunctiva.[35] Inflammatory cells are present in varying degrees of infiltration in pterygium samples.[6,7,8] These cells are located mainly in the stroma,[77] which is a sign of chronic inflammation[77,78] rather than hypersensitivity whereby inflammatory cells would be mainly in the epithelium.[7,79] It is not clear whether the degree of infiltration is related to pterygium size or the severity of inflammation. Knowledge of whether the inflammatory cell infiltrate is related to the

severity of inflammation would indicate whether inflammation is important for pterygium to occur or to recur.

Although the level of proinflammatory cytokines varied in different pterygia exposed to the same degree of UV light,[80] suggesting an intrinsic determinant of the severity of inflammation, it is not clear whether the severity of the inflammatory response is influenced by the duration of exposure to sunlight or it may be genetically determined. Some individuals may be deficient of T-lymphokine activated killer cell-originated protein kinase (TOPK), and its deficiency appears to intensify sunlight induced inflammation.[81]

Numerous fibroblasts[5,8] are present in pterygium and MMPs have been detected in pterygium fibroblasts and stroma.[3,72,73] MMP expression in fibroblasts and stroma may be due to DNA damage,[72] yet, one study did not show MMP expression in pterygium fibroblasts or stroma despite MMP detection in the limbal basal epithelial cells.[71] The same study detected tissue inhibitor of matrixmetalloproteinase 3 in inflammatory cells present in the connective tissue matrix, which may explain lack of MMP3 expression in the stroma. Whether these proteins are expressed or not by pterygium fibroblasts or stroma has not been reported in rural Africans.

Collagen degeneration, which is a sign of prolonged[5,33] rather than short term[82] exposure to sunlight is typical of pterygium,[5,8,27,77] however, it may be absent in some pterygia.[83] There is no report of whether collagen degeneration can be absent in pterygia of rural Africans.

2.2.4 Hereditary factors

Heredity has been implicated in pterygium formation by family history,[22,32] though, those histories were self-reported but not confirmed by an experienced observer. Some studies have implicated heredity by the presence of diagnosed pterygium-affected key relatives,[18,84,85,86] but, the families of pterygium probands were not compared with those of control probands. Moreover, previous family studies were case reports. Heredity may also be implicated by an early onset of a condition.[18,86,87,88]

A hereditary condition has a mode of inheritance.[84,89,90] Based on a study of 2 households autosomal dominant was found to be a likely mode of inheritance for the tendency to pterygium formation.[84] Nonetheless, this has not been verified with a large sample. Multifactorial mode of inheritance has also been reported to be likely because of a positive family history and a history of sunlight exposure;[32] it is not clear which mode or modes of inheritance is or are likely in pterygium occurrence.

A genetic predisposition to DNA damage has been reported in some pterygium patients,[29,91] however, those studies failed to explain the occurrence of pterygium in the patients not predisposed to DNA damage. It looks as if some individuals predisposed to pterygium occurrence are also prone to DNA damage. Moreover, the frequency of genetic conditions may vary between races;[92] it is not clear whether the tendency to pterygium occurrence is hereditary or acquired.

2.2.5 Factors in pterygium recurrence after excision

Young patient's age may be associated with pterygium recurrence after excision.[24,93] However, as young individuals rather than the old tended to have fleshy pterygia one study concluded that young age depended on pterygium fleshiness to be associated with recurrence after surgery.[25] There is no report comparing the patients age between those whose pterygia recurred and patients whose pterygia did not recur after excision of fleshy primary pterygia.

Excessive sunlight exposure may be a reason for pterygium recurrence.[23] But also, excessive sunlight may not be associated with post-surgical recurrence as recurrent pterygia did not show collagen degeneration.[5] Some pterygia may have a short recurrence time,[18] yet collagen degeneration occurs after a long duration of exposure,[33] suggesting that it is possible that a high exposure for a short duration could be a reason for pterygium to recur after surgery.[23] The duration of exposure of those whose pterygia recurred has not been compared with that of patients whose pterygia did not recur after surgery.

Large pterygia have been associated with recurrence.[26,94] However, adjunctive treatment was not applied in proportion to pterygium size[26] or the sample was small.[94] Another study failed to find a relationship between size and recurrence after surgery possibly due to a small sample size.[95] Unilaterality may be associated with recurrence however, this was concluded following a retrospective study.[96]

2.2.6 Pterygium pathogenesis

Numerous mechanisms including fibroblast damage,[5,97] limbal stem cell damage,[28,72] inflammation,[6,7] growth factors,[8,36,97,98,99,100] and genetic polymorphisms[29,91,101] have been described in the pathogenesis of pterygium. However, some of those mechanisms such as DNA damage[28,72] and its hereditary predisposition[29,91] or growth factor gene polymorphisms[101] do not occur in all pterygium patients. It is not known whether the factors which occur in all pterygium patients, such as sunlight exposure[19] and inflammation[6] and numerous fibroblasts[8] interact to develop pterygium. Collagen degeneration may be present in most of pterygia,[27] but, it is unlikely to be the reason for the conjunctiva to grow onto the cornea.[5] This is because even pingueculae have collagen degeneration,[27] moreover, some pterygia may not have collagen degeneration histologically.[83]

Damage to the DNA of the limbal stem cells might result in the migration of both the reserve and the transient amplifying cell, which perhaps causes limbal stem cell deficiency and dragging of the conjunctiva with its stroma onto the cornea.[31] Migration of damaged limbal cells may be facilitated by MMPs secreted by damaged limbal cells and damaged fibroblasts, which may fragment Bowman's membrane and cause collagen degeneration.[72,73] The studies which reported that MMPs facilitated migration of damaged limbal cells[72,73] fail to explain how Bowman's membrane is fragmented or collagen degeneration occurs in the absence of MMP expression in the pterygium fibroblasts or stroma.[71]

2.2.6.1 Sunlight and inflammation

Sunlight exposure induces oxidative stress in the conjunctiva.[76] ROS oxidize cell membrane lipids[102] by phosphorylation, which increases the level of phospholipids.[103] Phospholipids stimulate production of cyclooxygenase-2 (COX-2) and interleukin-8 (IL-8),[104] which are pro-inflammatory.[105,106] COX-2 mediates inflammation via prostaglandin E2 (PGE2), which COX-2 converts from oxidized lipids.[107] Sunlight exposure has been correlated with chronic conjunctivitis,[6,7,8,77] and COX-2[108] as well as PGE2[100] have been shown to be present in pterygia but absent in unexposed conjunctivas.

Dust, smoke, and pollens seem to cause ocular surface irritation, which together with sunlight damage may provoke chronic conjunctivitis that perhaps triggers pterygium to occur.[7] Mast cells have been reported in pterygium samples along blood vessels,[109] suggesting absence of an allergic phenomenon.[110] Yet, hypersensitivity has been proposed to be a factor in pterygium development because chronic inflammatory cells were infiltrated in the pterygium epithelium.[7] It is not clear whether hypersensitivity or irritation or inflammation are essential for the conjunctival fibrovascular proliferation to grow onto the cornea.

2.2.6.2 Inflammation and growth factors

Sunlight-provoked inflammation[35] may be associated with growth factors,[8] which are mitogenic to fibroblasts.[97,98] In spite of this, it is not known how sunlight, inflammation, and numerous fibroblasts are inter-connected to cause pterygium. A high infiltration of inflammatory cells in the stroma, positive for growth factors

suggests a high level of growth factor protein,[8] which previous studies seem to suggest to be the reason for pterygium to develop.[99,100] Nonetheless, one study showed that pterygium samples and controls may have similar high levels of expression of some angiogenic growth factors such as basic fibroblast growth factor (bFGF) but only pterygia express fibrogenic growth factors such as platelet derived growth factor (PDGF).[8] This suggests that pterygium begins due to the over-expression of fibrogenic growth factors rather than angiogenic growth factors. It may also be that pterygium is not associated with just a high expression of growth factors because a previous study has shown that the conditioned medium from cultured pterygium fibroblasts caused sluggish mitosis of cultured control fibroblasts yet it caused vibrant mitosis of pterygium fibroblasts.[97]

Although it looks as if angiogenic growth factors are not important for pterygium to occur[8] neovascularisation characterizes pterygium.[6] Pterygium rather than control fibroblasts have been shown to undergo vibrant proliferation,[97] and VEGF has been shown to be upregulated in pterygium rather than control stromal cells including fibroblasts, suggesting angiogenesis is secondary to fibroblast proliferation in pterygium. IL-8 and COX-2 induce angiogenic growth factors.[111,112] ROS may directly stimulate capillary growth[113] in addition to inducing angiogenic growth factors.[75]

2.2.6.3 Heredity and growth factors

Studies, which have shown over-expression of growth factors failed to explain why growth factor availability was prolonged.[8,36,97,99,100] Vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGFβ) polymorphisms are the same in

patients and un-affected individuals.[114] This suggests that the over-expressions of these growth factors in pterygium patients is unlikely to be due to them being genetically up-regulated. However, one study reported that female pterygium patients had VEGF gene 460 polymorphism.[111]

Pterygium fibroblasts are probably abnormal because they behave different than fibroblasts from individuals not affected by pterygium.[5,97] The abnormality is perhaps due to damage by sunlight,[5] which is supported by the overexpression of MMPs in fibroblasts.[72,73] On the other hand, since it may also be that MMPs are inhibited in pterygium fibroblasts, yet these proteins may be present in limbal epithelial cells,[71] this suggests that the abnormality causing the fibroblasts to proliferate vigorously is not acquired through sunlight damage as reported.[5,72,73] Heredity[18,22,32,83,85,86] perhaps is the reason why fibroblasts or growth factors behave different in pterygium cases than in controls but, this needs clarification.

The concept of heredity in pterygium development appears to be supported by a study that reported that keloid derived fibroblasts showed abnormal expression of multiple genes including those for type II receptors of growth factors.[115] Keloid, like pterygium is a fibrotic condition. As growth factors are up-regulated in cells other than fibroblasts such as endothelial and inflammatory cells;[8,99,100] and growth factors have been shown to be mitogenic to fibroblasts;[97,98] and the mitogenic action is regulated by inhibitors of growth factor activity[116,117] and of receptors;[118,119] and the inhibitors are genetically determined;[120,121] it may be that prolonged availability of growth factors[8,36,99,100] is due

to their lack of inhibition at gene level. However, literature search has not shown a lack of inhibition of growth factors occurs in pterygium.

2.2.6.4 Inflammation and pterygium

Inflammation activates TGFβ[122,123] which stimulates fibroblasts to synthesize collagen.[124,125] Collagen is deposited randomly and so it causes previously transparent tissues to become opaque.[126] Activated TGFβ also inhibits MMPs[127,128] thereby decreasing collagenolysis.[129] Collagen synthesis coupled with little degradation increases the amount of collagen. Therefore collagen may be the reason for the pterygium cap.

Factors contributing to pterygium size are not clearly understood. Prolonged action of growth factors has been documented,[8,36,99,100] and the cap is composed of collagen,[5] suggesting its size is related to collagen content. It is unclear if inflammation, which activates TGFβ[122,123] is also related to pterygium size.

2.2.6.5 Sunlight and growth factors

Growth factors are induced by sunlight also.[8,36,99,100,130] This occurs in cases as well as controls because pterygium patients may be exposed to sunlight for similar durations as the individuals not affected by pterygium.[14,15] The unexposed conjunctivas of pterygium patients do not express growth factors, which suggests that sunlight is necessary for growth factors to be produced.[99,100] This is supported by in vitro studies.[36,130] Even a short duration of exposure induces growth factors.[36]

Oxidative stress, which sunlight induces in the nucleus and cytoplasm of the epithelial and stromal cells of the ocular surface,[76] up-regulates genes for growth factors thereby causing growth factor synthesis.[75] Growth factors such as TGFβ may generate oxidative stress from the cell membrane in the process of translocation to the nucleus, or, inhibit anti-oxidant enzymes.[131] Oxidative stress also denatures proteins and oxidizes lipids.[102] Lipids are over-expressed in pterygia[132] and the presence of collagen degeneration[27] is a sign of prolonged matrix damage.[33] It has been reported fibroblasts were present in the stroma after fragmentation of Bowman's membrane, which suggests collagenolysis contributes to pterygium development.[5]

2.2.6.6 Growth factors and mitosis of fibroblasts and endothelial cells

Growth factors effect fibroblast mitosis[97,98] via receptors[133,134,135] at the fibroblast cell membrane. The signal for fibroblast mitosis is translocated from the growth factor type 1 receptor at the cell membrane to the nucleus by sma and mad (smad[136]) proteins.[133] sma means small,[137] and mad means mothers against decapentaplegic.[138] sma and mad proteins were originally isolated from *Caenorhabditis elegans* and *Drosophila melanogaster* respectively[137,138] and they are the same protein.[137]

The growth factor-receptor interaction is internalised and specific endocytic vesicles are formed,[133] in which receptor-regulated smad proteins in a series of steps including smad 1 and 5 activate the receptor leading to the initiation of a signal for the transcription of genes for fibroblast mitosis.[133,139] After an adequate signal a different type of endocytic vesicles is formed when the growth factor-receptor complex is internalised.[133] The inhibitory smad proteins in a series of steps including smad-7 in

these vesicles terminate the signal for transcription[116,117] independent of receptor deactivation.[116] Inhibitory smad proteins inhibit growth factors at the nucleus.[116,117,133]

Smad-7 stimulates smad ubiquitin regulatory factor-1 (smurf-1),[118] which degrades the receptor.[118,133] Smurf-1 may compete with smurf-2 to degrade receptors.[119] By degradation receptor inhibitors[118,119] interfere with growth factor translocation.[133] Activity of smad7 is independent of smurf activity,[116] but smurf action is stimulated by smad-7.[118]

Recent studies have shown that multiple genes were abnormaly expressed in pterygia and that the genetic abnormality was acquired.[140,141] However, those studies failed to exclude genetic inheritance as a possible cause of abnormal gene expression in pterygium because non-immortalised pterygium fibroblasts were compared with immortalised pterygium fibroblasts[140] or, pterygium was compared with normal superior bulbar conjunctiva from the same pterygium patient.[141] Immortalisations, as well as changes in pterygium different from those in normal conjunctiva from the same patient are due to acquired processes. Genotype in immortalised cells is different from that in non-immortalised cells of the same type;[142] whether inhibitory smad, and smurf proteins are involved or not in pterygium there is no literature showing that genetic expression in pterygia may be hereditary.

Although the inhibitory smads,[121] and smurf proteins[120] are genetically determined some growth factors may inhibit or stimulate other growth factors or receptors. For example, FGF may up-regulate PDGF receptors.[143] TGFβ, which is anti-

proliferative[144,145] may exert proliferative action by stimulating connective tissue growth factor (CTGF),[146] yet it may inhibit PDGF if bFGF is absent.[147] TGFβ may also up-regulate VEGF.[148]

Neovascularisation may characterise pterygia[6] and intra-epithelial capillaries have been identified in front of the pterygium head.[149] However, it is not clear why neovascularisation is limited to pterygium and its cap yet, angiogenic as well as fibrogenic growth factors are up-regulated.[8,99,100] Increased synthesis of VEGF[103] and stimulation of capillary growth[113] by oxidative stress[76, 150] may explain angiogenesis that perhaps causes pterygium.[149] VEGF causes neovascularisation in a frame-work of fibronectin.[151]

Because the size of pterygium decreased after treatment with bevacizumab (anti-VEGF) it was proposed that neovascularisation was necessary for pterygium to occur moreover, atrophic pterygia lacked vascularisation.[152] Nonetheless, it may be that neovascularisation is not necessary for pterygium onset because pterygium may recur after surgery irrespective of bevacizumab injection immediately after excision.[153] Repeated bevacizumab injections seem to be necessary to minimize recurrence but, they may not abolish recurrence after surgery.[154] This suggests that anti-VEGF therapy fails to correct the fundamental cause for pterygium development.

Physiological conditions such as the uterus and placenta in pregnancy whereby enlargement occurs have a high metabolic rate, which is manifested by angiogenesis.[155] A high metabolic rate also manifests as increase in phospholipids,[156]

which indicate the presence of ROS.[102] Since phospholipids[132] and prostaglandins[100] have been reported to be up-regulated in pterygium fibroblasts this shows that pterygium fibroblasts have a high metabolic rate[156] and oxidative stress is high.[102] Hence, pterygium angiogenesis occurs due to a high metabolic rate of pterygium fibroblasts.

2.2.6.7 Pterygium and depletion of limbal stem cells

Pterygium may occur due to depletion of limbal stem cells as they migrate in all directions.[31] Migration to the stroma causes epithelial cells to be transformed to fibroblasts.[157] Inflammation, which occurs in the exposed corneo-conjunctiva[6,77] may aggravate stem cells to migrate to the stroma.[158] The fibroblasts transformed from the migrant epithelial cells[157] might proliferate to develop pterygium[31] in individuals predisposed[158] to the inability of discs large factor-5 (Dlg5) to inhibit TGFβ receptor.[159] If the inability of Dlg5 to inhibit TGFβ were the reason for pterygium to occur most pterygia would be severely inflamed but, there is no report of whether most pterygia are severely inflamed.

2.2.7 Clinical anatomy of the ocular surface

The bulbar conjunctiva is loosely attached to the underlying Tenon's fascia peripheral to the surgical limbus after which the conjunctiva and Tenon's fascia fuse and they are firmly attached to the sclera till the outer border of the anatomic limbus.[160] From the limbus into the cornea the stroma is compact.[161] The conjunctival stroma contains fibroblasts,[129] blood vessels,[162] and chronic inflammatory cells.[163] Because few blood

vessels are present in unaffected nasal conjunctivas[164] pterygium is not the only reason for conjunctival vascularisation. Nevertheless, recurrent pterygia have more blood vessels than exposed normal conjunctivas.[164] The superficial episcleral plexus is found in the surgical limbus.[162] The conjunctival stroma is at the same level as the Bowman's membrane in the cornea.[2,31]

2.2.8 Overview of the patterns of genetic inheritance

Mendelian pattern of inheritance involves single genes[81,88,165] as opposed to two or more genes which seem to occur in maturity onset diabetes mellitus.[89] The genotype, but not the environment determines phenotype in Mendelian inheritance.[81,88,165] Dominant inheritance occurs when a defective allele, which is dominant is inherited.[165,166] A defective allele manifests an abnormal phenotype even when its presence is opposed by the normal allele in a heterozygous state.[165] In the homozygous state, the phenotype caused by the defective alleles is so severe that affected individuals perish before birth or early in life.[166] According to Mendelian principles the risk of an offspring of a heterozygous parent becoming affected is 50% and if both parents are heterozygous 25% of the offspring may be severely affected.[166] Incomplete penetrance sometimes occurs, which leads to a skipped generation.[90]

Recessive inheritance occurs when ineffective alleles are inherited.[81,88] In a heterozygous state of a single locus one normal allele is sufficient to cause normal phenotype, however, in a homozygous state the gene is so ineffective that an abnormal phenotype occurs.[81] According to Mendelian principles the risk of the offspring

becoming affected is 100% if both parents are homozygous recessive while it is 50% if one parent is homozygous and the partner is heterozygous and it is 25% if both parents are heterozygous.[81] No offspring becomes affected if one parent has normal paired alleles while the partner is heterozygous or homozygous recessive.[81] Double heterozygotes occur when 2 heterozygous genes that code for the same phenotype are in different loci.[88] The phenotype is abnormal because the presence of 2 ineffective alleles, which code for the same protein, although in different loci weakens the normal alleles.[88] According to Mendelian principles,[165,166] if two double heterozygotes mate the ratio between the unaffected and the affected offspring is 5:11.

Because the absence of a normal allele causes the defective allele to be lethal in dominant inheritance,[166] and males lack one of the alleles located in the Y-chromosome, Mendelian principles require that sex linked conditions are always recessive.[167] Males may be affected by inheriting a recessive allele from the mother whether she is affected or she is a carrier.[167] Affected fathers may transmit their recessive gene only to their daughters who may manifest the abnormal phenotype only if the mother is homozygous or heterozygous recessive;[167] however, females in sex linked inheritance are usually carriers.

In multifactorial mode of inheritance genetic and non-genetic interaction may occur[32,168] or, two or more genes may modify one another's effects[169,170,171,172] to produce a phenotype. Alleles are not defective[165] or ineffective[88] but, genes have to be switched on before transcription to messenger RNA after which protein synthesis may occur.[173,174,175] Digenic inheritance is the simplest form of multifactorial inheritance.[89]

Affected individuals tend to cluster in families.[170] In gene to gene interactions the polygenic model stipulates that the more determinant genes present the greater the tendency of transmission to subsequent generations[172] and the severer the phenotype.[171] Based on Mendelian principles[81,165,166] the genotypes of the mating individuals also contribute to determining transmission. For example, in digenic inheritance the ratio between the unaffected and affected offspring of partners whose two genes each has only one allele active (heterozygous inactive genes), which leads to a normal phenotype is 9:7. It is 4:12 if one parent is heterozygous inactive in each gene and the partner is homozygous inactive in both genes. A homozygous inactive gene leads to an abnormal phenotype as if it were homozygous recessive.[81]

The threshold model of multifactorial inheritance stipulates that the contributions by genes and non-genetic factors reach a threshold that leads to phenotype whereby sometimes, genes have the greater contribution and other times the non-genetic factors contribute more to the threshold.[168] Although a previous study seems to suggest that the threshold model is possible in pterygium occurrence because of the variation in the degree of exposure of pterygium patients to sunlight,[32] no study has suggested a possibility of the polygenic model. This may be because previous family studies have been case reports.[18,84,85,86] Mitochondria are cytoplasmic organelles and transmission in mitochondrial inheritance occurs only from mothers.[176] This is because during fertilization only the sperm nucleus fuses with the ovum.

Chapter 3: Materials and Methods

3.1 Ethics

The present study was conducted between August 2008 and February 2012. Before commencing the study ethical clearance was obtained from the human research and ethics committee of the University of the Witwatersrand Johannesburg, and the Polokwane/Mankweng Hospital Complex research ethics and publications committee. The protocol was approved by the Postgraduate Committee for higher degrees in the Faculty of Health Sciences of the University of the Witwatersrand Johannesburg. Permission to conduct this research in Limpopo Province was obtained from the Limpopo Department of Health and Social Welfare. Because this research included a clinical trial it was registered at www. nih.gov before commencing the research. The tenets of the declaration of Helsinki (2000) were adhered to in obtaining consent.

3.2 Sources of data

This study was conducted in the Eye Unit of the Mankweng Hospital, which is the tertiary referral Centre in Limpopo Province. The Eye Unit has both an Eye Clinic and a Refraction Clinic. Pterygium cases were selected from the Eye clinic. To minimize the possibility of controls having an eye disease these were selected from the Refraction Clinic. Control specimens for immunohistochemistry studies were obtained from the patients selected in the eye clinic who did not have pterygia and the eyes had been irreparably injured.

The Tropic of Capricorn (23.5 degrees south of the Equator) bisects Limpopo Province,[177] which makes this Province suitable for studies on the effects of UV radiation.[38,49] The surface area of Limpopo is 125755 square km. The areas inhabited by rural Africans are hardly paved and so, dust levels are high. Statistics South Africa estimated the midyear population of Limpopo to be 5227200 in 2009 and of this, 2436400 (46.6%) was 20-64 years old; 87% of the population was rural.[178]

The South African weather bureau reports that most of the year in Limpopo is sunny and dry;[177] on average the sun rises at 6am and sets at 6pm. Due to the year being mostly sunny radiated UV is large.[179] The UV index ranges from 4-10 depending on cloud cover in Limpopo.[180] Reflected UV is high because most of the areas are rocky or mountainous.[181] Because of poverty[51] most of the cooking and house warming in rural areas is by firewood, which generates smoke.

Three main ethnic groups live in Limpopo. These are, Pedi, Tsonga and Venda. Others include Tswana, Ndebele and few from other provinces. All these groups of people are Bantu speaking who have been reported to have migrated from East and Central Africa and arrived in Southern Africa as early as 500AD.[182] The Pedi who constitute the major group in Limpopo Province are generally traditional.[183] They have been practicing first degree cousin marriage traditionally so as to keep the wealth given as dowry to the parents of the bride by the groom within the extended family.[184] Reproduction between first degree cousins may promote the occurrence of hereditary conditions.[185,186] This population was suitable for studies on hereditary conditions.

3.3 Design

A prospective case controlled study matched for age and sex, which are potential confounders was designed to explore the epidemiological factors in pterygium occurrence. Because factors associated with pterygium occurrence in Limpopo Province are not known data from the first 71 cases and 71 controls were analyzed, to get a clue on significant factors in pterygium occurrence. Family history was the only factor positively associated with pterygium occurrence. As the present study lacked funds for molecular studies, which had been planned to determine whether genetic predisposition was acquired or hereditary, it became necessary to conduct family studies to verify the self-reported family histories. A follow-up prospective case controlled study of families matched in number of probands was conducted. A study of families confirms whether pterygium occurs in families and enables the calculation of the likely mode of inheritance.[90] Familial occurrence may be due to heredity or a shared environment.[169] Nevertheless, hereditary but not environmental conditions have a mode of inheritance.[89,90]

Comparison between cases and controls in the present study was necessary to determine whether differences exist, which may cause the cases to be affected by pterygium.[40] Matching ensured that the comparisons were valid.

To investigate whether UV radiation contributed to pterygium recurrence by damaging limbal stem cells thereby causing limbal stem cell deficiency, a randomized study was planned to compare recurrence rate of pterygium after CAT with that after LCAT.[54]

Randomization minimizes bias in distributing patients to the 2 procedures. A follow-up descriptive case-controlled study on the expression of damaged limbal basal epithelial cells was conducted to confirm whether limbal stem cell deficiency if present could be due to damaged stem cells.

The presence of inflammatory cells in the pterygium samples was confirmed and these cells were assessed to determine whether pterygium occurrence and its recurrence after surgery were related to the severity of inflammation. If inflammation was crucial for pterygium to occur most of the pterygia would be severely inflamed. The presence or absence of collagen degeneration was noted to determine whether the severity of inflammation was related to the level of exposure of the patient to sunlight because collagen degeneration is an objective measure of the duration of high sunlight exposure.[33]

3.4 Sample size

Three hundred participants (150 cases and 150 controls) were calculated to detect a 20% difference in family history between cases and controls with a power of 80% and a type 1 error probability of 0.05, assuming a base rate of 10% in controls. The assumption was based on an Australian study that reported positive family history rate of 8-12% in controls,[22] which is the only report comparing family history of pterygium between cases and controls. Nevertheless, 230 patients were interviewed because 30 of the first 150 did not have indication for surgery so, 80 additional patients who had indication for surgery were interviewed. These cases were not matched with controls.

Since rural Africans have been reported to obtain employment on farms[9] it is unlikely that only cases or controls were occupied outdoors and so, sunlight exposure was thought to be unsuitable to base sample size calculation on. As the determinants of pterygium occurrence in a rural African population were not known the study presented results on the epidemiology of pterygium in a rural African population, to the Ophthalmological Society of Southern Africa Congress in 2010. By then, data were available for 71 cases and 71 controls. Fifty one pedigrees of pterygium probands and 50 of controls were studied because a previous study on primary blepharospasm found that this number of pedigrees obtained many relatives sufficient for analysis.[90]

One hundred and seventy six patients, 88 per group, were calculated to detect a 15% difference in recurrence rate between CAT and LCAT procedures with a power of 80% and alpha value of 5%, and assuming a base recurrence rate of 20% in CAT. This recurrence rate was based on 21%, which has been reported by a study on a similar population.[95] A default rate of 12% was factored and so 200 patients were operated on. Because the study had planned to encourage the participants to return for follow-up by offering a contribution to transport costs a default rate of 12% was considered to be adequate for an acceptable participation rate.

3.5 Participant selection

Consecutive patients attending the out-patients department, males and females, who were 21-65 years old, were eligible to participate if they were rural black Africans. Age below 21 years could not legally consent. As nearly half of Limpopo's population

was 20-64 years old[178] the 21-65 year age group was a suitable sampling frame. The results from this study are representative of a rural African population.

The study selected the patients who had primary pterygium to be cases and those who did not have pterygium to be controls. As malignancy may masquerade as pterygium and this can confuse study findings, the patients selected did not have clinical evidence of ocular surface malignancy. And to prevent the possibility of pseudo-pterygium masquerading as pterygium, patients with scleral or peripheral corneal thinning were rejected. For a similar reason, patients with evidence of previous ocular surface trauma, surgery or inflammation, such as those with scars and Herbert's pits were excluded. Other causes of inflammation would confuse findings due to inflammation caused by sunlight damage.[35,77] Because the presenting complaint of the patients attending the refraction clinic was poor vision those having cataracts, glaucoma, corneal scars, retinopathies, or optic nerve disorders were excluded in cases and controls. To minimize other causes of dry eyes the patients who had blepharitis, lid deformities, or were using prescribed topical or systemic medications were excluded. Exclusion of the patients using prescribed eye drops ensured that chronic pterygium inflammation had not been treated and this increased the credibility of the findings on inflammatory cell assessment. This exclusion also minimized the possibility of the patients having allergic ocular surface conditions participating in the study as these are likely to be using chronic medication. Controls that were using contact lenses were rejected because contact lenses may cause dry eyes. As the individuals who were using chronic medication were excluded investigation on the effect of the status of health on pterygium occurrence was abandoned.

The families of the cases as well as controls that were visited were chosen if the probands agreed to a family visit and their key relatives were living within twenty kilometres of one another within Limpopo Province. Living close together in the same province minimized individual differences in the environmental exposure to UV radiation,[38] which made it unlikely that the environment was crucial for pterygium to occur. Moreover, living close together ensured that the relatives visited were key family members as the tradition of cousin marriage was practiced by the study population;[184] as the youngest age at diagnosis of pterygium presence in Africans has been reported to be ten years[7] this age was the minimum for family members to be examined.

The pterygium cases were offered surgery if there was an indication such as visual impairment due to the growth, a growth threatening the visual axis, frequent pterygium irritation, or cosmetic disfigurement.[9,13,14] The patients were selected if they accepted surgery or if they requested for operation to improve cosmetic appearance.

Pterygium specimens from the operated patients were investigated by routine histology and by immunohistochemistry. Histology was necessary to make sure that pterygium was being dealt with. The control specimens were obtained from the nasal corneo-conjunctiva of patients without pterygium who were undergoing evisceration for irreparably injured eyes; these controls were chosen because the location of the nasal corneo-conjunctiva is similar to that of nasal pterygia, which occur more frequently than temporal pterygia. The injury in the eyes chosen had spared the nasal corneo-conjunctiva and sclera, which minimized the possibility of MMP expression due to

trauma;[187] it was anticipated that the immunohistochemical markers would be universally negative in controls if DNA damage were associated with pterygium occurrence. The injured patients fulfilled the inclusion and exclusion criteria of the pterygium patients.

3.6 Interview

The age and sex were verified by the identity document and the participants were asked for their ethnic group. Structured questions, which ensured uniformity, were used in the interview. These were tested on the first 142 participants and they were effective except for questions on previous food eaten and on marital status. The participants expressed difficulty remembering the food eaten. The participants who were married traditionally, and their marriages had not been registered reported that they were unmarried hence, introducing bias. So, investigations on the effect of diet and marital status were abandoned. The questions used for interviews covered family history of pterygium; all the occupations ever held and habits regarding time spent outdoors in those occupations or leisure; exposure to dust while at work or, to smoke anytime; use of tobacco and of alcohol; and use of traditional eye medicine.

To facilitate recall family history of pterygium was assessed in a manner similar to that reported in a previous study;[32] the participants were shown three photographs of eyes with pterygium (Figure 3.1). They were then asked if they had ever seen a person with such a growth on the eye. If they answered affirmatively they were asked if that person

is or was a relative. And if so, the participants were asked for the type of relationship they had with that person.

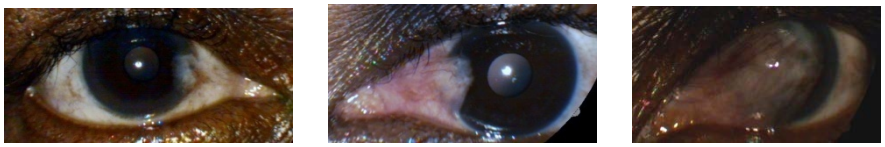


Figure 3.1: Three photographs of eyes with pterygium, used in the interviews

Sunlight exposure was evaluated by determining the proportions of outdoors occupations as a percentage of all occupations ever held. Each outdoors occupation was deemed to occur if 5hours or longer per day between 7am and 6pm in workdays were spent outside a building. The study chose 5hours because the average working time is 8am-10.30am, 11am-1pm, and 2pm-4pm. From 9am to 5pm high levels of UV radiation occur.[179] Workdays were at least 4 per week. The mean daily duration of exposure in a life time was calculated from the durations of exposures at work, weekends, and leisure days in all the occupations ever held. This was because some individuals may spend a short time outdoors during work days but spend a long time outdoors on weekends and leisure days;[32] the participants were asked whether they had ever used spectacles or sunglasses and if so, when the use started.

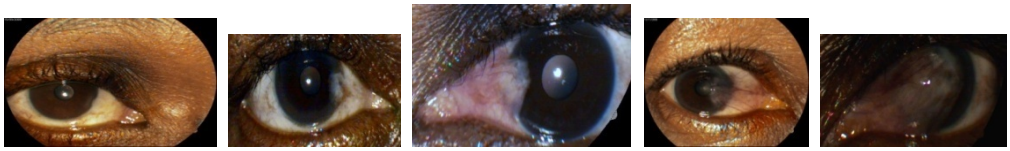
The effect of suspended solid particles in air was determined by asking whether the participant was usually exposed to dust while at work or to smoke at any time. The effects of tobacco, alcohol and traditional eye medicine were assessed by asking whether those substances had ever been used. Three participants who used traditional

medicine were questioned about its source, how it was formulated, and how it was used, and whether it was stored or used immediately after formulation.

3.7 Full eye examination

An unstable tear film suggested the presence of a dry eye,[21,188] which was assessed by measuring the tear film breakup time (TBUT). 1% fluorescein in a paper strip was used and thereafter, the previously reported procedure was followed;[188] the cornea was assessed after application of fluorescein for areas of dark spots or streaks between blinks. The mean of three measurements was the TBUT. If the TBUT was less than 10 seconds the tear film was considered to be unstable.

Whether pterygium occurrence was unilateral or bilateral was noted. The pterygia were assessed for fleshiness as previously reported.[25] Visibility of episcleral vessels through the growth was evaluated and graded into easily visible (grade 1) and not visible (grade 3). Anything in between was grade 2. Evaluation of the extent was done in a manner similar to that previously reported by Youngson.[189] Grade 1 growths had just crossed the limbus, grade 2 were approaching half of the corneal radius, grade 3 had just crossed this radius, grade 4 were approaching the corneal mid-point. And according to Carmichael (personal communication) grade 5 pterygia crossed the corneal mid-point. Figure 3.2 illustrates the grades of pterygium extent. Grades 1 and 2 pterygia were defined as small and grade 3 or larger as large. Grades 4 or 5 were sub-catergorized as extra-large growths.



Grade1 Grade 2 Grade 3 Grade 4 Grade 5

Figure 3.2: Photographs of eyes with pterygium showing grading of extent

3.8 Family studies

The ages of the family members were established from their national identification documents and the ages of the children younger than 16 years who did not possess those documents were confirmed by their parents. I examined the eyes of key relatives using light from a diagnostic lamp for presence of pterygium. Recent photographs of the relatives that were absent were also examined by me. Key relatives included siblings, parents, offspring, grandparents, and first uncles and aunts of the proband.

3.9 Comparison of CAT and LCAT procedures

Three hundred numbers, 150 even and 150 odd, in sealed envelopes were used for randomizing the patients into CAT if the patient picked an even number or LCAT if an odd number was picked. Surgery was performed under a microscope by two experienced surgeons (SF or MS) who followed the same technique.

The pterygium was excised as described previously;[54] the head and neck were dissected off using a curved crescent knife. Care was taken to leave no remnants of the

growth on the cornea. The body was excised at 4 mm from the limbus and at its superior and inferior borders with the un-stretched conjunctiva.

Free conjunctival auto transplant was performed in a way that was previously reported;[54] the conjunctiva at the upper part of the globe of the same eye was harvested using Vannas scissors. It was harvested 1 mm longer and wider than the bare sclera to cover so as to minimize the effect of possible graft retraction. The graft was rotated into the bed orienting its limbal side to the recipient limbus. 10/0 nylon sutures were used. Limbal conjunctival auto transplant also was done as described in an earlier report;[54] the conjunctival flap was harvested and the superficial limbus was dissected including 0.5mm of the peripheral cornea. The graft was sutured into the bed anatomically oriented, which ensured delivery of the harvested stem cells to the pterygium-affected limbus.

Subconjunctival dexamethasone (2 mg) was injected into the inferior fornix at the conclusion of surgery and topical ciprofloxacin 3mg/ml and prednisolone acetate 10mg/ml were applied 6 hourly postoperatively. The antibiotic was used for one week but the steroid was tapered over the next six weeks so as to minimize post-operative inflammation, which has been associated with recurrence.[190] Sutures were removed at 1 month.[23]

The patients were followed-up for a minimum duration of 6 months because a prospective study has reported that by six months 94% of recurrences in excised primary pterygia had occurred.[24] Recurrence or no recurrence was confirmed by two

Ophthalmologists (TS or BA) who were masked to the pre-operative status and type of surgery performed, which minimized bias. Recurrence was defined as the crossing of a wing-shaped fibrovascular growth of the conjunctiva onto the cornea at the site of previous pterygium.[24,25]

3.10 Histological examination and immunohistochemistry investigations

Samples that were found on histology to be crushed or fragmented were excluded. These are unsuitable for investigations on MMPs because traumatized tissues are likely to express MMPs in the stroma,[187] which would confound results on stromal MMP detection due to remodeling not related to trauma.[129] The controls were obtained from traumatized eyes however, the injury had not extended to the nasal corneo-conjunctiva which was the study area. Pterygium was confirmed by the presence of collagen degeneration,[27] numerous blood vessels[6] and fibroblasts,[8] and the absence of neoplasia. Fibroblasts were identified by their oblong shape.[8] These may be synthesizing little collagen.[5,191]

To confirm whether or not limbal stem cell damage was associated with pterygia immunohistochemistry using p53 and MMP1, 2 and 3 antibodies was done as damaged cells express these proteins.[28,71,72] Fibroblasts and stroma were observed for expression[72,73] or no expression of MMPs.[71]

Inflammatory cells were confirmed by immunohistochemistry using an antibody against leukocyte common antigen (LCA).[79] However, this may also detect lymphoma infiltrations whereby the leukocytes would be crowded[192] rather than be scattered as in inflammation.[6,8] The depth of the location of most of the inflammatory cells distinguished between hypersensitivity reactions at the epithelial surface[7,79] and inflammation due to sunlight damage whereby leukocytes are located mainly in the stroma.[33,35,77] Due to lack of equipment, which would consider the size of the section and distribution of cells inflammatory cells were assessed by counting all leukocytes in each specimen.

Inflammatory cells were not investigated in the controls because evisceration was performed at least 72hrs after injury when the conjunctiva had already undergone chemosis. Because of post-traumatic chemosis it was assumed that trauma would cause inflammatory changes,[35] which would confuse the findings on inflammatory cells.

After excisions of the pterygia and control tissues each sample was marked with alcian blue at one longitudinal border to facilitate identification of the direction along which to cut sections. The specimen was labeled at the tail/conjunctival end and placed deep surface facing cardboard paper to keep it flat. It was immediately inserted into 10% buffered formal saline and sent to the laboratory on the same day.

The samples were processed to paraffin blocks within 24hours and they were cut at 3 microns thick from within 300 microns of the mid-longitudinal meridian so that the chance of detecting target cells in different specimens was equal. To confirm the

diagnosis of pterygium (presence of fibroblasts[8] and blood vessels[6]), and to exclude neoplasia, which may also cause p53 expression,[34] the sections were stained with haematoxylin and eosin.

To determine the optimal dilution of primary antibodies, their concentrates were diluted with antibody diluent. The concentration that highlighted the cells best without precipitation on the section of the control tissue was chosen. The antibodies, clone, source, and their dilutions are shown in Table 3:1. One senior histotechnologist (DBM) processed the sections using the same technique in the same conditions.

Table 3.1: List of antibodies, their clones, sources and their dilution used in the immunohistochemical studies

Antibody	Clone	Source	Dilution
LCA	PD7/26 and 2B11	Dako	
P53	DO-7		
MMP1	3B6		1:50
MMP2	8B4	Santa Cruz	
MMP3	1B4		

The sections were placed on slides and incubated overnight at 60 degrees Celsius to fix them on the slides. They were then de-paraffinized in xylene and the xylene was removed by absolute alcohol. The sections were rehydrated using decreasing concentrations of alcohol and finally tap water. Thereafter, they were rinsed in distilled

water till all waxy debris was removed. The samples were immersed in target retrieval solution (TRS) at pH 9 then treated in a 700 watts microwave oven for 5 minutes. Subsequently, the samples were rinsed in Tris Buffered Saline (TBS) while stirring gently for 5 minutes. To block nonspecific staining due to endogenous peroxidase the samples were immersed in peroxidase blocking solution for 5 minutes and then rinsed in TBS for 5 minutes. The sections were incubated with calibrated primary antibodies for 30 minutes. And they were rinsed in TBS for 10 minutes. To provide binding sites for the chromogen, the sections were incubated with horseradish peroxidase (HRP) rabbit/mouse secondary antibody for 30 minutes then they were rinsed in TBS for 5 minutes before incubation with 3,3' diamino-benzidine-tetrahydrochloride (DAB) chromogen for 10 minutes followed by incubation with DAB substrate buffer for 10 minutes. The sections were again rinsed in TBS buffer for 5 minutes then counterstained with Mayer's haematoxyline for 30 seconds. Bluing of the sections was done in running tap water for 3 minutes. Subsequently, cover-slips were applied using Faramount aqueous mounting medium. The slides were read under the guidance of one experienced and masked Pathologist (JK).

The control tissues for primary antibodies are shown in Table 3:2. These tissues were positive with primary antibody and negative without. The sources of the reagents used are shown in Table 3:3.

Table 3.2: Control tissues used in immunohistochemical studies

Primary antibody	Control tissue
LCA	Tonsil
P53	
MMP1	Breast carcinoma
MMP2	
MMP3	

Table 3.3: Sources of reagents used

Reagent	Source
TRS	DAKO
TBS	
Peroxidase blocking solution	
Antibody diluent	
HRP rabbit/mouse secondary antibody	
DAB chromogen	
DAB substrate buffer	

3.11 Data analysis

As pterygium patients may minimize exposure to sunshine after diagnosis[193] the reference period for data collection after interview of each case and its control was set before pterygium occurrence was diagnosed in the case. Analysis was done by calculating the odds ratios (ORs) for the risk factors of pterygium occurrence by

conditional logistic regression models using age and gender as pairing variables. To get a clue on factors significant in pterygium occurrence in a rural African population univariate analysis was done on the data obtained from the first 71 cases and 71 controls.

The relative significance of factors in pterygium occurrence was assessed by univariate and a multivariate analysis on 150 case-control pairs, which included the initial 71 case-control pairs. The variables that were significant in univariate analysis were included in a multivariate analysis to test for independence of association. Because alcohol was used by only 8 cases and 4 controls alcohol use was excluded from statistical analysis.

Owing to the obviously large differences between pterygium and control families statistical calculations were not done. Since households were small and alleles may or may not be transmitted, a large family, which increases the credibility of the calculated mode of inheritance was obtained by combining the family members of pterygium probands.[90,194] One pedigree was used to illustrate likely modes of inheritance.[85,86]

According to the Mendelian principles the different modes of inheritance have specific ratios between the unaffected and affected family members.[81,166] As that ratio was not Mendelian and the computed equivalent ratio was not exact the nearest ratio equivalent to a Mendelian one was estimated, which was assumed to indicate the likely mode of inheritance. This assumption was based on the fact that mathematical calculations can yield decimals, yet decimals of individuals don't exist.

Genotype determines the phenotype that an individual may be prone to.[81,166,167,168,176] Because molecular studies were not done genotypes were suggested based on Mendelian principles.[81,165,166] To suggest the genotypes of pterygium patients genotypes of a mating couple whose proportion of unaffected and pterygium-affected offspring matched with the equivalent ratio were predicted. By applying Mendelian principles[81,165,166] to that mating couple the genotypes of the offspring were predicted.

To test whether CAT and LCAT were comparable demographic data such as age, sex, and occupation after surgery, as well as data on pterygium characteristics such as unilateral or bilateral growths, and extent were compared between patients undergoing CAT and those undergoing LCAT. To test whether or not demographic or pterygium characteristics affected recurrence these were compared between the pterygia that recurred (cases) and those that did not (controls). Multivariate analysis of variables significant in univariate analysis was done. Recurrence rates in CAT and LCAT were compared.

The number and percentage of pterygium specimens positive for p53 and MMPs in the limbal basal epithelial cells was compared between cases and controls. The inflammatory cells were counted manually due to lack of equipment. The size of the specimen was considered crudely. The inflammatory cell count was categorized and the distribution of pterygia of various grades among the different ranges of inflammatory cell counts was examined. The lower level of the lowest range whereby the inflammatory cell count of the largest pterygium fitted was regarded as the lower limit of high count. This is because smaller pterygia having the same range of count as

the largest pterygium are expected to have increasingly more inflammatory cells per pterygium size as the pterygium size decreases. It was practical that if the count was due to size of the growth small pterygia would have a low but not a high count while large pterygia would have a high but not a low count. The number and percentage of samples having the different categories of inflammatory cell infiltration were compared among pterygia of different extents to test whether the degree of cell infiltration was due to pterygium size. Also, that number and percentage of samples were compared among pterygia from patients exposed to UV light for various categories of duration. This was to test whether the daily duration of exposure influenced the severity of inflammation. The effect of the use of traditional eye medication on inflammation was tested by comparing the number of sections having the different categories of inflammatory cell count between use and no use of traditional eye medication.

Statistical significance of categorical variables in large samples was tested by the Chi-square test, so as to be able to predict outcomes from samples that have sizes outside the present. Small samples were tested by the Fisher's exact test as only the null hypothesis was of interest. Statistical significance in continuous variables was tested by the Student's t-test if the data was normally distributed otherwise; the data was categorized then tested. In recurrence after surgery age was categorized into <50 years (young) and ≥50 years (old) even if age was normally distributed, so as to make it comparable to a previous study.[24]

Significance in all calculations was set at a maximum p value of 0.05 or if an OR of 1 was outside the 95% confidence interval (CI). STATA 9 for Windows software (STATA Corporation, College Station, USA) was used to perform statistical calculations. Trends in the data as well as aberrations from the expected trends indicated significance in the assessment of inflammatory cells and mode of inheritance.

Chapter 4: Results

4.1 Factors significant in pterygium occurrence

Data on initial 71 cases and 71 controls were analyzed. The mean age ±standard deviation (SD) of the participants was 45 ±10.65 years with a range of 22-65 years. One hundred and two of the participants (71.8%) of 142 were female and 40 (28.2%), were male.

Family history of pterygium was present in 26 cases (36.6%) of 71 and 7 controls (9.8%) of 71; OR = 4.6; 95% CI = 1.8-11.6. Family history was more frequent in cases than controls.

An unstable tear film was present in five cases (7%) of 71 and 12 controls (17%) of 71; OR = 0.24; 95% CI = 0.06-0.93. The unstable tear film was more common in controls than cases.

4.2 Relative significance of factors in pterygium occurrence

One hundred and fifty cases and 150 age and sex matched controls were interviewed and their eyes examined. Spectacles or sunglasses had not been used by the 300 participants (100%). Table 4.1 presents demographic and environmental factors not significant. The most frequent age range was 40-49 years. The females were over 3.5 times as frequent as males.

Table 4.1: Demographic and environmental factors found to be non-significant

Factor		Cases (%) N=150	Controls (%) N=150
Age in years.	20-29	12 (8)	12 (8)
	30-39	23 (15.3)	23 (15.3)
	40-49	54 (36)	54 (36)
	50-59	51 (34)	51 (34)
	60+	10 (6.6)	10 (6.6)
Gender	Female	118 (78.6)	118 (78.6)
	Male	32 (21.3)	32 (21.3)
Education in years.	≤12	106 (70.6)	114 (76)
	>12	44 (29.3)	36 (24)
Ethnic group	Pedi	111 (74)	112 (74.6)
	Other	39 (26)	38 (25.3)
Outdoors occupation		80 (53.3)	80 (53.3)
Exposed to dust		124 (82.7)	126 (84)
Exposed to smoke		127 (84.7)	121 (80.7)
Ever used tobacco		31 (20.7)	26 (17.3)

Figure 4.1 compares the cases and controls in their mean daily duration of exposure to sunlight. A long daily duration of exposure was more frequent in cases as well as controls. The numbers of cases and controls exposed for long or short durations were similar.

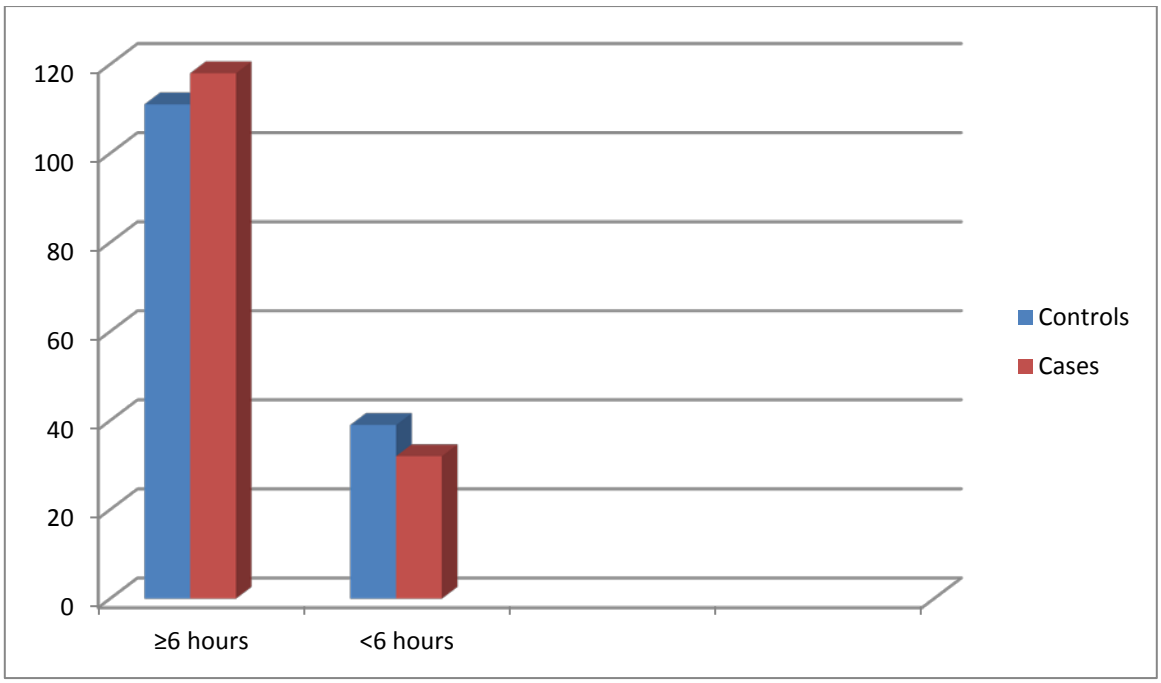


Figure 4.1: Numbers of cases and controls exposed to sunlight for a mean daily duration of ≥6 hours and those exposed for <6 hours

Table 4.2 presents the proportion, OR, and p value of variables significantly associated with pterygium occurrence from the univariate analysis. Family history of pterygium and the use of traditional eye medication were reported more frequently by pterygium patients. Unstable tear film was more frequent in controls.

Table 4.2: Risk factors found to be significant in univariate analysis

Factor	Cases (%) N=150	Controls (%) N=150	OR	P value*
Family history	46 (30.6)	15 (10)	3.81	<0.01
Used traditional eye medication	79 (52.6)	60 (40)	1.70	0.03
Unstable tear film	10 (6.6)	26 (17.3)	0.33	<0.01

* Chi-square test

Table 4.3 presents variables independently associated with pterygium occurrence. When all variables significantly associated with pterygium occurrence in univariate analysis were included in the same model in a multivariate analysis the ORs for family history and the use of traditional eye drops increased whereas the OR for the unstable tear film decreased. The significances of the ORs for family history and unstable tear film remained the same whereas the significance of the OR for the use of traditional eye drops increased.

Table 4.3: Factors found to be significant in multivariate analysis

Factor	Cases (%) N=150	Controls (%) N=150	OR	P value*
Family history	46 (30.6)	15 (10)	3.93	<0.01
Used traditional eye medication	79 (52.6)	60 (40)	2.03	<0.01
Unstable tear film	10 (6.6)	26 (17.3)	0.30	<0.01

* Chi-square test

Three participants who were quizzed about traditional medicine reported that it was prepared from leaves of a plant that the Pedi call *Mmale*. Fresh leaves were harvested and crushed immediately and placed in a basin, to which fresh water was added to make a suspension. The patient was asked to immerse his/her open eyes into the prepared medicine for several minutes to treat red eyes. The medicine was not stored. Figure 4.2 shows the frequency of use of traditional eye medication in cases and controls. Nearly half of the controls also used this medication.

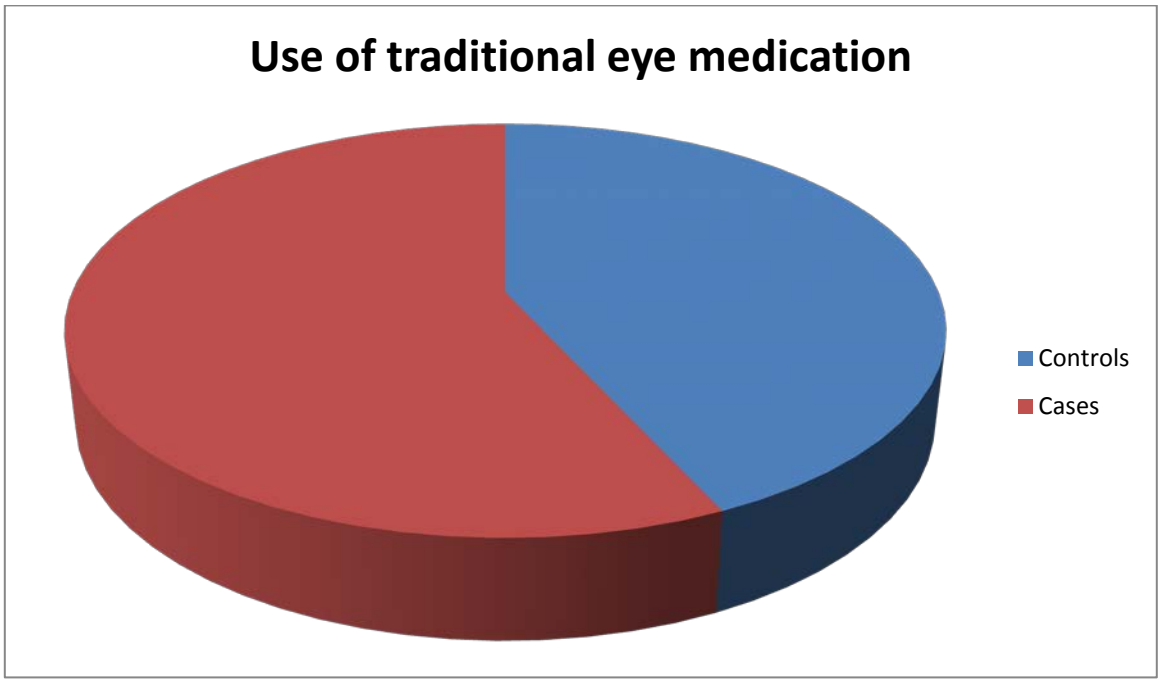


Figure 4.2: Frequency of use of traditional eye medication in cases and controls.

4.3: Familial factors

Fifty one families of pterygium probands and 50 of control probands were visited. The probands lived in the different regions of Limpopo Province. Three hundred and eighty

two combined relatives of pterygium probands including the probands and 394 combined relatives of control probands including the probands were examined. Fourteen family members of cases (3.6%) of 382 and 17 family members of controls (4.3%) of 394 were diagnosed by photographs. The age range of the individuals examined was from ten to 86 years. The proportion of family members aged 40 years or older was 71.9% in cases (275 of 382) and 72% in controls (284 of 394). The pterygium probands had 56 combined offspring aged less than 20 years and three of them (5.4%) were pterygium-affected. The cases had 2-14 individuals in a pedigree and the controls had 4-12. Thirty four pedigrees of cases (66.7%) of 51 and 33 control families (66%) of 50 had 6-9 individuals in a household, which was the median and also the most frequent range of family size. The pedigrees of pterygium probands and controls were comparable.

Figure 4.3 illustrates a pterygium pedigree. Pterygium affected more individuals in the second generation than in the third generation. Pterygium recurred within 3 months after surgery in the proband and his affected daughter was 16 years old.

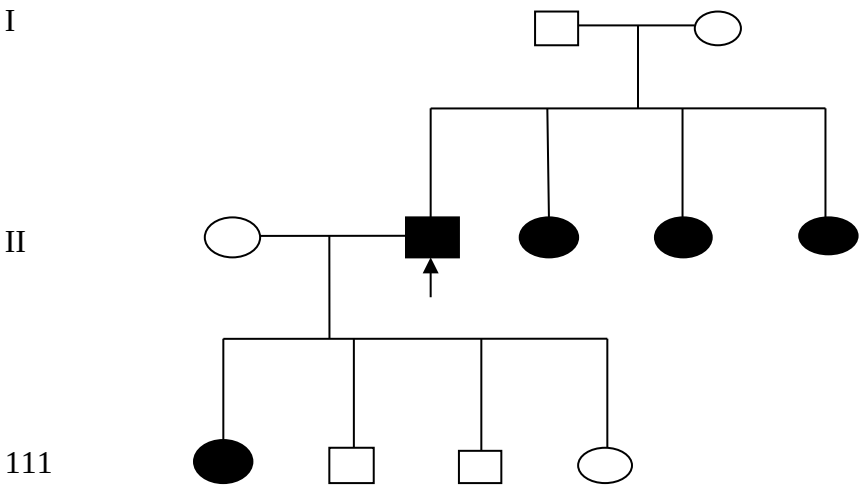


Figure 4.3: One pedigree whereby the proband was a pterygium patient

Oval empty = unaffected females; oval shaded = affected females; rectangles empty = unaffected males; rectangles shaded = affected male; the arrow indicates the proband.

One hundred and fifty seven combined relatives in the families of cases (41%) of 382 were pterygium-affected and 16 combined relatives in control pedigrees (4%) of 394 were pterygium patients. Pterygium-affected relatives were more frequent in the case pedigrees. The ratio of the unaffected to the pterygium-affected persons in the combined families of the cases was 1.4:1 and the equivalent ratio was 9.8:7, which was estimated at 9:7. The predicted genotypes of a mating couple whose unaffected and pterygium-affected offspring are in proportions that match with the 9:7 ratio are *AaBb* X *AaBb* whereby *A* or *B* stands for active alleles and *a* or *b* stands for inactive alleles. Those parents are not pterygium patients. Table 4.4 depicts genotypes of the offspring of the mating couple having the predicted genotypes *AaBb* X *AaBb*. The depicted genotypes of pterygium-affected offspring are highlighted by bold font. It appears that pterygium patients have at least one type of gene having both of its alleles inactive.

The proportion of patients having both alleles of one type of gene inactive was the same as that of patients having both alleles of the second type of gene inactive

Table 4.4: Suggested genotypes of affected and unaffected offspring

AaBb X *AaBb*

	AB	*Ab*	*aB*	*ab*
AB	*AABB*	*AABb*	*AaBB*	*AaBb*
Ab	*AAbB*	***AAbb****	*AabB*	***Aabb****
aB	*aABB*	*aABb*	***aaBB****	***aaBb****
ab	*aAbB*	***aAbb****	***aabB****	***aabb****

*Pterygium patient

Table 4.5 presents the number of pedigrees of cases and controls in relation to the number of family members and number of individuals affected in a family. Larger families tended to have more pterygium-affected persons. Groups of pterygium-affected relatives were more frequent in cases. Groups of 3-5 pterygium patients in a household occurred in 36 pterygium households (70.5%) of 51 compared with 1 control household (2%) of 50.

Table 4.5: Distribution of pterygium-affected individuals in families

		Case pedigrees: number of individuals in family									
		≤4	5	6	7	8	9	10	11	12	14
Number of individuals affected in a family	0	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*
	1	2*	3*	2*	1*	0*	0*	0*	0*	0*	0*
	2	1*	3*	0*	1*	1*	1*	0*	0*	0*	0*
	3	0*	0*	5*	2*	1*	3*	1*	0*	1*	0*
	4	0*	0*	7*	0*	1*	7*	1*	2*	0*	1*
	5	0*	0*	0*	1*	1*	0*	1*	0*	0*	1*
		Control pedigrees: number of individuals in family									
		≤4	5	6	7	8	9	10	11	12	14
Number of individuals affected in a family	0	3*	3*	5*	2*	7*	10*	4*	1*	2*	0*
	1	0*	1*	4*	2*	2*	1*	0*	0*	1*	0*
	2	0*	0*	0*	0*	0*	0*	1*	0*	0*	0*
	3	0*	0*	0*	0*	0*	0*	0*	0*	1*	0*
	4	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*
	5	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*

*Number of families

4.4 Factors in pterygium recurrence after surgery

Two hundred patients having pterygia of fleshiness grade 3 (fleshy) were operated on. After 10 patients were lost to follow-up, data were available for 190 patients who were followed-up for a minimum duration of 6 months. One hundred and one patients

(53.2%) of 190 underwent CAT but 89 participants (46.8%) of 190 underwent LCAT. Only one eye per patient was enrolled in the study. The age range was 22-65 years; mean ±SD was 46.6 ±10.8 years. Twenty nine participants (15.3%) of 190 had small pterygia. Twenty eight (96.6%) of 29 participants that had small pterygia were young. Thirty eight patients (20%) of 190 had extra-large growths. Twenty two (57.9%) of 38 patients that had extra-large pterygia were old.

Pterygium recurred after surgery in 52 patients (27.4%) of 190. The growth recurred in 38 patients (73.1%) of 52 within 3 months of excision (short recurrence time). It recurred after 3 months of surgery (long recurrence time) in 14 patients (26.9%) of 52. Two patients (5.3%) of 38 whose pterygia recurred within a short time after excision were probands in family studies. Each of these probands had one of their offspring, aged 16 years, pterygium-affected. Of the patients who had short recurrence times seven (18.4%) of 38 experienced post-surgical recurrence within 1 month. Table 4.6 shows a comparison of age and pterygium extent in patients who experienced a short post-surgical recurrence time and those who experienced a long recurrence time. A short recurrence time tended to be more frequent than a long recurrence time after surgery. The frequency of young individuals and that of old individuals who experienced a short recurrence time was similar. Also, the frequency of young patients and that of old patients who experienced a long post-surgical recurrence time was similar. The frequencies of small and large pterygia that recurred within a short time were similar, and the frequencies of small and large pterygia that recurred after a long time were similar. Of the 13 old patients who experienced recurrence, one (7.7%) age 51 years had a small pterygium, which recurred after 3 months following surgery. Of

39 young patients, who experienced post-surgical recurrence of pterygium, 20 (51.3%) had small growths. Sixteen (80%) of 20 young participants who had small pterygia that recurred experienced recurrence within 3 months after surgery.

Table 4.6: Comparison of age and pterygium extent in patients experiencing short and long post-surgical recurrence times

Variable	**Short recurrence time (%)** **N = 38**	**Long recurrence time (%)** **N = 14**
<50 years N = 39	29 (74.4)	10 (25.6)
≥50 years N = 13	9 (69.2)	4 (30.8)
<grade 3 extent N = 21	16 (76.2)	5 (23.8)
≥grade 3 extent N = 31	22 (71)	9 (29)

Of 38 participants who had extra-large pterygia, 18 (47.4%) experienced post-surgical recurrence. Figure 4.5 shows the distribution of extra-large pterygia that recurred after excision and those that did not in young and old patients. Extra-large pterygia were more frequent in old patients however; the proportions which recurred were similar to those which did not recur after excision in old and young individuals.

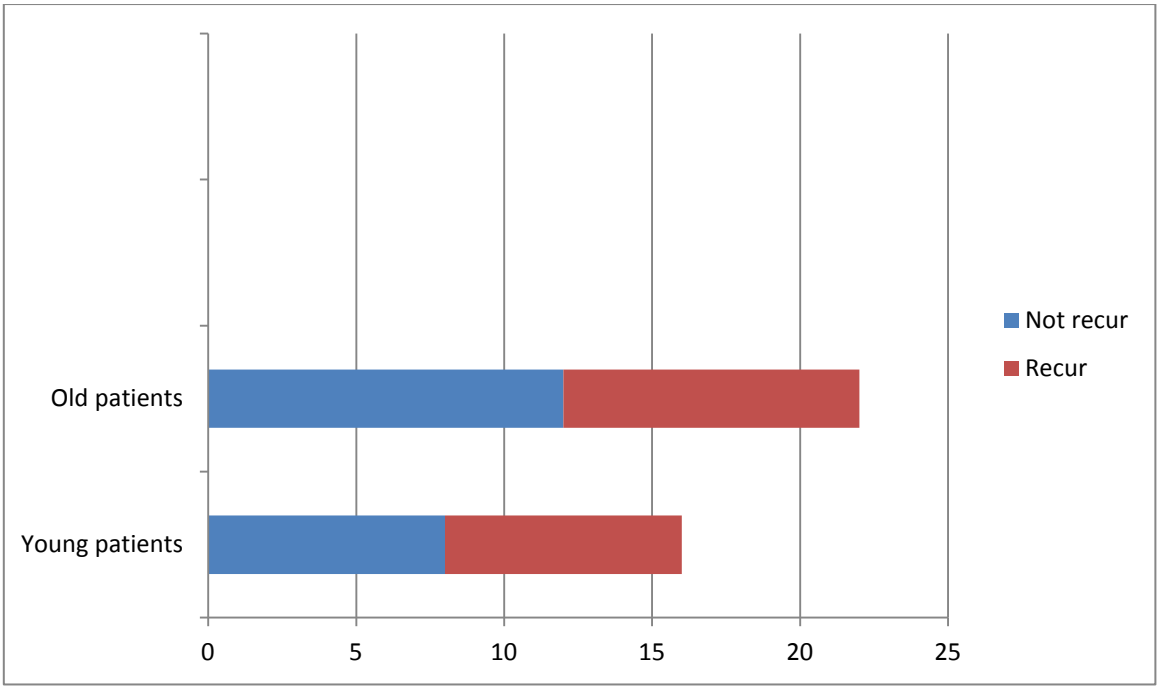


Figure 4.5: Distribution of extra-large pterygia in young and old patients. The horizontal axis represents the count of patients

Table 4.7 shows the univariate analysis of demographic and pterygium characteristics of the participants whose pterygium recurred after excision and those whose growths did not recur. Recurrence was more frequent than no recurrence in young patients and in small pterygia.

Table 4.7: Univariate analysis of demographic and pterygium characteristics in patients with recurrence after excision and those without recurrence

Variable	Recur (%) N=52	Not recur (%) N=138	OR	95% CI	P-value*
Age <50years.	39 (75)	72 (52)	2.75	1.35-5.59	<0.01
Age ≥50years.	13 (25)	66 (48)	0.36	0.18-0.74	
Female	42 (81)	113 (82)	0.92	0.41-2.09	0.86
Outdoors occupation	28 (54)	83 (60)	0.77	0.40-1.47	0.43
Bilateral	40 (77)	97 (70)	1.41	0.67-2.96	0.36
Extent grade 2	21 (40)	8 (6)	11.1	4.55-33.33	<0.01
Extent grade ≥3	31 (60)	130 (94)	0.09	0.03-0.22	

* Chi-square test

Table 4.8 shows the multivariate analysis of the factors significant in pterygium recurrence. The OR for recurrence after excision of pterygium in young patients decreased compared to that in univariate analysis and it was no more significant. The OR for post-surgical recurrence of small pterygia decreased but it remained significant.

Table 4.8: Multivariate analysis of the factors significant in pterygium recurrence after surgery

Variable	Recur (%) N=52	Not recur (%) N=138	OR	95% CI	P-value*
Age <50years.	39 (75)	72 (52)	1.54	0.70-3.36	0.28
Age ≥50years.	13 (25)	66 (48)	0.65	0.29-1.42	
Extent grade 2	21 (40)	8 (6)	9.1	4.54-33.33	<0.01
Extent grade ≥3	31 (60)	130 (94)	0.11	0.04-0.28	

* Chi-square test

Table 4.9 presents univariate analysis of the demographic and pterygium characteristics as well as the surgical outcome in the patients that underwent CAT or LCAT. The proportions of the patients having those variables were similar between the two procedures.

Table 4.9: Univariate analysis of demographic and pterygium characteristics of patients operated, and surgical outcome

Characteristic	CAT N=101	LCAT N=89	P-value
Mean age in years (±SD)	46.6 ±10.2	46.1 ±11.5	[+]0.72
Gender			
Male	18 (17.8%)	17 (19.1%)	[*]0.82
Female	83 (82.2%)	72 (80.9%)	
Occupation after surgery			
Indoors	43 (42.6%)	36 (40.5%)	[*]0.77
Outdoors	58 (57.4%)	53 (59.6%)	
Laterality			
Unilateral	29 (28.7%)	24 (27.0%)	[*]0.79
Bilateral	72 (71.3%)	65 (73.0%)	
Extent grade			
2	15 (14.9%)	14 (15.7%)	[*]0.87
≥3	86 (85.1%)	75 (84.3%)	
Surgical outcome			
Recur	29 (28.7%)	23 (25.8%)	[*]0.66
Not recur	72 (71.3%)	66 (74.2%)	

[+] Student t test; [*] Chi-square test

4.5 Expression of p53, MMPs, and LCA in pterygium

Fifty nine pterygium samples and seven corneo-conjunctival controls were investigated by immunohistochemistry using antibodies against p53 and MMP1, 2 and 3, and LCA. Forty five samples (76.3%) of 59 were from females while 14 samples (23.7%) of 59 were from males. The patients' age range was 23-64 years. Thirty three individuals (55.9%) of 59 were young while 26 individuals (44.1%) of 59 were old. Six controls (85.7%) of seven were from young individuals. All seven control specimens were from males. Spectacles or sunglasses had not been used by cases or controls. There was no neoplasia in pterygia and controls. Numerous fibroblast-like cells were identified in all 59 pterygium samples (100%). The majority of the fibroblast-like cells were thin in most of the samples. Collagen degeneration was seen in 58 pterygium specimens (98.3%) of 59 and five controls (71.4%) of seven. Blood vessels were noted in pterygium samples whereas hardly any blood vessel was seen in control sections.

Figure 4.6 shows limbal basal epithelial nuclear-immunostaining with p53 antibody. A similar result was obtained in the controls. p53 was detected in 11 pterygium specimens (18.6%) of 59 and five controls (71.4%) of seven; $p < 0.01$ Fisher's exact test. Up-regulation of p53 was more frequent in controls.

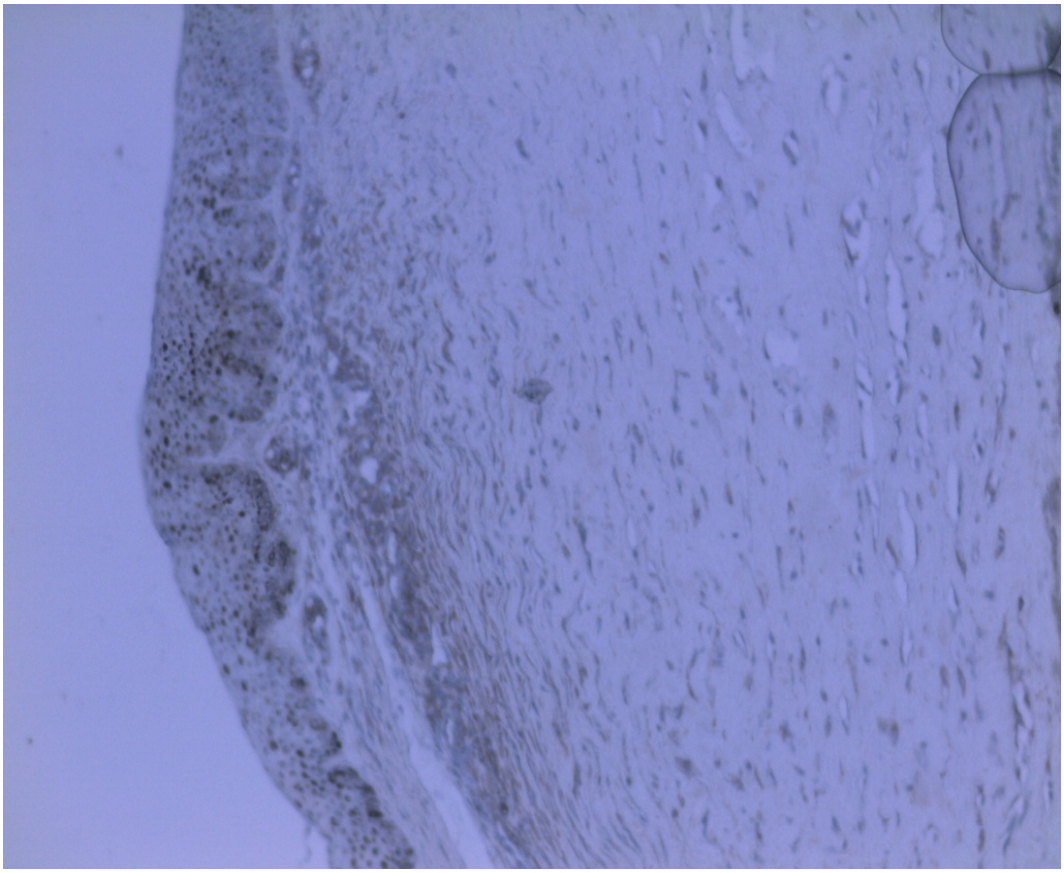


Figure 4.6: Pterygium section at the limbus, immune-stained with p53 antibody. Brown intra-nuclear reaction product was present indicating accumulation of p53 protein. A similar result was obtained in the limbus of controls (image not shown). The original was magnified X100.

Figure 4.7 shows MMP1 cytoplasmic immune-reaction product of limbal epithelial cells. A similar result was obtained in the positive sections for MMP2, 3, and respective controls. MMP1 was positive in 14 cases (23.7%) of 59 and five controls (71.4%) of seven; p = 0.02 Fisher's exact test. MMP2 was present in 16 pterygium sections (27.1%) of 59 and five controls (71.4%) of seven; p = 0.03 Fisher's exact test. MMP3 was overexpressed in 16 pterygium sections (27.1%) of 59 and five controls

(71.4%) of seven; p = 0.03 Fisher's exact test. MMP expression was more frequent in controls. MMPs were not detected in fibroblasts or stroma of pterygia or control samples. MMPs and p53 co-localized in ten p53 positive samples (90.9%) of 11.

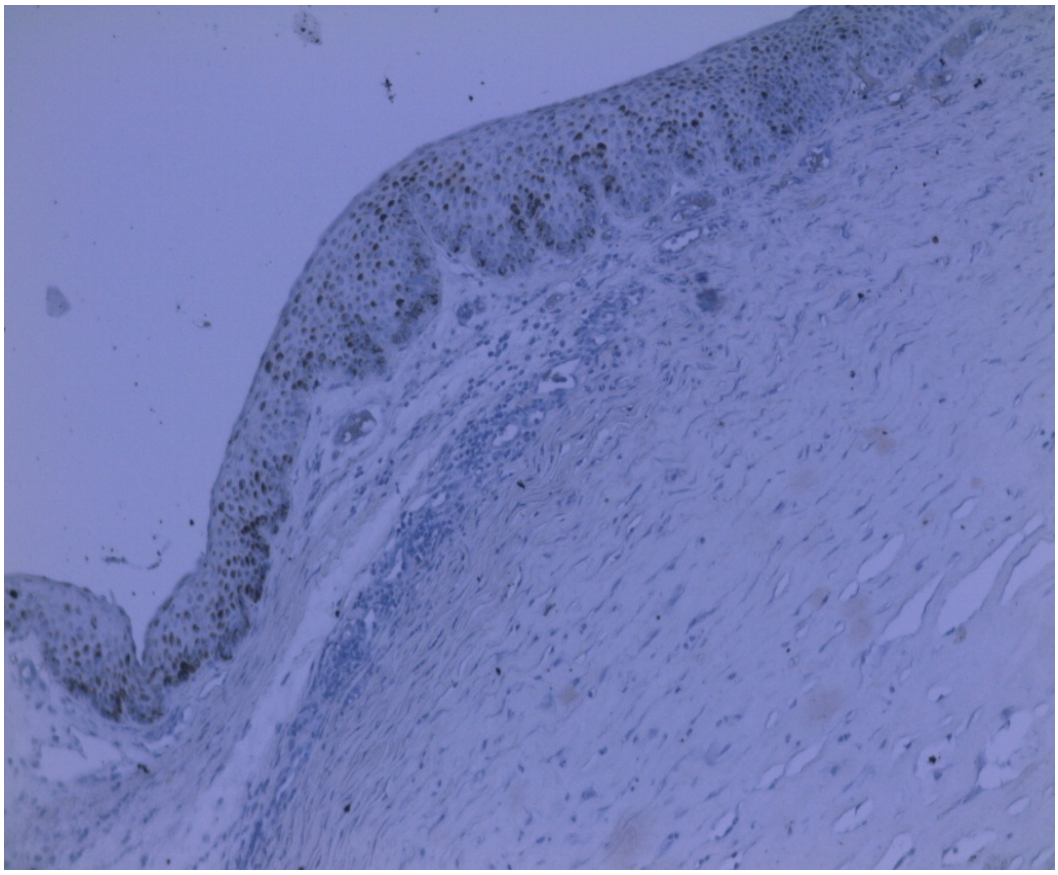


Figure 4.7: Section of pterygium at limbus. Immune-staining with antibody against MMPs. Basal cell cytoplasmic brown reaction product was found indicating a positive result. A similar result was obtained for MMP2, 3, and the respective controls (images not shown). The original was magnified X100.

LCA positive cells were scattered unevenly in all samples. These cells were located mainly in the stroma and away from the superficial epithelium (Figure 4.8). Expression

of LCA within the blood vessels was not considered. Eighteen pterygia (30.5%) of 59 were small and 41 pterygia (69.5%) of 59 were large. Table 4.10 shows the number of samples of various grades of pterygium extent having inflammatory cell counts of different ranges. The inflammatory cell count in five small pterygia (27.8%) of 18 was high (>200 cells) and in 22 large pterygia (53.8%) of 41 was low (≤200 cells); $p = 0.25$ Fisher's exact test. The proportion of samples with high cell counts tended to increase with pterygium extent. Thirty one sections were from patients exposed for 6 or more hours per day and 17 (54.8% of 31) had low cell counts. Twenty eight sections were from patients exposed for less than 6 hours per day and 10 (35% of 28) had high cell counts. Twenty growths (33.5%) of 59 recurred after surgery. Ten samples (50%) of 20 had high cell counts, and ten specimens (50%) of 20 had low counts; $p = 0.40$ Fisher's exact test. The tendency of pterygia with high cell counts to recur was similar to that of pterygia with low cell counts to recur after excision. Twelve pterygia that recurred after surgery (60%) of 20 were large growths. Five of the large growths that recurred (41.7%) of 12 had low inflammatory cell counts.

Table 4.10: The number of pterygia of various grades having inflammatory cell counts of different categories

Cells	Grade of pterygium			
	2 (N = 18)	3 (N = 27)	4 (N = 12)	5 (N = 2)
1-100	9 (50%)	12 (44.4%)	4 (33.3%)	0 (0%)
101-200	4 (22.2%)	5 (18.5%)	1 (8.3%)	0 (0%)
201-300	4 (22.2%)	6 (22.2%)	3 (25%)	2 (100%)
301-400	0 (0%)	3 (11.1%)	3(25%)	0 (0%)
401+	1 (5.6%)	1 (3.7%)	1 (8.3%)	0 (0%)

Twenty eight pterygium specimens (47.5%) of 59 were from patients who used traditional eye medication. Table 4.11 shows the number of pterygium samples with different categories of inflammatory cell infiltrations, obtained from patients who had used traditional eye medication and those who had not. Low infiltrations were more common. Samples with low infiltrations from patients who had used traditional eye medicine were more than those from patients who had not used. Specimens with high infiltrations from individuals who had used traditional eye medication were less than those from individuals who had not used this remedy.

Table 4.11: Distribution of pterygium samples having different degrees of inflammatory cell infiltrations in patients using traditional eye medicine and those not using

CELLS	TRADITIONAL EYE MEDICATION		P*
	NOT USED N =31	USED N = 28	
≤200	16 (51.6%)	19 (67.9%)	0.33
>200	15 (48.4%)	9 (32.1%)	

*Fishers exact test

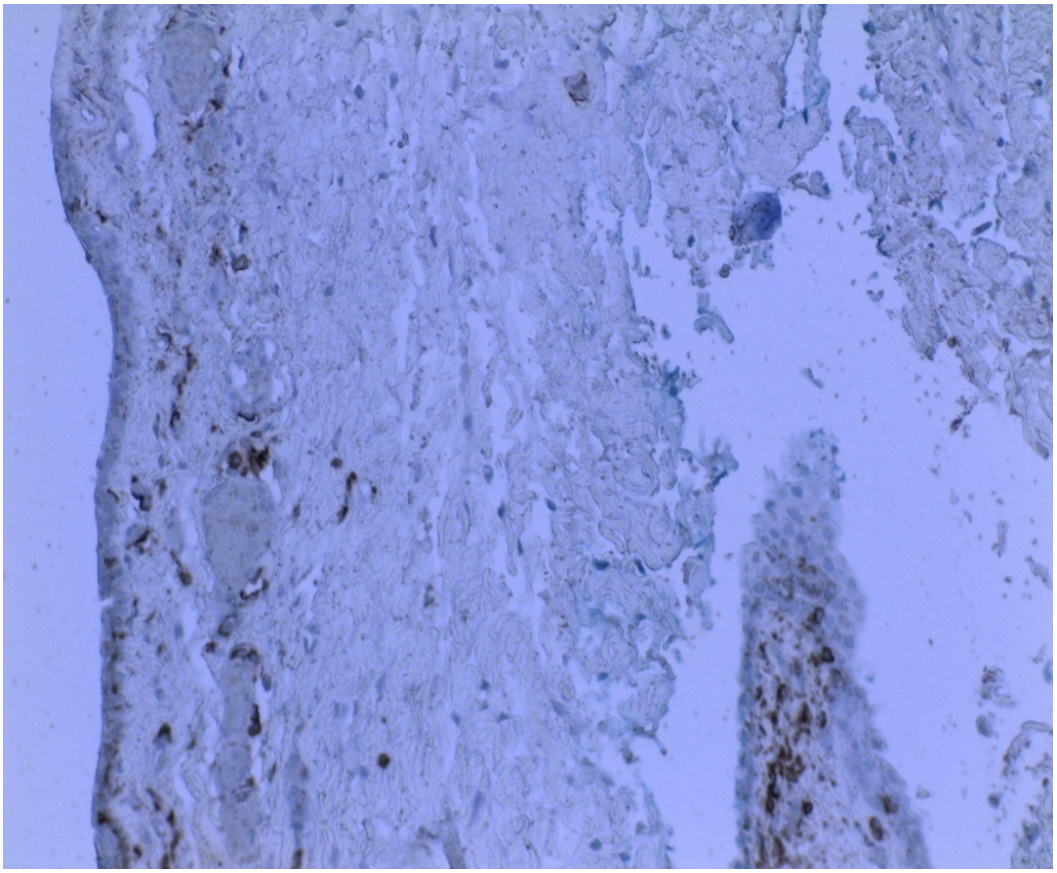


Figure 4.8: Photomicrograph of a pterygium section immune-stained with leucocyte common antigen (LCA) showing brown staining of the cell membranes indicating the presence of inflammatory cells. The original was magnified X200.

Chapter 5: Discussion

Summary of the new findings in a rural African population

The following are the original findings of the present study.

1. Hereditary predisposition was crucial for pterygium to occur.
2. The mode of inheritance was found to be multifactorial and followed the polygenic model.
3. There was some evidence that the inflammatory cell count was a true reflection of inflammation in the pterygia rather than pterygium size but this finding would require to be confirmed by a more refined technique. Also a low cell count was usually found in the specimens examined rather than severe inflammation. The findings indicate that inflammation was just a trigger for collagen conservation in pterygium patients. There was some evidence that exuberant collagen contributes to develop pterygium.
4. MMPs appeared to be inhibited in control conjunctival fibroblasts.
5. The data supported the view that sunlight exposure was most likely only a trigger for pterygium to occur in individuals who had other factors such as hereditary predisposition. There was some support for sunlight exposure to be only a trigger for inflammation to occur. There was also some support for prolonged exposure to be just a trigger for collagen degeneration.
6. Limbal stem cell deficiency did not appear to be a factor in the recurrence of primary pterygium after surgery.

5.1: Hereditary predisposition is crucial for pterygium to occur

Family history of pterygium, which implicates heredity[22,32] was associated with pterygium occurrence. It was independent of the use of traditional eye medication and of an unstable tear film. The positive family history rate in the cases is consistent with a previous Australian study that reported a family history rate of 38% in patients.[22] It is also consistent with an earlier South African study that reported a positive history rate of 30-35% in predominantly Caucasian pterygium patients.[32] The positive rate in controls agrees with an Australian study that found a family history rate of 8-12% in controls.[22] The association of family history with pterygium patients suggests familial occurrence of pterygium, which may be due to heredity or a shared environment or both.[169]

Having groups of diagnosed pterygium-affected relatives was associated with the pedigrees of pterygium probands. This association supports previous case-reports[18,84,85,86] and so it confirms familial occurrence of pterygium. This is similar to a previous study that reported that individuals affected by primary blepharospasm were associated with relatives diagnosed as cases of primary blepharospasm.[90]

I found the use of traditional eye medication to be the only environmental factor associated with pterygium. It was independent of family history and the presence of an unstable tear film. However, it is unlikely that traditional eye treatment directly causes pterygium because 40% of age and gender matched controls also used this remedy obtained from the same practitioners and prepared in the same way and applied in the

same manner. Traditional medicine was used to treat the symptom of red eyes so; pterygium patients were more likely than controls to use it since pterygia may cause eyes to be red. Moreover, it may be that traditional eye medication neutralized free radicals[67] without introducing these to the ocular surface since it was freshly prepared, used immediately, and it had no added substances. Added preservatives may introduce free radicals or alter pH, which make topical medicines to become toxic to the ocular surface.[63]

An unstable tear film was not associated with pterygium occurrence. The lack of association was independent of family history of pterygium and the use of traditional eye treatment. The present study supports an earlier study, which found a lack of association between pterygium occurrence and an unstable tear film.[61] But, it disagrees with others that found pterygium patients to be associated with unstable tear film.[21,60] Although plant derived traditional eye medicine from China might stabilize the tear film,[68] it is unlikely that the current controls were more likely to have dry eyes because they were less likely to use traditional eye medication; dry eyes were associated with controls independent of the use of traditional medicine. The transient blurring of eye sight related to dry eyes possibly led some of the controls to seek healthcare from the refraction clinic, which gives the impression that an unstable tear film was protective against pterygium. However, since an unstable tear film was infrequent it is unlikely to be a shared environment that caused familial occurrence of pterygium. This is consistent with a previous study, which showed that an unstable tear film was unlikely to cause pterygium.[62]

Although excessive sunlight exposure is widely believed to be the main factor associated with pterygium occurrence,[19,38,40,41] this study failed to find a relationship between pterygium occurrence and excessive exposure to sunlight. Some pterygium cases had short durations of exposure to sunlight, which is consistent with earlier reports.[32,47] However, the majority of the patients had heavy exposure most likely because of a low level of education, which led these patients to spend a long time outdoors[19,41,43] probably employed on farms.[9] Low levels of education in black South Africans has previously been reported.[51] Zhao et al failed to show independent association between pterygium and high exposure to UV light.[47] The present study compared cases with controls matched for age and gender, and the cases and the controls lived in the same province, which is sunny and dry.[177] That may explain why the findings of this study differ from the common belief, which is that pterygium occurs because of excessive exposure to sunlight.[19,38,40,41]

The affected and unaffected relatives lived close together in rural areas. The families of cases and controls had similar proportions of individuals aged 40years or more, suggesting similar life time durations of exposure to sunlight. The affected offspring of pterygium probands who were aged less than 20 years were much fewer than the unaffected, yet both groups of offspring had similar exposure to sunlight as they were scholars. Therefore, it is most likely that there is or are other factors besides sunlight, which collectively contribute to pterygium development. Since pterygium occurred irrespective of the level of exposure it is most likely that sunlight is only a trigger.

The majority of the participants were exposed to dust and to smoke. These variables had no relationship with pterygium occurrence. Poverty may explain the high rates of dust and smoke exposures, even sunlight exposure.[51] Poverty may also explain the high frequency of females as the males may have a tendency to migrate to seek for employment.[9] Smoking was infrequent and it is most likely to be unrelated to pterygium occurrence, which was also reported in Myanmar.[44] It is unlikely that exposure to dust or smoke, or smoking is the shared environment that contributed to the familial occurrence of pterygium.

Heredity is the predisposing factor for the familial occurrence of pterygium, as previously proposed by Booth[22] and Carmichael.[32] It explains the tendency of larger families to have more affected members, similar to previous reports on primary blepharospasm[90] and on multiple adenomatous polyposis.[194] Heredity explains the small number of affected offspring of pterygium probands compared to the unaffected offspring. The difference in number of affected and unaffected offspring of pterygium probands is because alleles may or may not be transmitted. The majority of the participants were under 50 years of age, which is similar to a previous African report that found that pterygium-affected individuals were frequently aged 31-40 years.[48] Young age at diagnosis suggests rapidly growing pterygium,[18] and it supports a hereditary basis for its occurrence.[18,86,88] Although pterygium cases were diagnosed in the families of control probands predisposition to pterygium is hereditary. Those controls are the unaffected relatives of pterygium patients.

The ratio between the unaffected and pterygium-affected individuals in the combined pedigrees of pterygium probands shows that pterygium patients were crowded in a family, which suggests multifactorial mode of inheritance.[170,171] According to the Mendelian principles, the ratio of 9:7 between the unaffected and pterygium-affected relatives suggests that two types of inactive genes (determinant genes) coding for two different proteins interact to predispose an individual to pterygium phenotype. Interaction of two types of genes is the simplest form of multifactorial inheritance.[89]

The presence of a mode of inheritance supports genetic inheritance[89,90] to be the predisposing factor for the familial occurrence of pterygium. Although this study could not demonstrate determinant genes it disagrees with previous studies that reported that multiple genes were abnormally expressed in pterygia and that the genetic abnormality was acquired, which explained pterygium but; those studies failed to rule out genetic inheritance.[140,141] The disagreement arises from the fact that the present study compared variables between pterygium patients and individuals unaffected by pterygium.

Multifactorial mode of inheritance in pterygium formation has been reported before, however, the threshold model was suggested.[32] The present study shows that multifactorial inheritance follows the polygenic model[170,171,172] because the number of affected individuals varied between families, even families of a similar size; and the pterygium recurrence time varied between patients, suggesting different speeds of progression;[18] and some cases who had experienced short recurrence times of pterygia after surgery had offspring younger than 20 years of age, suggesting the presence of

many determinant genes;[171] and an old individual had a small pterygium that recurred after a long time following excision, suggesting that it was growing slowly,[18] perhaps due to presence of few determinant genes.[171]

Considering single pedigrees seems to be misleading. For example, the present pterygium pedigree suggests that autosomal dominant with incomplete penetrance[90] is a possible mode of inheritance because two generations were confirmed to be affected.[85,86] The first generation was skipped[90] and the second and third generations were affected. The pedigree also suggests that autosomal recessive mode is possible because the first generation and the spouse of the proband might be carriers.[81]

Autosomal dominant mode of inheritance in pterygium occurrence has been reported before.[84,85,86] It was based on single pedigrees, however, due to the possibility of two modes of inheritance in one pedigree, which shows lack of a consistent Mendelian pattern single pedigrees are unsuitable for the determination of the mode of inheritance because the sample size is small.[90,194] It is clear from the present pedigree that mitochondrial mode of inheritance is extremely unlikely because all the female parents did not have pterygium.

The first generation and the wife of the proband seem to have two different types of heterozygous inactive genes,[89] which perhaps determined their lack of pterygium. Because the first generation who were without pterygium produced more affected offspring than those of the second generation comprising the proband and his unaffected spouse, this would seem to oppose the possibility of the polygenic model in

multifactorial inheritance, which states that the risk of transmission increases as the determinant genes increase.[170] Nevertheless, as the growth in the proband had a short recurrence time, and his daughter, aged 16 years was affected by pterygium, which suggest the presence of rapidly progressive pterygium[18] the polygenic model is likely. The polygenic model states also that the severity of a phenotype depends on the proportion of the determinant genes;[171] severe pterygia are clinically aggressive. They threaten vision quickly[9] and they manifest as rapid regrowth after excision.[18]

5.2 Pterygia have numerous fibroblasts and blood vessels, exhibit collagen degeneration, and they are infiltrated with chronic inflammatory cells

Numerous fibroblasts characterized pterygium samples, which is similar to previous reports.[6,7,8] It supports an earlier study which showed that pterygium fibroblasts other than controls multiplied vibrantly in the presence of high concentration of growth factors.[97] Although sunlight exposure causes fibroblasts to occur,[8] the pterygia this study examined were from individuals exposed to UV to different extents, which indicates that long daily durations of exposures is not the reason for numerous fibroblasts to occur in pterygia. The short duration of exposure in some pterygium cases is consistent with a previous study that reported that even a short burst of exposure to UV radiation induced fibrogenic growth factors in pterygia, which lasted for a long duration after radiation had stopped.[36] As heredity was fundamental for pterygium to occur, and numerous fibroblasts characterized pterygia heredity may be the reason for fibroblasts to become numerous.

Collagen degeneration was present in virtually all pterygium samples and most of the controls. This is similar to a previous report.[27] One pterygium sample did not manifest collagen degeneration histologically.This is similar to a previous study which reported that collagen degeneration may not be manifest in some pterygia.[83] Since the cases and controls were exposed for long periods, yet, collagen degeneration may be absent histologically these shows that there is another factor besides sunlight that is necessary for collagen degeneration to manifest; prolonged exposure is most likely only a trigger for collagen degeneration.

Chronic inflammatory cells in varying degrees of infiltration were present in pterygium samples. This is similar to previous reports.[6,7] The inflammatory cell infiltrate was mainly in the stroma, which is consistent with an earlier report,[77] but it is contradictory to a study that found infiltration to be mainly in the epithelium.[7] The difference may be due to the fact that this study excluded those who had clinical evidence of chronic ocular surface inflammation and those who were using prescribed topical eye medication. It is clear that hypersensitivity is not a contributor to the inflammation related to pterygium.

The inflammatory cell count indicates severity of inflammation rather than pterygium size because some small pterygia had high counts while some large pterygia had low inflammatory cell infiltrations. Because mild or severe inflammation occurred irrespective of pterygium size and inflammation tended to be mild rather than severe these indicate that inflammation is not the only reason for pterygium to occur. These also indicate that inflammation is probably just a trigger for pterygium to develop in

those having other reasons. As the inflammatory cell count tended to increase with pterygium size, this suggests that inflammation contributes to pterygium enlargement. However, as some large pterygia had low counts, even those that recurred post-surgically suggesting they were growing; this shows that inflammation is unlikely to be the main reason for pterygium size.

Because some pterygia with long daily durations of exposure to UV light had low cell counts, whereas some with short daily durations of exposure had high counts, these shows that the long daily duration of exposure is not the reason for pterygium inflammation to be severe. This seems to be similar to a previous study that reported that the level of pro-inflammatory cytokines varied in pterygia exposed to the same intensity of light.[80] Sunlight is probably only a trigger for inflammation to occur.

MMPs were inhibited in fibroblasts and stroma of pterygium cases and controls despite being expressed in limbal basal epithelial cells of some pterygia and controls. This is most likely to be due to inflammation, which is known to inhibit MMPs in fibroblasts while stimulating these cells to synthesize collagen.[122,124,125,127,128] Collagen produced by stimulated fibroblasts is deposited randomly and it causes tissues to become opaque;[126] this explains pterygium fleshiness and cap. Since the cases and controls were exposed to UV light, and these had interpalpebral conjunctivitis, yet fleshy conjunctiva grew onto the cornea only in cases these suggest collagen production was exuberant in pterygium patients; exuberant collagen extends beyond the inflamed area. It is most likely that the control conjunctivas were inflamed because MMPs were inhibited in fibroblasts.[(122,123,127,128)] Besides, UV exposure is known to cause

inflammation of normal conjunctiva.[35] It is unlikely that the cornea in cases rather than controls was inflamed because these were similarly exposed to UV. As the fibroblasts were numerous in pterygia collective productions is the most likely reason for abundant collagen.

Because pterygium inflammation varied in severity, yet fleshiness was the same in all pterygia, these indicate inflammation just stimulates collagen production and conservation; it appears that mild inflammation causes as much exuberant collagen as severe inflammation does. As MMP expression in fibroblasts is a sign of sunlight induced damage,[72] it is unlikely that pterygium fibroblasts are numerous due to damage or, abundunt collagen is due to damaged fibroblasts. MMPs were inhibited in pterygium fibroblasts.

Inhibition of MMPs in fibroblasts implies these proteins are unlikely to be the cause of collagen degeneration as previously reported.[72,73] The present study agrees with a study that reported that MMPs were underexpressed in pterygium fibroblasts and stroma.[71] The lack of use of spectacles by the present participants may be a reason for underexpression of MMPs in fibroblasts. Spectacles protect against UV light.[19,45]

Many blood vessels were found in pterygium samples but hardly any in controls. Previous studies also reported numerous blood vessels in pterygia.[6,7] The presence of numerous fibroblasts is the likely explanation for profuse angiogenesis.[97] Although sunlight may cause neovascularisation[8,100] it is unlikely that profuse angiogenesis is

due to sunlight exposure because controls were exposed for similar durations as the cases yet controls were minimally vascularized.

5.3 Post-surgical recurrence is due to pterygium progression

Young age seems to be associated with pterygium recurrence after surgery; however, the association failed after controlling for pterygium size. This supports a previous study that showed that young age was associated with post-surgical recurrence, but, the association did not persist after controlling for pterygium fleshiness, which was frequent in the young;[25] in the current study it seems that fleshiness was related to recurrence in the young but, it appears that it was protective in the old. The present study failed to find a relationship between pterygium fleshiness and recurrence probably because all pterygia were fleshy. Due to the inconsistency of the effect of fleshiness on recurrence in different age groups it is unlikely that mere pterygium fleshiness is necessary for pterygium to recur or not to recur.

Fleshiness as well as extent and age may be dependent on pterygium progression to be connected with post-surgical recurrence. As all pterygia were fleshy, and these pterygia had different extents in all age groups, this indicates that grade 3 degree of fleshiness is achieved before the potential in extent.

Pterygium recurrence after surgery seems to be associated with small size of the growth and large pterygium size seems to be protective against recurrence independent of age. This contradicts previous studies, which reported that post-surgical recurrence

was associated with large pterygium size.[26,94] The current study employed conjunctival grafting as adjunctive treatment. The grafts were larger than the size of the defect to cover after excision of the growth. The sample involved a large number of participants. These facts may explain the disagreements with the previous studies, which used the same size of radiation applicator in large and small pterygia,[26] or, derived conclusions from a small sample size.[94] Under-application of adjunctive treatment such as radiotherapy leaves some abnormal tissue untreated, which may perpetuate pterygium.

It is most likely that the majority of small pterygia which qualified for surgery according to the present study's criteria were still growing,[24] which means fibroblasts were still proliferating vibrantly.[97] Although large pterygium size seems to be protective against post-surgical recurrence some large pterygia recurred in all age groups. This is most likely to be due to pterygium progression.[24] Post-surgical recurrence of large or small pterygia irrespective of chronological age seems to contradict previous studies that reported that young age was associated with pterygium recurrence after surgery independent of other significant factors.[24,96] Post-surgical recurrence of large or small pterygia regardless of chronological age shows that age or pterygium size does not cause recurrence after surgery. This is supported by the current observation that extra-large pterygia, which seem to suggest growing pterygia,[26] tended to lack a relationship with post-surgical recurrence irrespective of whether they occurred in the young or old persons.

The ineligibility of most old patients having small pterygia for surgery led the present study to find that the association of young age with post-surgical recurrence was

dependent on small pterygium size. It also led to the finding that small pterygium extent was associated with recurrence independent of age. Small pterygia that did not recur in the young were most likely to be regressive.[24] The present study selection criteria, which is a reflection of the patients who undergo pterygium surgery in this population led to the discovery that the patient's age or pterygium size despite association with recurrence after surgery are unlikely to cause pterygium recurrence. The essence of post-surgical recurrence is pterygium progression[24] or sustained fibroblast genesis.[97]

The majority of the pterygia that recurred after surgery, had short recurrence times and pterygia in young patients tended to recur rapidly. This supports a previous study that observed that young individuals in a family experienced fast recurrences of excised pterygia.[18] Short recurrence time indicates fast growing pterygium; it is unlikely that young age causes pterygium to recur rapidly as some large pterygia in the old also had short recurrence times. Patients having fast growing pterygia are most likely to seek healthcare promptly because such pterygia are clinically aggressive[18] and they may threaten vision quickly.[9] Poverty[51] and the availability of traditional medication[64,65] may explain why some old patients having large fast growing pterygia failed to obtain healthcare while they were still young. Some old patients having large slow growing pterygia perhaps sought healthcare because of severe visual impairment.

The present study suggests that pterygium onset in most affected persons is at a young age. However, onset at a young age does not mean that all pterygia in the young are small. Some young patients had large pterygia, suggesting rapid growth;[18] similarly,

having small pterygia does not mean young age. An old individual had a small pterygium, which recurred after a long time following excision, suggesting slow growth;[18] many cases irrespective of age had large pterygia, which suggests that pterygium progression occurs in the majority of the ptergium-affected individuals seeking surgery in the present population. There was no relationship between pterygium size and recurrence time.

The current study failed to confirm excessive sunlight exposure to be related to post-surgical recurrence of pterygium. This contradicts an earlier study, which assessed exposure only in patients who experienced recurrence;[23] the present study compared the duration of exposure to UV light after operation in recurrence after surgery and no recurrence. The present finding is consistent with that of a study that reported that recurrent pterygia had no histological signs of collagen degeneration;[5] sunlight irrespective of its duration of exposure after pterygium excision is unrelated to post-surgical recurrence. The severity of inflammation had no relationship with pterygium recurrence after excision. This supports a previous report, which showed that clinically inflamed pterygia were not associated with post-surgical recurrence.[24]

5.4 Limbal stem cell deficiency is unlikely to be the cause of pterygium recurrence after excision.

Free conjunctival autotransplant and LCAT procedures appear to have similar recurrence rates, which has been reported before.[54,96] Due to a large sample, and a prospective study, the similar post-surgical outcome suggests that pterygium

recurrence is unlikely to be caused by limbal stem cell deficiency. This is supported by the lack of association of damaged limbal stem cells with pterygium samples.

The present study disagrees with a recent study that reported that LCAT was superior to CAT probably because that study was a meta-analysis of previous studies.[55] That meta-analysis included case-controlled studies and studies on outcome of surgery on recurrent pterygia. Since heterogeneity estimates have been reported to be uncertain,[57] this may be why Zheng et al[55] reported superiority of LCAT. Such difference between meta-analysis and high powered studies like the present one has been reported before,[56] so, the current state of affairs is likely to be that LCAT is similar to CAT in the treatment of primary pterygium.

Damaged limbal stem cells may migrate in all directions[31] and these may be transformed into fibroblasts in the conjunctival stroma.[157] The transformed fibroblasts may proliferate in predisposed individuals to form a fibrotic mass.[158,159] However, it seems that such a fibrotic mass is unlikely to be the reason for pterygium to occur. Damaged limbal cells were not associated with the pterygia examined. Localized limbal stem cell deficiency was not confirmed in pterygium recurrence since the recurrence rates after surgery were similar whether CAT or LCAT was used. It is unlikely that the susceptibility to the proliferation of transformed fibroblasts (lack of Dlg5)[158,159] is the predisposition to pterygium occurrence and recurrence.

Chapter 6: Proposed model of pterygium development

6.1 Summary

The proposed model of pterygium development is described under hereditary predisposition, sunlight exposure and inflammation, which are the themes arising from this thesis. Heredity is fundamental for pterygium to occur. Sunlight is only a trigger for pterygium occurrence. Inflammation triggers pterygium existences in those predisposed by heredity and are exposed to sunlight.

Figure 6.1 depicts the proposed model of pterygium development. The flow chart shows that heredity may interfere with the regulation of fibroblasts leading to the occurrence of numerous fibroblasts. Sunlight exposure triggers pterygium development only in individuals predisposed by heredity influencing fibroblast production. Angiogenesis is caused by sunlight, however; profuse neovascularization follows the presence of numerous fibroblasts. Sunlight is the trigger for inflammation, which stimulates the fibroblasts to synthesise and conserve collagen. Sunlight in addition triggers collagen degeneration. As the fibroblasts are many collective productions causes exuberant collagen. Exuberant collagen is the means by which inflammation triggers pterygium existence. Pterygium probably develops from abundant collagen, numerous fibroblasts, many blood vessels, degenerated collagen, and inflammatory cells.

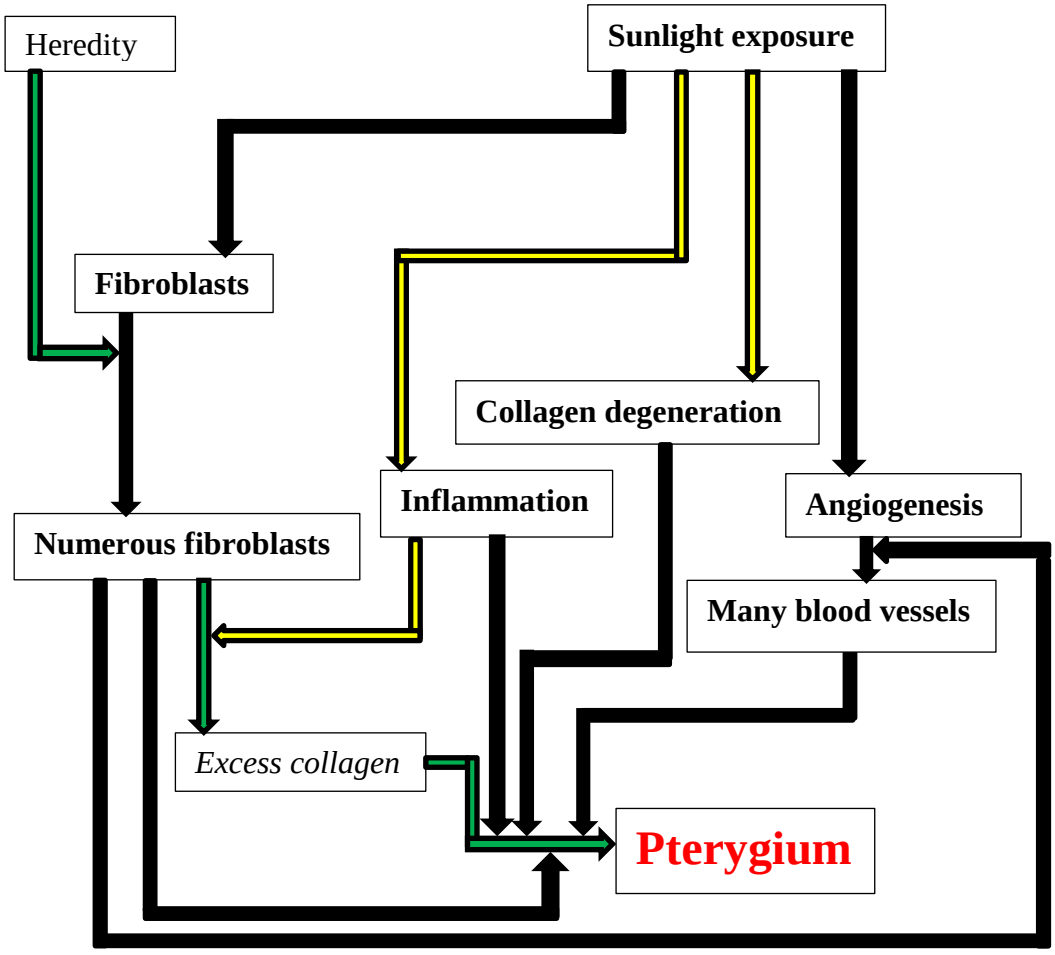


Figure 6.1: Proposed model of pterygium development. Normal font indicates an association verified by this study. Bold font indicates known associations. Font in italics shows an association suggested by this study. Bold green arrows show pathways that this study suggests cause pterygium occurrence. Bold yellow arrows indicate paths that this study suggests trigger pterygium to occur. Bold black arrows show well-known causative pathways.

Chapter 7

7.1: Conclusions

This study has established the determinants of pterygium occurrence and has suggested a determinant of pterygium recurrence after surgery in a rural African Population. It has offered some understanding into the pathogenesis of pterygium. The study rejects the null hypothesis so accepts the alternative that pterygium occurrence is associated with affected relatives and the use of traditional eye medication. Pterygium occurrence is determined by multiple modifiable factors, which interact to develop pterygium.

Multiple factors including hereditary predisposition, sunlight exposure, and inflammation interact to initiate pterygium development. A hereditary predisposition is crucial for pterygium to occur in a rural African population. This follows multifactorial mode of inheritance having the polygenic model. Post-surgical recurrence is apparently due to pterygium progression.

Sunlight exposure may be high in the majority of individuals; however, despite a low level of exposure in some individuals it is only a trigger for pterygium occurrence in those predisposed. Sunlight exposure irrespective of its duration also just triggers inflammatory cell infiltration in the exposed conjunctiva; inflammation whatever its severity just triggers collagen production and conservation in individuals exposed to sunlight. Sunlight also, only triggers development of blood vessels. Prolonged sunlight exposure is only a trigger for collagen degeneration.

An unstable tear film is unlikely to be associated with pterygium, and damaged limbal basal epithelial cells are unlikely to be related to pterygium. Collagen degeneration, which is typical of pterygium, is unrelated to matrixmetalloproteinases in the setting of persistent inflammation.

Recurrence after excision is unlikely to be caused by pterygium fleshiness, extent, or inflammation. Young individuals may frequently have pterygia that recur, even recur rapidly after surgery; however, age per se is not the reason for recurrence or rapid recurrence. Limbal stem cell deficiency is unlikely to be the reason for pterygia to recur after surgery.

7.2: Recommendations

Studies to identify the molecular nature of a hereditary predisposition to pterygium are necessary. This will lead to trials on whether genetic modification causes pterygium to regress and perhaps not to recur after surgery. Genetic counseling would be directed at individuals at risk of pterygium development.

The minimum duration of exposure to sunlight that induces pterygium in predisposed persons needs to be investigated. An investigation on the minimum duration of exposure that induces inflammatory cell infiltration is also recommended. Sun protection would then be advised appropriately to discourage pterygium development in predisposed individuals. Although it requires chronic medication control of

inflammation medically, appears to be an attractive option to prevent pterygium existence.

This study did not investigate clinical features such as Stoker's line, punctate staining of the head and body, induced corneal astigmatism, and vascularization, which may be related to pterygium. These need investigation to determine whether they might indicate pterygia that have a risk of recurrence after excision.

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Appendix 1: Participant assessment form

Case record form-1

_____

Participant's number:

Mark X. Where appropriate: **Or Fill in space.**

Personal information: Status = Marital status;

Age					
Gender					
Ethnicity					
Status					

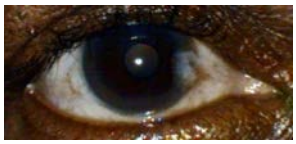
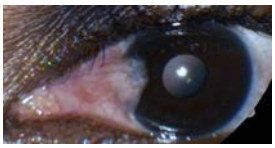
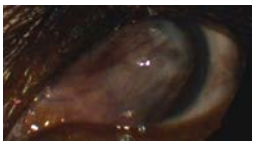
What is your occupation?

How long have you been in present occupation?

What were your previous occupations?

Hereditary factors:

Have you ever seen any person with a growth on the eye as shown in the photograph?

Has any member of your family ever had or have such a problem?

Lifestyle information:

Tobacco:

Do you smoke?	
What do you smoke?	
How much do you smoke per day?	
For how many years have you been using smoking?	
When did you stop smoking?	

Alcohol:

Do you use alcohol?	
What do you drink?	
How much do you drink per day?	
For how many years have you been using alcohol?	
When did you stop drinking?	

Health questions:

Have you consulted a health practitioner or traditional healer before?

How many times per year, in average, do you consult?

What was the purpose of consultation?	Illness	Other
Do you use traditional eye medicine?		
How frequently do you use medicine from health practitioners?		

Environmental factors:

Sunshine exposure:

On a usual day, from what time to what time do you get exposed to the sun?

How many usual days do you have in a week?

For how long have you had such usual days?

What was the exposure to the sun before?

On other days, from what time to what time do you get exposed to the sun?

How many other days do you have in a week?

Are there weeks (example holidays) when you do not get such exposure to the sun?

How many such weeks are there in a year?

In such weeks how long per day are you exposed to the sun?

Sun protection:

Do you wear spectacles or sun glasses while in the sun?

If sometimes, which times? …….

Dust:

Do you usually get exposed to dust?

How many days per week do you get exposed?

Are there weeks in a year when you are not exposed in the usual way?

How many such weeks are there in a year?

How many days are you exposed to dust in one unusual week?

Smoke:

Are you usually exposed to smoke?

How many days per week do you get exposed?

Are there weeks in a year when you are not exposed in the usual way?

How many such weeks are there in a year?

How many days are you exposed to smoke in one unusual week?

Case record form-2

Participant's number:

Mark X. Where appropriate: **Fill in space.**

Ocular surface disease:

Ocular surface examination:

	Right	**Left**
Papillae		
Conjunctival Scars		
Herbert's pits		
Conjunctival xerosis		
Bitot's spots		
Allergy		
Other chronic Conjunctivitis		
Superficial peripheral Pannus		

Tear film:

	Right	Left
Meniscus	Concave	Concave
	Straight	Straight
	Normal	Normal
Mucus debris	Present	Present
	Absent	Absent
Corneal filaments	Present	Present
	Absent	Absent
TBUT	< 6 Seconds	< 6 Seconds
	6 – 9 seconds	6 – 9 seconds
	10 Seconds or more	10 Seconds or more

Pterygium factors:

When did you notice the growth?

Has the pterygium grown?

Laterality	Right		Left		Double
Inflammation	Yes	No	Yes	No	
Grade of fleshiness	1		2		3
Grade of extent	1	2	3	4	5

Preoperative special examination:

Central Keratometry in diopters		**Visual Acuity**	
Right eye	**Left eye**	**Right eye**	**Left eye**

Eye examination:

	Right	**Left**
Corneal scar		
Cataract		
ISNT rule		
Discs		
Retina		
IOP		

Type of surgery:

Eye operated: Right Left.

CAT	**LCAT**

Case record form-3

Participant number:

Pedigree

For affected male insert ■

Unaffected male □

Affected female ●

Unaffected female ○

Slanted line across if photograph diagnosed eg ∅

Proband ↗

Generation I (age in yrs)

Generation II (age in yrs)

Generation III (age in yrs)

Case record form-4

Participant's number:

Mark X. Where appropriate; **fill Blank space; leave N/A.**

Post operative examination:

Date	Visit	Recurrence in mm	Astigmatism	VA	Complications
	Day 1	N/A	N/A		
	Week 1	N/A	N/A		
	Month1				
	Month3		N/A		
	Month6				

Key

Complication: n = number of complications

BK = band keratopathy

C = conjunctivalization of cornea

CD = corneoscleral dellen

CE	=	corneal edema
CS	=	corneal scar
D	=	discharge
DE	=	delayed epithelialization
EC	=	epithelial cysts
K	=	bacterial keratitis
H	=	haematoma under graft
LG	=	loss of graft
GE	=	graft edema
GH	=	graft hyperemia
GN	=	graft necrosis
GR	=	graft retraction
P	=	pannus
PD	=	persistent epithelial defect
SU	=	stromal ulcer
TG	=	Tenon’s granuloma
C	=	conjunctivalization of cornea
SU	=	stromal ulcer

Case record form-5

Participant's number:

Histology:

Neoplasia:

Positive	Negative

Collagen degeneration:

Positive	Negative

Fibroblasts:

Numerous	Few

Blood vessels:

Many	Few

Chronic inflammatory cells:

Present	Absent
Cell count	

Immunohistochemistry of damaged limbal stem cells:

p53:

Positive	Negative

MMP1:

Positive	Negative

MMP2:

Positive	Negative

MMP3:

Positive	Negative

MMP expression in fibroblasts

MMP1:

Positive	Negative

MMP2:

Positive	Negative

MMP3:

Positive	Negative

Appendix 2: Informed consent form

Protocol number:

Name of study: **DETERMINANTS OF PTERYGIUM OCCURRENCE AND RECURRENCE IN A RURAL AFRICAN POPULATION.**

Date of version of this consent: **24/02/2008**

Researcher**: Dr. P Anguria.** Phone number: **0765396392**

Protocol approved by Wits HREC on: ……

I hereby confirm that I have been informed by Dr P Anguria, about the nature, conduct, benefits and risks of clinical study number: ……., titled determinants of pterygium occurrence and recurrence in a rural African Population.

- I have also received, read and understood the written information (Participant information leaflet) regarding the clinical study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research I agree that the data collected during this study can be processed in a computerized system by Dr P Anguria.
- I may at any stage, without prejudice withdraw my consent and participation in the study.

- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

PARTICIPANT:

Printed name **Signature** **Date** **Time**

I, ----------, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY DOCTOR:

Printed name **Signature** **Date** **Time**

TRANSLATOR: ---------- **(Designation):**

Printed name **Signature** **Date** **Time**

WITNESS:

Printed name **Signature** **Date** **Time**

Appendix 3: **Verbal participant informed consent form**

Protocol number:

Name of study: **DETERMINANTS OF PTERYGIUM OCCURRENCE AND RECURRENCE IN A RURAL AFRICAN POPULATION.**

Date of version of this consent: **24/02/2008**

Researcher: **Dr. P Anguria.** Phone number: **0765396392**

Protocol approved by Wits HREC on: ……

- I the undersigned ……. Have read and have explained fully to the participant, named ……. And/or his/her relative/friend/legal representative……, the participant information leaflet.
- The account I have given has explained both the possible risks and benefits of the study as well as the alternative treatments available for his/her illness. The participant and/or his/her relative/friend/legal representative understand these.
- The participant and/or his/her relative/friend/legal representative indicated that he/she understands that the participant will be free to withdraw from the study at any time for any reason and without jeopardizing his/her subsequent treatment.

I hereby certify that the participant and his/her relative/friend/legal representative, acting on his/her behalf, have agreed to participate in this study.

PARTICIPANT:

Printed name **Thumbprint** **Date** **Time**

STUDY DOCTOR:

Printed name **Signature** **Date** **Time**

TRANSLATOR: ……. (DESIGNATION)

Printed name **Signature** **Date** **Time**

PARTICIPANT'S RELATIVE/FRIEND/LEGAL REPRESENTATIVE:

…… (RELATIONSHIP)

Printed name **Signature/Thumbprint** **Date** **Time**

WITNESS:

Printed name **Signature** **Date** **Time**

Appendix 4: Informed consent form–control participant

Protocol number:

Name of study: **DETERMINANTS OF PTERYGIUM OCCURRENCE AND RECURRENCE IN A RURAL AFRICAN POPULATION.**

Date of version of this consent: **24/02/2008**

Researcher**: Dr. P Anguria.** Phone number: **0765396392**

Protocol approved by Wits HREC on: ……

I hereby confirm that I have been informed by Dr P Anguria, about the nature, conduct, benefits and risks of clinical study number: ……., titled determinants of pterygium occurrence and recurrence in a rural African Population.

- I have also received, read and understood the written invitation (Appendix 7a: control participant invitation letter) regarding the clinical study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research I agree that the data collected during this study can be processed in a computerized system by Dr P Anguria.
- I may at any stage, without prejudice withdraw my consent and participation in the study.

- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

PARTICIPANT:

Printed name **Signature** **Date** **Time**

I, ----------, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY DOCTOR:

Printed name **Signature** **Date** **Time**

TRANSLATOR: ---------- **(Designation):**

Printed name **Signature** **Date** **Time**

WITNESS:

Printed name **Signature** **Date** **Time**

Appendix 5: Informed consent form–evisceration control participant

Protocol number:

Name of study: **DETERMINANTS OF PTERYGIUM OCCURRENCE AND RECURRENCE IN A RURAL AFRICAN POPULATION.**

Date of version of this consent: **24/02/2008**

Researcher**: Dr. P Anguria.** Phone number: **0765396392**

Protocol approved by Wits HREC on: ……

I hereby confirm that I have been informed by Dr P Anguria, about the nature, conduct, benefits and risks of clinical study number: ……., titled determinants of pterygium occurrence and recurrence in a rural African Population.

- I have also received, read and understood the written invitation (Appendix 7b: control participant invitation letter) regarding the clinical study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research I agree that Dr P Anguria can process the data collected during this study in a computerized system.
- I may at any stage, without prejudice withdraw my consent and participation in the study.

- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

PARTICIPANT:

Printed name **Signature** **Date** **Time**

I, ----------, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY DOCTOR:

Printed name **Signature** **Date** **Time**

TRANSLATOR: ---------- **(Designation):**

Printed name **Signature** **Date** **Time**

WITNESS:

Printed name **Signature** **Date** **Time**

Appendix 6: Participant information leaflet

Study number:

Name of study: **DETERMINANTS OF PTERYGIUM OCCURRENCE AND RECURRENCE IN A RURAL AFRICAN POPULATION.**

Researcher: Dr P Anguria. Phone number: 0765396392

Institution: Mankweng hospital

Protocol approved by Wits HREC on: ……..

Date and time of informed consent discussion: …….. ……

Dear Sir/Madam

Good day, I Dr Peter Anguria, an eye specialist working at Mankweng Hospital would like to invite you to take part in the study called: "determinants of pterygium occurrence and recurrence in a rural African population". Please read this information leaflet about the study. If there is anything you do not understand, please ask me.

PURPOSE OF THE STUDY:

- You have been found suffering from a disease called pterygium that is a wing shaped fleshy growth on the eye. It is partly caused by sun damage to the eye. Pterygium is stubborn in the sense that it recurs frequently; and your case requires surgery.
- The purpose of this study is to determine what causes pterygium to occur and recur; and which type of operation is best in South African setting.

LENGTH OF THE STUDY AND NUMBER OF PARTICIPANTS:

- This study will be performed in Mankweng hospital
- Approximately 355 people will participate in this study.
- The participants will be between the ages of 21 and 65 years
- The total amount of time required for your participation will be a maximum of 7 months. You will be asked to visit me 7 times during the study.

PROCEDURES:

- **Visit 1**

If you agree to participate, I will ask you health related questions; perform general examination and additional eye examination. Eye photographs to determine accurately the extent and severity of pterygium and to document it will be done. Eye photographs will be repeated at the 5^{th} and 7^{th} visits to measure the degree of improvement of your eye after operation. General and additional eye examinations will not hurt beyond the sting of the eye drop used for eye examination.

I will collect 1 teaspoon (5mls) of blood from you to be sent to the laboratory for gene tests related to pterygium. I will provide you with a leaflet to tell you about gene tests and explain it to you. We shall together with you, prepare a date of operation to remove the pterygium. To improve the accuracy of the results, I will ask a nurse to allocate you to one of the operating procedures, according to the random number that you will pick (like a spinning coin). Neither you nor I will know in advance the procedure you will be allocated to. 2 operations are currently done. Both successful but we are not sure which is best in South African setting.

- **Visit 2**

You will be requested to sign the hospital consent form before going to theatre for the operation to remove the pterygium from your eye. A local anaesthetic by eye drops and injection around the pterygium will prevent pain during operation and immediately after. The gap left by the pterygium will be patched with either a membrane called conjunctiva or conjunctiva with limbus. The patch will be obtained from the upper part of the same eye.

Your operated eye will be covered with an eye pad to protect it, for one day.

You will receive 2 sets of eye drops to take home to apply to the operated eye, each 4 times a day. If you feel pain, you will swallow pills that you usually take for pain.

The pterygium removed from your eye will be sent to the laboratory for tests related to pterygium during this study. Your information that identifies you as a participant in this study will not be disclosed. If the pterygium is no longer needed for this study, it will be destroyed.

- **Visit 3**

The next day after operation, you will be examined to ensure that you are recovering well.

- **Visit 4**

1 week after operation you will be examined to ensure that you have recovered from the operation.

1 eye drop called Ciloxan will be stopped but you will continue with one, called Predforte for a total of 6 weeks.

- **Visits 5, 6 and 7**

These visits will take place 1 month, 3 months and 6 months respectively, after operation. The purposes of these visits are to detect any recurrence of pterygium because recurrence is known to be commonest during this period; and to monitor the healing process. Interview and eye examination will be performed during each visit; the eyes will be photographed at month 1 and 6. Once during your visits, I will ask Dr Shawn Thompson to check your eye so as to ensure accuracy of the result of the study.

DISCOMFORT:

- Eye drops that are used routinely for examination and for treatment usually sting slightly on application but this lasts for about half a minute.
- Because operation involves removing the surface of the front part of your eye, you may feel sore on the eye for a few days up to a maximum of 4 days. Use of an eye pad however will help reduce the intensity and duration of this discomfort. Pain pills as well will relieve soreness.

RISKS OF OPERATION:

- The risks of operation are not because of this research but are the possible risks that can occur as a result of usual treatment of pterygium.
- There is a slight possibility of infection on the eye. Your protection is that a doctor who knows and has been performing operations to remove pterygium for a long time will do the operation under conditions free from small living things that can cause infection. Also, you will receive antibiotics both at the conclusion of the operation and

after, to take home. This is aimed at preventing the possibility of infection. If infection occurs, it will be treated.

- There is also a possibility of the clear front part of your eye, slightly loosing some of its clarity, both as a healing process and as a result of pterygium. This may result in you feeling glare especially after removing a large pterygium. But the clarity of this part of the eye and glare may improve with time and with treatment to control healing. The treatment is by one of the drops you will receive.
- There is a slight possibility that your eye may be slow to heal. This may result in you experiencing decreased eyesight, tearing, discomfort on exposure to light, pain in the eye and redness of the eye. If slow healing is persistent, it is possible to encourage healing.
- The pterygium may recur. Up to 39% chance of recurrence is reported in the medical literature.

UNFORESEEN RISKS OF OPERATION:

- Removal of pterygium from the eye is usually done followed by patching with conjunctiva or limbus with conjunctiva, without serious problems. However, you should immediately contact me if you feel anything unusual about your eye during your taking part in this study. Your return to normal will be watched closely and all actions to prevent things going wrong will be taken to make sure there are no problems after operation.

BENEFITS:

- The possible benefit to you from your taking part in this study may be everlasting removal of your pterygium.
- However, you may not benefit from this study.
- Your taking part in this study will add to medical knowledge that may help other people who, like you, suffer from pterygium.

ALTERNATIVE FORMS OF TREATMENT;

- Other possible treatment for pterygium is use of eye drops to relieve the eye irritation associated with pterygium.
- If you choose not to take part in this study, you will still receive the best current care from your usual doctor. This may or may not include the ways of treatment used in this study.

BENEFITS AND RISKS OF ALTERNATIVE TREATMENT:

- If you choose medical treatment, you will avoid all the possible discomforts and chances of things going wrong in operation, but there is a chance that the pterygium will continue to grow and decrease your eyesight. Also the chance of the front part of the eye losing its clarity is higher when operation is delayed till the pterygium is big.

YOUR RESPONSIBILITY:

- Tell the truth when you give your health history so as to make the results of the study accurate.

- Follow all the advice given after operation including applying eye drops and to return for check up.
- Report any pain, redness in the eye or decrease in eyesight quickly. This will aid in early treatment of possible problems.

RIGHTS AS A PARTICIPANT IN THIS STUDY:

- Your taking part in this study is entirely by free choice and you can refuse to take part or stop at any time without giving any reason. But you must tell me if you wish to stop taking part after operation has already been done. I will watch your return to normal after operation or if you wish, be watched by your usual eye doctor. Your health is the first priority.
- Even if you refuse or stop taking part in this study, you will get other eye care.
- If you do not give accurate history or do not follow the actions asked in the study I may remove you from the study at any time.
- If any new information that can affect your willingness to continue taking part in this study comes during the study, I will tell you so that you can choose to continue or stop taking part in the study.

EMERGENCY CARE AND HOSPITALISATION:

- If anything about your eye happens without warning and you look for quick care or admission to hospital during this study, please tell the treating doctor that you are taking part in this study and that I must be told.

FINANCIAL ARRANGEMENTS:

- You will not pay for the laboratory tests done in this study.
- You will however be expected to pay for the usual hospital costs which are not related with this study but to the treatment you will get from hospital.

REIMBURSEMENT FOR STUDY PARTICIPATION:

- You will not be paid for taking part in this study but R50 to help with your transport and refreshments will be given to you each time when you come for the visits at 1 week, 1 month and 3 months. At 6 months, you will get R100.

ETHICAL APPROVAL:

- This clinical study protocol has been handed in to the University of the Witwatersrand, Human Research Ethics Committee (HREC) and the Pietersburg-Mankweng Hospital Complex Research, Ethics and Publications Committee. Both committees have granted written approval.
- The study has been structured in accordance with the requirements of the South African principles of good clinical practice (second edition, 2006) and in accordance with the declaration of Helsinki (last updated: October 2000), which deals with the recommendations guiding doctors in biomedical research involving human participants. A copy of each may be obtained from me should you wish to view them.
- I do not have any financial or personal interests in organizations sponsoring this study.

SOURCE OF ADDITIONAL INFORMATION:

For the duration of the study, you will be under my care. My names are Dr Peter Anguria

If at any time between your visits you feel that any of your symptoms are causing any problems or you have any questions about your eye during the study, please do not hesitate to contact me at 0765396392. In my absence, contact Dr F Stegmann at 0828009316.

- If you want any information regarding your rights as a research participant, or have complaints regarding this research study, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC), which is an independent committee, established to help protect the rights of research participants at 011-717 2229.

For research information you may contact me at 0765396392.

CONFIDENTIALITY:

- All information obtained during the course of this study, including hospital records, personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study.

The University of the Witwatersrand, Human Research Ethics Committee (HREC) and the Pietersburg-Mankweng Hospital Complex Research Ethics and Publications Committee might inspect this information. Therefore you hereby authorize me to release your medical records to them.

- They will utilize these records only in connection with carrying out their obligations relating to this clinical study.

- Any information uncovered regarding your state of health as a result of your taking part in this study will be held in strict confidence. You will be informed of any finding of importance to your health or continued participation in this study but this information will not be disclosed to any third party in addition to the ones mentioned above without your written permission. The only exception to this rule will be cases of communicable diseases where a legal duty of notification of the Department of Health exists. In this case, you will be informed of my intent to disclose such information to the authorized state agency.

Appendix 7: Invitation letter for control participant

Dear Sir/Madam

Good day, I Dr Peter Anguria, an eye specialist working at Mankweng hospital would like to invite you to take part in a study called: "determinants of pterygium occurrence and recurrence in a rural African population". The aim of this study is to find out why a wing shaped fleshy growth on the eye, called pterygium, appears and re-appears.

You are not suffering from pterygium but your taking part will add to medical understanding why some people suffer from pterygium.

During your taking part, you will be asked health related questions, examined and your eyes tested. A total of 5mls (1 teaspoon) of blood will be removed from your arm and sent for gene tests related to pterygium. What we find out about you will be compared with what we find out in people suffering from pterygium. Removing blood from the arm is sore like an injection. There is a slight chance that an infection may occur in the arm at the place of removal of blood. Your protection is that a doctor who knows and has been removing blood for a long time will remove the blood under conditions free from small living things that cause infection. Also, infection can be treated if it occurs. Your taking part in the study will be for only 1 hr. You will not be asked to come back for more questioning or tests. You will not benefit from this study but your taking part will add to medical understanding that may help others suffering from pterygium. No one without your permission will know what you will tell me and what I will find out in examining you and testing your eyes during this study. You will not pay for the tests. I will provide you with a leaflet to tell you about the gene tests and explain it to you. You may take home the leaflet and the unsigned permission forms to think about and to talk about with your relatives, friends and may be, regular doctor, before choosing to take part.

If you choose to take part, you will be asked to sign a consent form so as to make sure that you have understood the aim of the study, the way the study will be done, the chances that things can go wrong and that you agree to go ahead with taking part in the study.

Even if you agree to take part in the study, you will still keep the freedom to stop taking part at any time, for any reason and still get the best care available. I will give you a copy of the signed consent form to keep.

Yours truly,

Dr. P Anguria. **Date: _____**

Appendix 8: **Invitation letter for control participant undergoing evisceration.**

Dear Sir/Madam

Good day, I Dr Peter Anguria, an eye specialist working at Mankweng hospital would like to invite you to take part in a study called: “determinants of pterygium occurrence and recurrence in a rural African population”. The aim of this study is to find out why a wing shaped fleshy growth on the eye, called pterygium, appears and re-appears.

Unfortunately you have injured your eye so badly that it is not possible to repair but remove it. The eye would normally be disposed of (incinerated) but I am asking your permission to perform studies related to pterygium on your injured eye after it has been removed. Your taking part in the study will add to medical understanding of the causes of pterygium occurrence and recurrence.

During your taking part, you will be asked health related questions, examined and your eyes tested. 1 teaspoon (5mls) of blood will be removed from your arm and sent for gene tests related to pterygium. What we shall find out about you will be compared with what we find out in people suffering from pterygium. Removing blood from the arm is sore like an injection. There is a slight chance that an infection may occur in the arm at the place of removal of blood. Your protection is that a doctor who knows and has been removing blood for a long time will remove the blood under conditions free from small living things that cause infection. Also, infection can be treated if it occurs. Your taking part in the study will be for only 1 hr. You will not be asked to come back for more questioning or tests. You are being asked to give the front part of your damaged eye after it has been removed, for tests related to pterygium. You will not benefit from this study but your taking part will add to medical understanding that may help others suffering from pterygium. No one without your permission

will know what you will tell me and what I will find out in examining you and testing your eyes during this study. You will not pay for the tests. I will provide you with a leaflet to tell you about the gene tests and explain it to you. You may take home the leaflet and the unsigned consent forms to think about and to talk about with your relatives, friends and may be, regular doctor, before choosing to take part.

If you choose to take part, you will be asked to sign a consent form so as to make sure that you have understood the aim of the study, the way the study will be done, the chances that things can go wrong and that you agree to go ahead with taking part in the study.

Even if you agree to take part in the study, you will still keep the freedom to stop taking part at any time, for any reason and still get the best care available. I will give you a copy of the signed consent form to keep.

Yours truly,

Dr. P Anguria. **Date: _____**

Appendix 9: Ethical approval from the University of the Witwatersrand

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Anguria

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080414**
PROJECT	Determinants of pteryguim occurence and recurrent in a rural African population
INVESTIGATORS	Dr P Anguria
DEPARTMENT	Ophthalmology
DATE CONSIDERED	08.04.25
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.06.09 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof TR Carmichael

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 10: Ethical approval from Polokwane/Mankweng Hospital Complex

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA
DEPARTMENT OF HEALTH AND SOCIAL DEVELOPMENT

ETHICS COMMITTEE
CLEARANCE CERTIFICATE
UNIVERSITY OF LIMPOPO
Polokwane/Mankweng Hospital Complex

PROJECT NUMBER: 005/2008

RESEARCHER: DR P. ANGURIA

TITLE : DETERMINANTS OF PTERYGIUM OCCURRENCE AND RECURRENCE IN A RURAL AFRICAN POPULATION

DATE: 23 May 2008

Prof. A.J. Mbokazi

Chairman of Pietersburg Mankweng
Hospital Complex Ethics Committee

Note: The budget for research has to be considered separately. Ethics Committee is not providing any funds for projects.

1

Appendix 11: Approval of title

Faculty of Health Sciences
Medical School, 7 York Road, Parktown, 2193
Fax: (011) 717-2119
Tel: (011) 717-2745

Reference: Ms Tania Van Leeve
E-mail: tania.vanleeve@wits.ac.za
09 July 2008
Person No: 337837
PAG

Dr P Anguria
P.O. Box 55226
Polokwane
0700
South Africa

Dear Dr Anguria

Doctor of Philosophy: Approval of Title

We have pleasure in advising that your proposal entitled "*Determinants of pterygium occurence and recurrence in a rural African population*" has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix 12: Permission to conduct research in Limpopo Province

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH & SOCIAL DEVELOPMENT

Enquiries: Malomane FL
Ref: 4/2/2

7 August, 2008
Dr Peter Anguria
Eye Unit
Mankweng Hospital
SOVENGA

Dear Dr Peter Anguria

Determinants of Ptergium occurrence and recurrence in a rural African population

- Permission is hereby granted to Dr Peter Anguria to conduct a study as mentioned above in Limpopo Province at Mankweng Hospital
- The Department of Health and Social Development will expect a copy of the completed research for its own resource centre after completion of the study.
- The researcher is expected to avoid disrupting services in the course of his study
- The Researcher/s should be prepared to assist in interpretation and implementation of the recommendations where possible
- The Institutions management where the study is being conducted should be made aware of this,
- A copy of the permission letter can be forwarded to Management of the Institutions concerned

HEAD OF DEPARTMENT
HEALTH AND SOCIAL DEVELOPMENT
LIMPOPO PROVINCE

Private Bag x 9302 Polokwane.
18 College Str. Polokwane 0700. Tel.: (015) 293 6000 Fax: (015) 293 6211 Website: http/www.limpopo.gov.za
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Appendix 13: Permission to conduct research at Mankweng Hospital

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH AND SOCIAL DEVELOPMENT

PIETERSBURG/MANKWENG HOSPITAL COMPLEX

MEMO

TO : DR PETER ANGURIA
EYE WARD (MANKWENG HOSPITAL)

FROM : DR KHOABANE
ACTING SENIOR MANAGER

DATE : 21 JULY 2008

TOPIC : PERMISSION TO CONDUCT RESEARCH AT MANKWENG HOSPITAL

---

1. Your letter dated 08th July 2008 to conduct research at our Institution has been received, and contents accepted.

2. You are hence granted permission to conduct Research, as long as such implementation does not compromise service delivery.

Thanks

Dr Khoabane
Acting Senior Manager
(Mankweng Hospital)

DEPARTMENT OF HEALTH & WELFARE
MANKWENG HOSPITAL COMPLEX
05 AUG 2008
PRIVATE BAG X1727
SOVENGA 0727
LIMPOPO PROVINCE

Publications related to this thesis

Relationships of heredity and dry eye with pterygia in black African patients

Peter Anguria, Sam Ntuli, Trevor Carmichael

To the Editor: Pterygium is a wing-shaped fibrovascular growth of the conjunctiva across the limbus onto the cornea.[1] Although a hereditary influence on pterygium formation is recognised[1] and a report of possible genetic influence in white South Africans has been published,[2] little is known about these factors in black African populations.

Pterygia are widely believed to be due to excessive exposure to sunlight,[3,4] and a long duration of ultraviolet radiation has been reported to be responsible for its development.[5] The use of tobacco,[6] a poor tear film,[7] lower levels of education and skills,[6] ethnicity,[8] exposure to dust,[8] malnutrition[4] and chronic ocular surface inflammation[8] have been reported or proposed as associations with pterygium occurrence. The relative importance of these factors has not been described in rural black Africans. This report is confined to investigating possible genetic factors and dry eye, which were the main associations found.

Ethical clearance was obtained from the institutional research ethics committees. We prospectively interviewed 71 patients and 71 age- and sex-matched controls in the Mankweng Eye Unit in Polokwane. Patients with ocular surface malignancy and pseudo-pterygium were excluded.

Consent was obtained and participants were shown three photographs of eyes with pterygia (Fig. 1) and asked questions similar to those used in a previous study.[2] The patients were shown the photographs and asked if they had ever seen a person with such a growth on the eye. If they replied in the affirmative, they were asked if that person was a relative and if so what their relationship with the person was. Tear-film stability was assessed using a previously reported procedure;[7] after application of 5% fluorescein, the cornea was assessed for areas of dark spots or streaks in between the blinks.

The mean age of the participants was 45 years (standard deviation 10.65), with a range of 22 - 65 years. There were nearly three times as many females as males in the series.

There was a positive family history of pterygium in 36% of cases and 10% of controls. A positive history was almost 5 times as common in cases as in controls. This was statistically significant ($p<0.05$, chi-square test).

The tear film was unstable in 7% of cases and 17% of controls. An unstable tear-film was 4 times more common in controls than in cases, and this was significant ($p<0.05$, chi-square test).

A positive family history implies hereditary predisposition.[2] It was the only positive factor associated with the formation of pterygia in this study. In Australia 38% of patients admitted for pterygium surgery compared with 8 - 12% of controls admitted for other conditions had a family history of the growth.[9] In South Africa 30 - 35% of urban predominantly white individuals who had attended an ophthalmic practice because of pterygium had a positive family history.[2]

An unstable tear film (dry eye) was not associated with pterygium in this study, and this lack of association has previously been reported in Bantu patients.[10] Pterygia may cause irritation with concomitant tearing that female patients have found problematic.[11] The epiphora caused by ocular irritation may explain the finding of more dry eye in controls than cases. The association of dry eye with pterygium has been reported from Turkey[7] and Nigeria,[12] so the issue is unresolved.

The inherited predisposition that appears to underlie the formation of pterygium in black African patients requires further study.

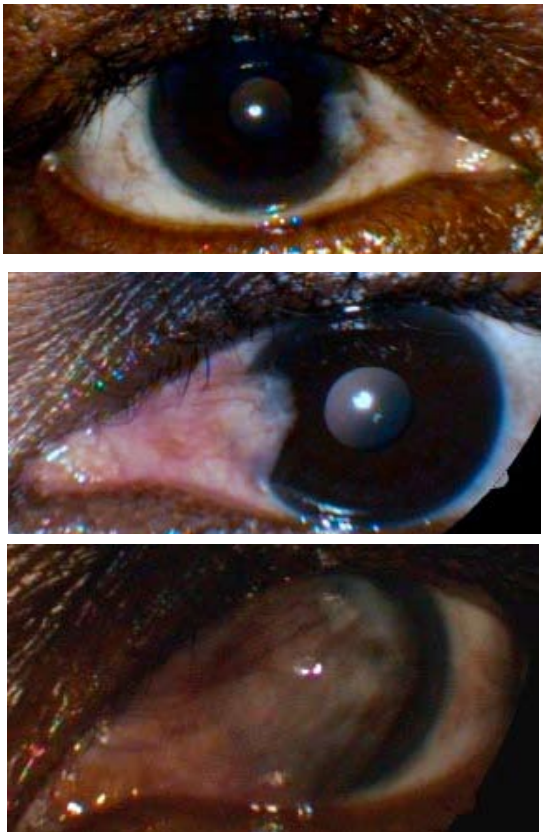


Fig. 1. Photographs of eyes with pterygium used for the study.

Division of Ophthalmology, Department of Neurosciences, University of the Witwatersrand, Johannesburg
Peter Anguria, MB ChB, MMed Ophth
Trevor Carmichael MB BCh, FCS (SA), PhD (Med), MSc (Med)

University of Limpopo, Polokwane campus
Sam Ntuli, BSc Stat, BSc (Hon) Stat, MSc Biostat

Corresponding author: *P Anguria (irarak58@gmail.com)*

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Accepted 18 October 2010.

Traditional eye medication and pterygium occurrence in Limpopo Province

P Anguria, S Ntuli, B Interewicz, T Carmichael

Background. The relative importance of environmental and hereditary factors in the occurrence of pterygium in African blacks has not been reported.

Aim. To investigate the relative significance of factors associated with pterygium occurrence.

Methods. This was a prospective case-controlled study where 150 pterygium patients and 150 controls participated. Interviews were conducted, eyes examined and multivariate analysis done. The families of 51 pterygium cases and 50 controls were examined for presence of pterygium.

Results. Of 150 cases and 150 controls, 79 (52.6%) and 60 (40%) used traditional eye drops (odds ratio (OR) 2.03; p=0.009. Ten cases (6.6%) and 26 controls (17.3%) had unstable tear film (OR 0.30; p=0.007. Forty-six cases (30.6%) and 15 controls (10%) reported a positive family history (OR 3.93; p<0.001). Groups of 3 - 5 pterygium cases in a household occurred in 36 of 51 pterygium families (70.5%) v. 1 of 50 controls (2%).

Conclusions. Pterygium occurrence was associated with the use of traditional eye drops, a positive family history and having groups of diagnosed pterygium-affected relatives. However, unstable tear film seemed protective against pterygium occurrence.

S Afr Med J 2012;102(8):687-690. DOI:10.7196/SAMJ.5930

Blacks in Africa use traditional medicine widely[1] but there is no report of whether its topical use in the eyes is associated with pterygium occurrence. Excessive exposure to ultraviolet (UV) light based on location,[2] occupation,[3] and length of time spent outdoors[4] is regarded as the main association of pterygium occurrence. However, a previous report has shown that pterygium cases were more frequent in the Karoo than the Transkei, areas that receive similar levels of UV light.[5] Moreover, climatic droplet keratopathy and pingueculae, which are also associated with excessive sunlight exposure, did not occur together with pterygium significantly.[5] Contrary to expectation, pterygium prevalence was found to be low in patients attending a referral hospital in Rwanda, which is close to the equator and at a high altitude.[6] Those reports suggest that there was another factor besides UV light in pterygium occurrence.

Exposure to dust has been reported to be associated with pterygium[3,5] and heredity may also be a factor;[8,9] however, single pedigrees were investigated, or the participants self-reported their family histories.

A relationship between dry eyes and pterygium has been claimed,[10] yet has also been contradicted.[9] Dry eye, whether associated with pterygium or not, has not been reported to be independent of other factors related to pterygium occurrence. Tobacco use has been reported to be associated,[11] but also as being protective,[12] and to have no relationship[13] with pterygium presence. There is no report on a relationship between tobacco use and pterygium among blacks.

As the relative importance of hereditary and environmental factors in the occurrence of pterygium in blacks has not been reported, we aimed to determine the relative significance of the main factors associated with pterygium occurrence in rural blacks. Our objectives were to investigate whether environmental factors such as use of traditional eye drops, UV exposure, dust exposure, tobacco use, dry eye and family history were associated with pterygium, and whether pterygium clusters in rural black families.

Division of Ophthalmology, Department of Neurosciences, University of the Witwatersrand, Johannesburg
Peter Anguria, MB ChB, MMed Ophth
Trevor Carmichael, MB BCH, FCS (SA), PhD (Med), MSc (Med)

University of Limpopo, Polokwane Campus, Ga-Rankuwa
Sam Ntuli, BSc (Hon) Stat, MSc Biostat
Bozenna Interewicz, MD, PhD

Corresponding author: *P Anguria (irarak58@gmail.com)*

Methods

This was a prospective case-controlled study, matched for age and sex, in Mankweng Hospital, Sovenga, which receives patients from all over Limpopo Province. The tropic of Capricorn crosses the province, which is predominantly rural, and is sunny and dry for most of the year.[14]

Ethical clearance was obtained from the two institutions' research ethics committees, and the tenets of the Declaration of Helsinki (2000) were followed in obtaining consent.

Eligible participants were consecutive black patients aged 21 - 65 years who were born and living in a rural area. They were selected from the Outpatients Department in the Eye Unit. Those attending the eye clinic had primary pterygium; the controls, who were attending the refraction clinic, did not have pterygium and were not contact lens users. Cases and controls with previous ocular surgery or trauma, blepharitis or lid deformities, ocular surface malignancy, corneal scars, cataracts, maculopathy, glaucoma, optic atrophy or swelling were excluded. After obtaining consent, the selected individuals were recruited. A sample size of 300 (150 pterygium cases and 150 controls) was calculated to give a 20% difference in family history between cases and controls with a power of 80% and an alpha value of 0.05, assuming a base rate of 10% in controls. Between August 2008 and 2011, 150 pterygium cases and 150 controls were interviewed using structured questions that had been used in an earlier study.[9] A full eye examination was done. Demographic factors such as ethnic group and level of education were documented. The risk factors assessed were: use of traditional eye drops, occupational exposure to sunlight and dust, tobacco use, dry eye and family occurrence of pterygium.

Use of traditional eye drops was recorded. UV light was assessed by determining the daily mean hours of exposure to sunlight, which was calculated from time spent working outdoors and leisure time outdoors. The proportion of outdoor occupations as a percentage of all occupations was calculated. Exposure to dust while at work and whether tobacco had ever been used was verified.

Familial occurrence was initially assessed by asking for family history. Such history was considered present if the pterygium patient or control said that a relative had or ever had a lesion on the eye that looked similar to a pterygium as shown to them in photographs.

The presence or not of dry eyes was evaluated by measuring the tear film break-up time (TBUT) as previously reported.[9] The cornea was assessed for dark spots or streaks after application of fluorescein. The mean of three time intervals between blinks for the appearance of these areas was the TBUT, and a value <10 seconds was considered to indicate an unstable tear film (dry eye).

Follow-up family studies on selected cases and controls, who accepted a family visit and whose relatives were living within 20 km of each other within the rural areas of Limpopo Province were conducted. Families that did not possess diagnostic photographs of absent key members were excluded.

The key relatives of 51 pterygium probands and 50 controls were visited and the eyes of those >9 years old were examined by one ophthalmologist (PA) using a diagnostic lamp, for presence of pterygium. Nine years was chosen as a cut-off age because this is the youngest age reported at which pterygium has been diagnosed in black patients.[5] Photographs of absent family members were examined by PA. Key relatives included siblings, parents, offspring, grandparents, first uncles and aunts, and the propositus.

Data on variables that were reported to be present in a patient and the control partner during the period before the person was diagnosed as having pterygium, and data from eye examination, as well as data from family studies, were considered. Descriptive statistics for pterygium cases and controls were summarised. Odds ratios (ORs) for the risk factors of pterygium were calculated by a conditional logistic regression model. In all models, a 1:1 pair-matched analysis was performed using age and gender as pairing variables. Statistical significance was tested by the chi-square test, and variables that were significant in the univariate analyses were included in a multivariate analysis. In all the analyses, significance was set at $p \leq 0.05$. STATA 9 for Windows software (STATA Corporation, College Station, USA) was used for statistical calculations.

To obtain a larger sample size for analysis in family studies, the key relatives of the case patients and those of the controls were combined into two separate large families.[15] The difference in proportion of pterygium-affected individuals between the combined families of the cases and those of the controls was compared. The proportion of pedigrees having a group of pterygium-affected individuals was compared between cases and controls.

Results

One hundred and fifty pairs of cases and controls were interviewed and their eyes examined. Table 1 presents demographic factors and environmental factors not found to be significant. The most frequent age range was 40 - 49 years. There were over 3½ times as many females as males. Education and ethnicity were similar among the cases and the controls.

Table 2 presents the count (percentage) and odds ratio (OR) of variables significantly associated with pterygium occurrence from the univariate analyses. The use of traditional eye drops and family history of pterygium were reported more frequently in pterygium patients. Dry eye was more frequent in controls.

Table 3 summarises a joint model of pterygium susceptibility with all variables listed in Table 2. All remained independently significant of one another. The pedigrees of 51 pterygium cases and 50 controls were visited. The families were located in the different regions of Limpopo Province; 382 individuals were examined in the combined families of pterygium cases and 394 in controls. Fourteen (3.6%) of 382 family members of pterygium probands, and 17 (4.3%) controls, were diagnosed by means of photographs. The age range of the family members was from 10 to 86 years, and the proportion of individuals aged ≤40 years was 71.9% (275 of 382) in pterygium cases and 72% (284 of 394) in controls. The pterygium probands had 56 combined offspring aged ≤20 years, of whom 3 (5.4%) were affected. Two - 14 individuals in a family were examined among the case pedigrees, and 4 - 12 in control pedigrees. Thirty-four (66.7%) of 51 case families and 33 (66%) of 50 control families had 6 - 9 individuals in a household, which was the median and also the most frequent range of family size. We found that 157 (41%) of 382 combined key relatives in cases were pterygium-affected, and 16 (4%) of 394 in the combined control pedigrees were pterygium-affected.

Table 4 presents the number of pedigrees of cases and controls in relation to the number of family members and number of individuals affected in a household. Larger families tended to have more

Table 1. Demographic factors and environmental factors not significant

Factor	Cases (%)	Controls (%)
Age		
20 - 29	12 (8)	12 (8)
30 - 39	23 (15.3)	23 (15.3)
40 - 49	54 (36)	54 (36)
50 - 59	51 (34)	51 (34)
60+	10 (6.6)	10 (6.6)
Sex		
Female	118 (78.6)	118 (78.6)
Male	32 (21.3)	32 (21.3)
Education		
≤12 years	106 (70.6)	114 (76)
>12 years	44 (29.3)	36 (24)
Ethnic group		
Pedi	111 (74)	112 (74.6)
Other	39 (26)	38 (25.3)
Outdoor occupation	80 (53.3)	80 (53.3)
Sunlight ≥6 hr/day	118 (78.7)	111 (74)
Dust exposure	124 (82.7)	126 (84)
Tobacco use	31 (20.7)	26 (17.3)

Table 2. Risk factors found significant in univariate analysis

Factor	Cases (%)	Controls (%)	OR	*p*
Traditional eye drops	79 (52.6)	60 (40)	1.70	0.03
Family history	40 (30.6)	15 (10)	3.81	<0.001
Dry eyes	10 (6.6)	26 (17.3)	0.33	0.007

Table 3. Multivariate analysis of significant factors

Factor	Cases (%)	Controls (%)	OR	*p*
Traditional eye drops	79 (52.6)	60 (40)	2.03	0.009
Family history	40 (30.6)	15 (10)	3.93	<0.001
Dry eyes	10 (6.6)	26 (17.3)	0.30	0.007

Table 4. Distribution of pterygium-affected individuals in families

		Case pedigrees: number of individuals in family								
	≤4	5	6	7	8	9	10	11	12	14
No. of individuals affected in a family	0	0	0	0	0	0	0	0	0	0
	1	2	3	2	1	0	0	0	0	0
	2	1	3	0	1	1	1	0	0	0
	3	0	0	5	2	1	3	1	0	1	0
	4	0	0	7	0	1	7	1	2	0	1
	5	0	0	0	1	1	0	0	1	0	1
		Control pedigrees: number of individuals in family								
	≤4	5	6	7	8	9	10	11	12	14
No. of individuals affected in a family	0	3	3	5	2	7	10	4	1	2	0
	1	0	1	4	2	2	1	0	0	1	0
	2	0	0	0	0	0	0	1	0	0	0
	3	0	0	0	0	0	0	0	0	1	0
	4	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0

pterygium-affected persons. Groups of pterygium-affected family members were more frequent in cases. Groups of 3 - 5 pterygium cases in a household occurred in 36 (70.5%) of 51 pterygium families v. 1 (2%) of 50 controls.

Discussion

The use of traditional eye drops was the only significant environmental factor related to the occurrence of pterygium, and this was independent of family history and the presence or absence of dry eyes. Traditional eye drops are prepared from the leaves of a plant that the Pedi call *Mmale*, and were used several times a day for several weeks when eyes were acutely red (personal communication in 2011 from 3 users). As nearly half of the controls reported use of these traditional eye drops, yet did not have pterygium, it is unlikely that traditional eye drops directly caused pterygium. It has been reported that blacks who used traditional medicine believed in it[1] and that they also followed certain traditions[16] such as cross-cousin marriage,[17] which tends to promote hereditary conditions in an extended family.[18] The use of traditional eye medicine implicates an hereditary predisposition to pterygium occurrence in this study.

Family history of pterygium, which suggests heredity,[9] was found to be the most significant, and independent risk, factor for pterygium occurrence, which has not been reported before. Diagnosed pterygium-affected key relatives were associated with pterygium occurrence, so confirming familial occurrence, which is a similar finding to that in a report on primary blepharospasm.[15] The association of diagnosed pterygium-affected relatives with pterygium cases has not been reported before. Since alleles may or may not be transmitted, this explains why some families had 1 or 2 affected individuals. The presence of pterygium-affected individuals in some families of control probands seems to oppose a hereditary effect; however, those families were of pterygium patients in whom the probands were unaffected. Having both affected and unaffected individuals in a family does not mean that the disease is not hereditary.

Familial occurrence may be due to heredity or a shared environment or both. Since excessive sunlight exposure and dust exposure were not confirmed to be associated with pterygium cases; and the affected and unaffected family members lived close together; and the affected offspring of pterygium probands, who were aged ≤20 years were much fewer than the unaffected, yet both groups of offspring had similar exposure to sunlight as they were scholars; and the families of cases and controls lived in the same province that has been reported to be sunny and dry;[14] and the age range of the individuals in the families of cases and controls was similar, suggesting a similar lifetime duration of exposure; we conclude that it is unlikely that excessive sunlight exposure and dust exposure were the shared environment that determined pterygium occurrence. Heredity may be the predisposing factor for the familial occurrence of pterygium.[19] Moreover, heredity explains the tendency of larger families to have more affected members.[15,20] Because some patients were exposed for short durations, sunlight despite low exposure may be only a trigger for pterygium to occur in predisposed individuals. As the level of education was equally low among patients and controls, and the available employment was mainly on farms (as reported before[21]), these may be reasons why controls were as excessively exposed to sunlight and dust as the cases.

An unstable tear film was infrequent in this study. This condition was negatively associated with pterygium presence, and was independent of use of eye drops and of family history. The blurring of eyesight owing to dry eyes was a reason for some controls to seek refraction. The majority of participants were female, perhaps because of male migration from rural areas to urban centres to find work, as previously reported.[9]

Conclusions

The use of traditional eye drops appears to be associated with pterygium occurrence in rural blacks while an unstable tear film, suggesting dry eyes, appears to be protective. Heredity, which manifested as a family history of pterygium, confirmed by having diagnosed affected relatives, was significant in pterygium occurrence. Investigations on a molecular basis for hereditary predisposition to pterygium occurrence are recommended as this will facilitate strategies to prevent pterygium.

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Chronic inflammatory cells and damaged limbal cells in pterygium

*Anguria P[1], Carmichael T[1], Ntuli S[2], Kitinya J[3]

1. Department of Neurosciences, Division of Ophthalmology, University of the Witwatersrand Johannesburg, South Africa.
2. Department of Community Health, University of Limpopo Polokwane Campus, South Africa
3. Department of Pathology, University of Limpopo Polokwane Campus, South Africa

Abstract

Background: Chronic inflammation in pterygium occurrence has not been explained. Whether damaged limbal basal epithelial cells are associated with pterygium occurrence in black Africans is not clear.

Objective: To explain chronic inflammation in pterygium, and to clarify whether damaged limbal basal epithelial cells were associated with pterygium occurrence in black Africans.

Methods: Chronic inflammatory changes and damaged limbal basal epithelial cells were assessed in 59 samples.

Results: Chronic inflammatory cells were present in 59 pterygia. Inflammatory cell count in 5 (27.8%) of 18 small pterygia was >200 (high) while in 22 (53.7%) of 41 large growths was <200 (low); p = 0.25. The proportion of pterygia with high counts tended to increase with pterygium extent. Twenty (33.9%) of 59 pterygia recurred after surgery. Ten (50%) of 20 samples had high cell counts and 10 (50%), low counts; p = 0.40.

P53 expression was detected in 11 (18.6%) of 59 pterygium samples and 5 (71.4%) of 7 controls; p = 0.007. MMP 1 staining was present in 14 (23.7%) of 59 sections and 5 (71.4%) of 7 controls; p = 0.02. MMP2 in 16 (27.1%) cases and 5 (71.4%) controls; p = 0.03. MMP3 was overexpressed in 16 (27.1%) of 59 cases and 5 (71.4%) controls; p = 0.03.

Conclusions: Mild chronic inflammation has a tendency to be more frequent than severe inflammation in pterygia. It is clear that damaged limbal basal epithelial cells are unlikely to be related to pterygium occurrence.

Key words: Pterygium, Inflammatory cells

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Introduction

Ultraviolet light, which is believed to cause pterygium[1] may induce chronic inflammatory cells in the conjunctiva[2] or damage limbal stem cells.[3] Because chronic inflammatory cells were shown to be present in pterygium samples, it was reported that chronic inflammation contributed to pterygium occurrence.[4] However, there is no report of whether the level of infiltration is related to the severity of inflammation and to pterygium occurrence, or, to pterygium size. In addition, there is no report of whether the degree of inflammatory cell infiltration is related to the grade of fleshiness, pterygium recurrence after surgery, or chronicity of sunlight exposure.

Matrix remodeling may be by matrix metalloproteinases (MMPs)[5] or due to sunlight.[6] MMPs have been detected in the fibroblasts and stroma of pterygium samples,[7] suggesting that these MMPs were remodeling the pterygium stroma,[5] but, this was attributed to damaged limbal basal epithelial cells.[7] Another study did not find MMP expression in the pterygium fibroblasts or stroma yet the limbal basal epithelial cells were damaged.[8] Whether MMPs are expressed or not by pterygium fibroblasts or stroma has not been corroborated.

Limbal stem cell damage by UV light may manifest as MMP[7,8] or p53[9] expression. Although p53 detection rate in pterygium samples may be high,[10] it has also been reported to be low.[11] P53 expression in pterygia from black Africans has been reported to be low however, the sample was small.[12] There is no report of whether or not MMPs are expressed by limbal basal epithelial cells of black African pterygia.

The presence of chronic inflammatory cells in pterygia has not been elucidated and whether the presence of damaged limbal basal epithelial cells is associated or not with pterygium occurrence in black Africans is not clear. Hence, this study was aimed to explain the presence of chronic inflammatory cells in pterygium and to clarify whether the presence of

***Corresponding author:**
Dr. Peter Anguria
Department of Neurosciences
Division of Ophthalmology
University of the Witwatersrand Johannesburg
P.O. Box 55226 Polokwane 0700
South Africa
Tel +27152676551
E mail irarak58@gmail.com

damaged limbal basal epithelial cells is associated or not with pterygium occurrence in black Africans. The objectives were to describe the expression of chronic inflammatory cells and damaged limbal basal epithelial cells in pterygium specimens.

This report shows that the degree of chronic inflammatory cell infiltration in pterygium samples tends to be low, and that damaged limbal basal epithelial cells are not likely to be associated with pterygium occurrence.

Methods

This was a descriptive study of pterygium samples obtained from the patients who were participating in the study on pterygium recurrence after surgery. The pterygia were those that caused corneal astigmatism or obstruction of or threatened to obstruct vision, or caused disfigurement, or were repeatedly irritating. None of the patients had received topical corticosteroids or non-steroidal anti-inflammatory treatment before surgery.

Ethical clearance was obtained from the 2 institutional research ethics committees before starting the study and the tenets of the declaration of Helsinki (2000) were followed in obtaining consent.

Fifty nine sections excised 4mm from the limbus, that were not fragmented or crushed were selected because trauma can release MMPs into the extracellular space,[13] which could be mistaken to cause pterygium remodeling.[5,7] Seven control sections were obtained from the nasal corneo-conjunctiva of patients that were undergoing evisceration for irreparably injured eyes. The lacerations had not extended to the nasal corneo-conjunctiva. We assumed that if damaged limbal basal epithelial cells were associated with pterygium, barely any control would have damaged cells. Chronic inflammatory cells were not investigated in the controls because control eyes were eviscerated more than 72hrs after injury and this was assumed to allow tissue infiltration with chronic inflammatory cells[14] thus to confuse the findings. The control patients' selection criteria were similar to those of the pterygium cases.[15] The sections were marked and laid flat to facilitate orientation as previously reported.[7]

One experienced histotechnologist processed the samples in a standard way. The sections were cut at 3 microns thick from within 300 microns of the mid longitudinal meridian of the pterygia so that the chance of detecting target cells in different specimens was similar. Haematoxylin and eosin (H & E) staining was used because it highlights routinely fibroblasts, blood vessels and collagen degeneration (characteristics of pterygium[4]), and neoplastic cells. Collagen degeneration served as an objective measure of excessive sunlight exposure.[16] And neoplastic cells, which may also express p53[12] as well as MMPs[5] may be confused with benign damaged cells.[7]

Chronic inflammatory cells were investigated by immunohistochemistry using an antibody against leukocyte common antigen (LCA) and damaged limbal basal epithelial cells, using antibodies against p53 and MMP1, 2 and 3. Extra-epithelial MMP expression was studied using the respective antibodies. The antibodies, source and dilutions are shown in table 1. Standard immunohistochemistry procedures were followed.[10] The sections were incubated overnight then de-paraffinized by xylene. Xylene was removed by decreasing concentrations of alcohol and the specimens washed in distilled water before immersion in target retrieval solution (Diagnostech, Dako, Johannesburg, South Africa). The specimens were treated in a microwave oven then rinsed in Tris Buffered Saline (TBS) (Diagnostech, Dako, Johannesburg, South Africa) before immersion in peroxidase blocking solution (Diagnostech, Dako, Johannesburg, South Africa). The samples were rinsed in TBS and incubated with primary antibodies after-which they were washed in TBS. They were incubated with horseradish peroxidase rabbit/mouse secondary antibody (Diagnostech, Dako, Johannesburg, South Africa) then rinsed in TBS before incubation with 3,3' diamino-benzidine-tetrahydrochloride (DAB) chromogen (Diagnostech, Dako, Johannesburg, South Africa). This was followed by incubation with DAB substrate buffer (Diagnostech, Dako, Johannesburg, South Africa). The specimens were bathed in TBS then counterstained with Mayer's haematoxylin. The slides were preserved using standard histological methods. One experienced and masked Pathologist (JK) read the slides.

Table 1: Antibodies used and their dilutions

Antibody	Clone	Source	Dilution
LCA	PD7/26 and 2B11	Dako	1:50
P53	DO-7		
MMP1	3B6	Santa Cruz	
MMP2	8B4		
MMP3	1B4		

Data analysis

Cells expressing LCA were counted and the count compared in all pterygia, and in samples of pterygia that recurred. Trends were examined. Trends in the expressions of p53 and MMPs were also examined. The trends or deviations from expected trends indicated significance.

Statistical significance was tested by the Fisher's exact test and $p<0.05$ was considered to be significant. Statistical calculations were done using STATA 9 for Windows software (STATA Corporation, College Station, USA).

Results

Fifty nine pterygia were investigated by immunohistochemistry. Forty five were from females while 14, from males. The patients' age range was from 23-64 years. Thirty three (55.9%) of 59 individuals were <50yrs old (young individuals[17]) while 26 (44.1%) were >50yrs old (old persons[17]). Spectacles or sunglasses had not been used. All pterygia were opaque. Neoplasia was confirmed absent, and fibroblasts and blood vessels were identified in 59 (100%) specimens. Collagen degeneration was absent in one (1.7%) of 59 specimens.

LCA positive cells were found to be scattered unevenly in all samples. These cells were located mainly in the stroma and away from the superficial epithelial cells (figure1). Intravascular cell expression of LCA was not considered. Eighteen (30.5%) of 59 pterygia were grade 2 (small) and 41 (69.5%), > grade 3 (large). The inflammatory cell count in 5 (27.8%) of 18 small pterygia was >200 (high) and in 22 (53.7%) of 41 large pterygia, was <200 (low); p = 0.25 Fisher's exact test. The proportion of samples with high cell counts had a tendency to increase with pterygium extent. Twenty (33.9%) of 59 pterygia recurred after surgery. Ten (50%) of 20 samples had high cell counts and 10 (50%), low counts; p = 0.40 Fisher's exact test.

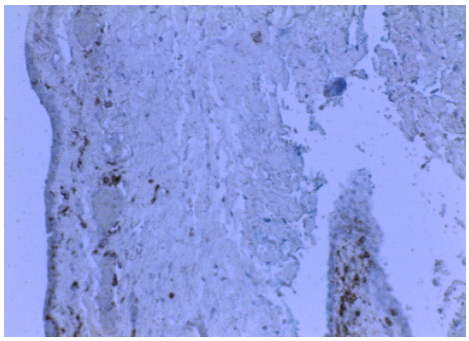


Figure 1: Photomicrograph of a pterygium section immunostained with LCA showing scattered brown reaction product indicating the presence of chronic inflammatory cells X200

Figure 2 shows MMP1, 2 and 3 cytoplasmic immune-reaction product of limbal basal epithelial cells. A similar result was obtained in the positive controls. MMP 1 was present in 14 (23.7%) of 59 pterygium sections, and 5 (71.4%) of 7 controls; p = 0.02 Fisher's exact test. MMP2 in 16 (27.1%) cases, and 5 (71.4%) controls; p = 0.03 Fisher's exact test. MMP3 was overexpressed in 16 (27.1%) of 59 cases, and 5 (71.4%) of 7 controls; p = 0.03 Fisher's exact test. MMPs were not detected in fibroblasts or stroma of pterygia or controls.

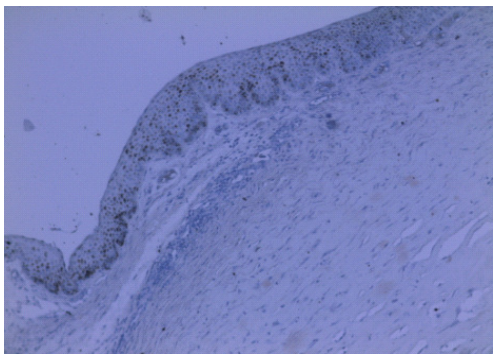


Figure 2: Section of pterygium at limbus. Immunostaining with an antibody against MMP1. Basal cytoplasmic brown reaction product was found indicating a positive result. X100. A similar result was obtained for MMP2, 3, and the respective controls (images not shown).

Figure 3 shows limbal basal epithelial nuclear-immunostaining with p53 antibody. A similar result was obtained in the controls. P53 was detected in 11 (18.6%) of 59 pterygium samples, and 5 (71.4%) of 7 controls; p = 0.007 (Fisher's exact test). MMP and p53 expressions were co-localised in 10 (90.9%) of 11 p53 positive pterygium samples.

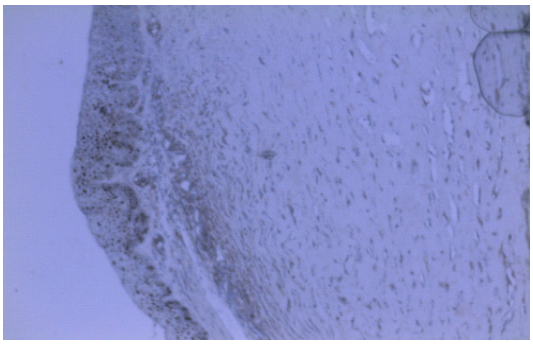


Figure 3: Section of pterygium at limbus. Immunostaining with p53 antibody. Brown reaction product was found in the nuclei of basal cells indicating accumulation of p53 protein. X100. A similar result was obtained in the limbus of controls (image not shown)

Discussion

Leukocyte common antigen has been used to identify T lymphocytes in conditions of persistent inflammation.[18] However, LCA is also present in lymphocytes whether involved in antigen/antibody reaction at an epithelial surface,[19] or, lymphoma.[20] Because LCA positive cells were located in the stroma and away from the surface epithelial cells, and were scattered rather than in masses, it is most likely that LCA positive cells in the present study indicate chronic pterygium inflammation[4] other than ocular surface hypersensitivity reactions[19] or conjunctival lymphoma.[20]

Although the inflammatory cell count was correlated with pterygium extent, the cell count clearly indicates severity of inflammation rather than pterygium extent because some small growths had high counts while some large ones had low counts. As the majority (59%) of the samples had low cell counts this suggests that inflammation may not be crucial for pterygium to be present or to be opaque. In addition, chronic pterygium inflammation appears not to be important for pterygium to recur after surgery, which is consistent with a previous report.[17] It looks as if the severity of chronic inflammation is independent of the degree of exposure to sunlight because some pterygia with collagen degeneration had high cell counts, while others also with collagen degeneration had low counts. These observations seem to be consistent with a previous report that showed that the severity of expressed proinflammatory cytokines varied between pterygia exposed to ambient light.[21] The apparent independence of the severity of inflammation, independent of the level of exposure suggests that an intrinsic determinant of the degree of inflammation possibly exists.

Some individuals may be deficient of T-lymphokine activated killer cell-originated protein kinase (TOPK) and its deficiency appears to intensify sunlight induced inflammation.[22] This would seem to suggest that a hereditary predisposition may be the reason that inflammation was severe in some pterygia.

Failure to detect MMP expression in fibroblasts or stroma contradicts a previous report,[7] perhaps due to persistent exposure to sunlight without wearing spectacles which may reduce UV radiation reaching the eyes.[23] UV light induces proinflammatory cytokines[21,24] and growth factors[24] in the conjunctiva. In the setting of inflammation, fibroblasts synthesize collagen rather than secrete MMPs moreover, transforming growth factor-beta inhibits MMPs.[14] Lack of MMP expression in fibroblasts or stroma shows that matrix remodeling observed in pterygia is unlikely to be due to MMPs. Rather, it is possibly due to sunlight damage.[6,16]

This study has shown that damaged limbal stem cells are not a factor in pterygium occurrence, which is also reported by Tsai et al.[11] However the study by Pelit et al showed the contrary.[10] We think this may be because damaged limbal stem cells are not being eliminated by natural killer cells.[25] Immunodeficiency would impair NK cells thereby increasing the expression of damaged cells.[26]

Conclusions

The inflammatory cell count in pterygia shows severity of inflammation. Mild inflammation tends to be more frequent than severe inflammation. Pterygium inflammation seems to have no relationship with pterygium recurrence after surgery. It is clear that inflammation occurs in the setting of sunlight exposure without UV protection. However, the degree of inflammation seems not to be controlled by the duration of sunlight exposure. Pterygium occurrence obviously, is not linked to damaged limbal basal epithelial cells.

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Young patient's age determines pterygium recurrence after surgery.

Anguria P[1], Ntuli S[2], Carmichael T[1]

1. Department of Neurosciences, Division of Ophthalmology, University of the Witwatersrand Johannesburg, South Africa.

2. Department of Community Health, University of Limpopo Polokwane Campus, South Africa

Abstract

Background. It is not clear whether demographic or pterygium characteristics or limbal stem cell deficiency determine pterygium recurrence after surgery.

Purpose. To determine whether the demographic, pterygium characteristics, or limbal stem cell deficiency determine pterygium recurrence after excision.

Methods. Of 190 patients operated and followed-up for 6 months, 101 and 89 underwent free conjunctival autotransplant (CAT) or limbal conjunctival autotransplant (LCAT) respectively. The age, gender, occupation, grade of pterygium extent and degree of fleshiness, and laterality were compared between recurrent and no recurrent pterygia. Multivariate analysis was performed to determine the predictors of pterygium recurrence. Recurrence rates after surgery were compared between CAT and LCAT.

Results. The age range of the 190 patients was 22-65 years, mean ±SD 46.4 ±10.8 years. Pterygium recurred in 52 (27.4%). Thirty-nine (75%) of 52 patients with pterygia that recurred were aged <50 years (young) vs. 72 (52%) of 138 young patients with no recurrence; odds ratio (OR) = 1.54; 95% confidence interval (95% CI) = 0.70-3.36; p = 0.28. Thirty-one (60%) of 52 participants with post-surgical recurrent pterygia had large pre-operative pterygium (grade ≥3) vs. 130 (94%) of 138 patients with large pterygia that did not recur; OR = 0.11; 95% CI = 0.04-0.28; p <0.001. Of 101 patients undergoing CAT, 29 (28.7%) experienced recurrence vs. 23 (25.8%) of 89 undergoing LCAT; p = 0.66.

Conclusions. Young age seems to be associated with pterygium recurrence after excision followed by conjunctival graft. Large pterygia were protective.

Key words: Young age; pterygium extent; pterygium recurrence.

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Introduction

Young age may be associated with pterygium recurrence after excision,[1,2,3] and recurrence has been observed in young members of one family.[4]

Pterygium fleshiness rather than young age has also been associated with recurrence.[5] However, these results are derived from studies that involved small numbers of the patients with fleshy primary pterygia, treated with free conjunctival graft (CAT).[5] The extent of primary pterygium on the cornea seems to have no relationship with pterygium recurrence after surgery however, due to the small study sample, it is not clear whether pterygium extent is related or not with recurrence.[6] Another study found that recurrence after surgery was associated with a large pterygium extent but, it is possible that some large pterygia in that study were inadequately treated by radiotherapy as an adjunct to excision because the size of the radiation applicator was the same for small and large pterygia.[7]

The effect of excessive exposure to sunlight on pterygium recurrence after surgery also remains controversial. Although exposure was not compared between recurrence and no recurrence, one report blamed excessive sunlight exposure for pterygium recurrence[7] whereas another study concluded otherwise because recurrent pterygia did not show collagen degeneration.[9] Limbal stem cell deficiency may be a possible reason for pterygium,[10] and this prompted a comparison of recurrence rates between CAT and limbal conjunctival autotransplant (LCAT).[11] However, the efficacy of CAT and LCAT in the treatment of primary pterygium has not been compared in a prospective randomised study with a large sample.

This study was aimed to determine whether demographic factors, pterygium characteristics, or limbal stem cell deficiency determine recurrence after excision of primary pterygium followed by conjunctival graft.

Corresponding author:
Anguria Peter
Department of Neurosciences, Division of Ophthalmology, University of the Witwatersrand
PO Box 55226 Polokwane, 0700 South Africa
Email: irarak58@gmail.com

Methods

A prospective randomised study was designed. Clearance was obtained from the 2 institutional research ethics committees and the clinical trials register number NCT 00713180 at nih.gov was obtained before starting the study. The tenets of the Declaration of Helsinki (2000) were followed in obtaining consent.

One hundred seventy six patients (88 per group) were needed to detect a 15% difference in recurrence rates between CAT and LCAT at an alpha value of 5% and a power of 80%, assuming a base recurrence rate of 20% in CAT. This assumption was based on a reported recurrence rate of 21% following CAT in a similar population.[6] Because the present study factored a default rate of 12%, 200 patients were operated-on.

The 200 patients comprised 120 who had participated in an earlier epidemiological study and 80 others who were interviewed and examined in the same way as those in the epidemiological study.[12] The indications for surgery were corneal astigmatism, obstruction or threatened obstruction of vision, disfigurement, or frequent inflammation.[13] No patient had received topical anti-inflammatory treatment before surgery. Participants were recruited and randomised to CAT or LCAT as adjunctive treatment to pterygium excision. Age, sex, occupation, pterygium extent and degree of fleshiness,[5] and laterality were recorded. Pterygium extent was assessed as previously described by Youngson.[14] Grade 1 was a growth that had just crossed the limbus; grade 2 was approaching half of the corneal radius; grade 3 crossed half of the radius; grade 4 extended up to the corneal centre; and according to Carmichael (personal communication August 2007), grade 5 crossed the corneal centre.

Between 2008 September and 2011 July, the patients underwent pterygium excision and treated as reported earlier.[11] Only one eye per patient was enrolled in the study. The pterygia were excised at 4mm from the limbus and at the superior and inferior borders of the growth. The head was dissected off using a crescent knife. The grafts, which were harvested 1mm larger than the host pterygium were sutured-in using 10/0 nylon. Post-operative treatment consisted of topical ciprofloxacin 3mg/ml four times daily for one week, and prednisolone acetate 10mg/ml four times daily for 4 weeks. Sutures were removed at 1 month following surgery.[8] The patients were followed-up for possible recurrence for a minimum duration of 6 months because a prospective study has reported that 94% of recurrences occurred within 6 months.[1] Recurrence was defined as a wing-shaped re-growth of fibrovascular tissue at the site of previous pterygium,[5] which was confirmed by a masked ophthalmologist.

Data analysis

Demographic and pterygium factors were compared in the patients whose pterygia recurred and those in whom pterygia did not recur after excision. The odds ratios (ORs) for recurrence and their 95% confidence intervals (CI) and significances were calculated using Chi-square test. Multivariate analysis was performed that only included factors that significantly determined pterygium recurrence in univariate analyses. Recurrence rates were compared between CAT and LCAT using Chi-square test. Statistical significance was tested by Student t test in continuous variables and Chi-square test in categorical variables. $P \leq 0.05$ was considered to be significant. STATA 9 for widows software (STATA Corporation, College Station, USA) was used for statistical calculations.

Results

All 200 patients had pterygia of fleshiness degree 3 (thick pterygia). After 10 patients were lost to follow-up, data were available for 190 patients who were followed-up for a minimum duration of 6 months. Their age range was 22-65 years with a mean ±SD of 46.4±10.8 years. Pterygium recurred after excision in 52 (27.4%) of the 190 participants. Seven pterygia (13.5%) of 52 recurred within 1 month, 31 (59.6%) between 1 and 3 months, and 14 (26.9%), between 3 and 6 months. Of 38 pterygia that recurred within 3 months, 29 (76.3%) occurred in young patients (<50yrs) Similarly, 10 (71.4%) patients who experienced pterygium recurrence more than 3 months after surgery were young individuals.

Table 1 shows the results of univariate regression analysis of the demographic and pterygium characteristics in participants whose pterygia recurred and those in whom pterygia did not recur after surgery. Recurrence was more frequent than no recurrence in young participants. Recurrence was less frequent than no recurrence after excision of large pterygia.

It also presents the results of multifactorial analysis of factors that were significant predictors of pterygium recurrence in univariate regression analysis.

The OR for developing pterygium recurrence in young patients decreased and was no more significant compared to the OR in univariate regression. In contrast, the OR for grade 3 or larger pterygia recurrence increased and remained significant.

Table 1: Univariate and multivariate analysis of demographic and pterygium characteristics in patients with recurrence and those with no recurrence.

			Crude			Adjusted		
Variable	**Recur (%)**	**Not recur (%)**	**OR**	**95% CI**	**p-value**	**OR**	**(95%)**	**p-value**
Age								
<50yrs	39 (75)	72 (52)	2.75	1.35-5.59	*0.005	1.54	(0.70-3.36)	*0.28
Gender								
Female	42 (81)	113 (82)	0.92	0.41-2.09	*0.86			
Occupation								
Outdoors	28 (54)	83 (60)	0.77	0.40-1.47	*0.43			
Laterality								
Bilateral	40 (77)	97 (70)	1.41	0.67-2.96	*0.36			
Extent								
Grade ≥3	31 (60)	130 (94)	0.09	0.03-0.22	*<0.001	0.11	0.04-0.28	*<0.001

*Chi-square test

One hundred and one (53.2%) of 190 participants underwent CAT whereas 89 (46.8%) underwent LCAT. Each participant was operated in only one eye. Table 2 shows the results of univariate regression analysis of the demographic and pterygium characteristics, as well as surgical outcome in participants who underwent CAT or LCAT. The proportions of the patients were similar in the two groups with regard to demographic and pterygium characteristics.

Table 2: Demographic and pterygium characteristics of patients operated, and surgical outcome

Characteristic	**CAT surgery**	**LCAT surgery**	**p – value**
Age (mean ± SD)	46.6 ±10.2	46.1 ±11.5	+0.72
Gender			
Male	18 (17.8%)	17 (19.1%)	*0.82
Female	83 (82.2%)	72 (80.9%)	
Occupation			
Indoors	43 (42.6%)	36 (40.5%)	*0.77
Outdoors	58 (57.4%)	53 (59.6%)	
Laterality			
Unilateral	29 (28.7%)	24 (27.0%)	*0.79
Bilateral	72 (71.3%)	65 (73.0%)	
Extent grade			
2≥3	15 (14.9%)	14 (15.7%)	*0.87
	86 (85.1%)	75 (84.3%)	
Surgical outcome			
Recur	29 (28.7%)	23 (25.8%)	*0.66
Not recur	72 (71.3%)	66 (74.2%)	

+Student t-test; *Chi-square test

Discussion

Despite recurrence of some large pterygia, large pterygia were protective against recurrence independently of the patients' old ages, which has not been reported before. This result contradicts an earlier study that reported that recurrence after surgery was associated with large pterygium extent.[7] The contradiction is most likely to be due to the application of adjunctive treatment in a proportion larger than the bare sclera after pterygium excision in the present study.

Young age was not confirmed to be associated with recurrence independently of pterygium extent, which is consistent with a previous study that failed to confirm an association between young age and recurrence independently of pterygium fleshiness.[5] However, the present study failed to confirm that fleshiness was associated with recurrence[5] perhaps because all the pterygia in the present study were fleshy. It looks as if thick pterygia that qualified for surgery were common in this study population. And it seems that fleshiness protected old patients from pterygium recurrence after excision followed by conjunctival graft. Yet fleshiness seems to have failed to protect young individuals from pterygium recurrence. It is unlikely that pterygium fleshiness was important for the growth to recur or not to recur after surgery.

The present study did not corroborate previous studies that showed that young age is associated with recurrence independently of other significant factors.[1,2] This is probably because this study excluded most small pterygia in old individuals due to a lack of indication for surgery, hence, the study found that large pterygium size was protective.

Pterygium progression is the reason for recurrence after surgery.[13] Young age implicates growing pterygium in this study. Pterygium progress may be intrinsically controlled because of two possible explanations in the present study thus. Most of the patients had participated in an earlier study that found heredity to be associated with pterygium occurrence.[12] Most of the recurrences occurred within three months after surgery, the majority of which were in young individuals, which is consistent with a previous report that observed that aggressive pterygia recurred within a short period after excision in young members of one family.[4] Pterygium progression may explain the varying recurrence times after surgery. It is possible that pterygia with shorter recurrence times grow faster than those with longer recurrence times, the discrepancy being dependent on the difference in concentration of growth factors.[15]

Although young age may be implicated in pterygium growth pterygia in nearly 65% of young individuals did not recur. It is possible that those pterygia had stopped growing thus; young age is not tantamount to progress. Since MMPs are underexpressed in fibroblasts and stroma of individuals with chronic solar conjunctivitis,[16] suggesting presence of transforming growth factor-beta,[17] we imagine that a decrease in available growth factors to a level that is insufficient to promote fibrovascular proliferation that causes pterygium, yet enough to suppress MMPs may well explain lack of pterygium progress. Fibroblast mitotic rate has been shown to be proportional to the level of growth factors.[15]

It is maybe that pterygia that had stopped growing at a young age would be still small and thick later in life and would not recur after surgery.[1] However, it is clear that most pterygia in the present study had grown larger. Since some large pterygia recurred after excision irrespective of the patients' age, this suggests that growth continued in those large pterygia and that pterygium growth is independent of chronological age. Notwithstanding sunlight exposure in all patients whose pterygia recurred, sunlight is unlikely to determine recurrence. However, sunlight irrespective of its duration of exposure might be just a trigger for recurrence after surgery.

CAT and LCAT procedures appeared to have similar recurrence rates, which has been reported before.[2,11] Because the present study had a large sample, and it was prospective, the similar post-surgical outcome suggests that recurrence after excision of primary pterygium is unlikely to be due to limbal stem cell deficiency. This is consistent with our previous report on the follow-up investigation of pterygium samples from the present study's participants, which showed a lack of association between damaged limbal stem cells and pterygium.[18] As we are finalizing this paper we are not aware of a study that has reported that LCAT was superior than CAT in the treatment of primary pterygia.

Conclusions

Young patient's age determines pterygium recurrence after surgery, and large pterygium extent appears to be protective. Recurrence after excision of primary pterygium seems unlikely to have a relationship with limbal stem cell deficiency. For the sake of simplicity and to avoid potential damage to normal limbal stem cells at the donor area, free conjunctival grafting is to be

preferred over limbal conjunctival grafting for treatment of primary pterygium.

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·Review·

The role of heredity in pterygium development

Peter Anguria[1], James Kitinya[2], Sam Ntuli[3], Trevor Carmichael[1]

[1]Department of Neurosciences, Division of Ophthalmology, University of the Witwatersrand Johannesburg, 7 York Road, Park Town 2193, South Africa
[2]Department of Anatomic Pathology, University of Limpopo Polokwane Campus, Private Bag X9316 Polokwane 0700, South Africa
[3]Department of Public Health Medicine, University of Limpopo Polokwane Campus, Private Bag X9316 Polokwane 0700, South Africa
Correspondence to: Peter Anguria. Department of Neurosciences, Division of Ophthalmology, University of the Witwatersrand Johannesburg, Polokwane 55226, South Africa. irarak58@gmail.com
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Abstract

• Several risk factors, which include heredity, ultra-violet (UV) light and chronic inflammation, contribute to pterygium development. However, there is no report integrating these factors in the pathogenesis of pterygium. The aim of this review is to describe the connection between heredity, UV, and inflammation in pterygium development. Existing reports indicate that sunlight exposure is the main factor in pterygium occurrence by inducing growth factor production or chronic inflammation or DNA damage. Heredity may be a factor. Our studies on factors in pterygium occurrence and recurrence identify that heredity is crucial for pterygium to develop, and that sunlight is only a trigger, and that chronic inflammation promotes pterygium enlargement. We propose that genetic factors may interfere with the control of fibrovascular proliferation while UV light or (sunlight) most likely only triggers pterygium development by inducing growth factors which promote vibrant fibrovascular proliferation in predisposed individuals. It also just triggers inflammation and collagenolysis, which may be promoters of the enlargement of the fibrovascular mass. Pterygium probably occurs in the presence of exuberant collagen production and profuse neovascularisation.

• **KEYWORDS:** pterygium; fibrovascular proliferation; heredity; sunlight; inflammation; growth factors
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INTRODUCTION

Pterygium, which is a wing-shaped fibrovascular growth of the conjunctiva across the limbus onto the cornea[1,2], is divided into the head that invades the anterior cornea, the neck that includes the superficial limbus, and the body that overlies the sclera [3]. The cap, which is the first sign of pterygium [4,5] is the halo infront of the pterygium head. It is deep to the epithelium [6]. Pterygium can impair vision and it can be cosmetically unacceptable [7]. Recurrent pterygium after surgery may be more aggressive than the primary growth [8]. Despite the problems related to pterygium [7,8] prevention of its occurrence and its recurrence after surgery have not been successful because the pathogenesis of pterygium is not clear.

This review describes the ocular surface anatomy relevant to pterygium, and it discusses the literature related to the current theories on pterygium pathogenesis, and it outlines the modes of genetic inheritance. It summarizes our recent studies on factors in pterygium occurrence and post-surgical recurrence and it concludes by proposing a model of pterygium development.

Ocular Surface Anatomy Relevant to Pterygium The bulbar conjunctiva is loosely attached to the underlying Tenon's fascia before the surgical limbus, thereafter the conjunctiva and Tenon's fascia, fused, adhere to the episclera [9]. The superficial episcleral plexus is found in the surgical limbus [10]. From the limbus, centrally, the tissues are compact [11]. Fibroblasts and blood vessels as well as inflammatory cells are located in the conjunctival stroma, which is at the same plane as the limbal stroma and Bowman's membrane of the cornea[3,6,12,13,16].

Current Theories on Pterygium Pathogenesis Inflammation [14] and fibrovascular proliferation [15-18] may be factors in pterygium occurrence. DNA damage [19-21] has been reported to initiate pterygium development. Hereditary predisposition [8,22-24] may be the underlying factor for pterygium occurrence. Ultra violet (UV) light has been shown to induce proinflammatory cytokines, chronic inflammatory cells, and growth factors [16,17,25,26]. It also may damage DNA in predisposed individuals [21,23,24,27]. However, integration of factors associated with pterygium occurrence has not been reported.

Sunlight Exposure All individuals may be exposed to UV light, which generates reactive oxygen species (ROS) from the ocular surface [28]. Excessive exposure is widely believed

to be the reason for pterygium to occur [29,30]. However, some studies have shown that pterygium may be infrequent in individuals highly exposed or, a low exposure may be frequent in pterygium patients [5.31-34]. Excessive sunlight exposure perhaps is also related to pterygium recurrence after surgery[35].

Excessive exposure to sunlight has been correlated with collagen degeneration although collagen degeneration has been discredited as a mechanism of pterygium pathogenesis[4]. Collagen degeneration may be present in primary pterygia but, some primary pterygia may not show collagen degeneration histologically [36,37]. This degeneration is not manifested in recurrent pterygia, suggesting short durations of exposure to UV light[4,38]. It seems that the level of sunlight exposure may not be important for pterygium to occur or to recur.

Inflammation It has been shown that UV light induces pro-inflammatory cytokines in pterygia, however, the degree of induction varied in pterygia exposed to the same level of UV light, suggesting that the level of exposure to sunlight may not be important for inflammation to be severe [25,39]. It might be that the severity of inflammation is genetically controlled. Some individuals may be deficient of T-lymphokine activated killer cell-originated protein kinase (TOPK) and its deficiency appears to increase sunlight induced inflammation[40].

Reactive oxygen species phosphorylate cell membrane lipids, which manifests as increased products of lipid metabolism [41,42]. These lipid products include prostaglandin E-2 (PGE-2), which has been reported in pterygia [18]. Oxidized phospholipids stimulate production of cyclooxygenase-2 (COX-2) enzyme and interleukin-8 (IL-8), which are pro-inflammatory [43,44]. Inflammatory cells are present in all pterygium samples, which indicates inflammation[5,14,15,45,46].

Inflammation has been proposed to be the final step in the formation of pterygia, however, that inflammation was thought to be a type of hypersensitivity because the leukocytes were mainly located in the epithelium [5]. It is not clear whether hypersensitivity is crucial for pterygium to be formed. Although inflammatory cells are present in pterygia older studies [5,14] did not indicate whether the inflammatory cell infiltration was related to the severity of inflammation or to the size of pterygium or to the level of exposure to sunlight. It is not clear how inflammation may be the final step in pterygium formation.

Inflammation activates transforming growth factor-beta (TGF –β) thereby stimulating the fibroblasts to synthesize collagen [47-50]. Transforming growth factor-beta also inhibits MMPs[51,52]. Collagen is deposited randomly (fibrosis), which causes tissues to become opaque[53]. Inhibition of MMPs tends to decrease collagenolysis, however, collagen degeneration characterizes pterygium[12,15,36]. Collagen degeneration is a sign of prolonged collagenolysis, which may be caused by ROS[41,54]. Although one previous study failed to detect MMPs in pterygium fibroblasts several studies have reported MMP expression, which seems to suggest that collagen degeneration in pterygia is due to MMPs [3,20,55,56]. As all pterygia have inflammatory cell infiltrations it may be that MMPs are not expressed in pterygium fibroblasts despite limbal stem cell damage, but, this needs to be corroborated. Inflammation also induces angiogenic growth factors[18,55,57,58]. Moreover, TGF –β up-regulates vascular endothelial growth factor (VEGF), which in a frame-work of fibronectin stimulates neovascularisation as collagen synthesis proceeds[53,59,60].

Fibrovascular proliferation Ultraviolet light, even of a short duration may induce growth factors such as basic fibroblast growth factor (bFGF), TGF-β, platelet derived growth factor (PDGF), VEGF, connective tissue growth factor (CTGF) and heparin binding epidermal growth factor-like epidermal growth factor (HB-EGF)[15-17]. Oxidative stress induces those growth factors in the fibroblasts, endothelial cells and inflammatory cells in the stroma. It also induces those growth factors in the conjunctival epithelium [15-18,61]. Growth factors promote vibrant proliferation of fibroblasts in pterygia but in controls, the same level of growth factor proteins causes sluggish mitosis [62,63]. This seems to suggest that vibrant fibroblast mitosis is unlikely to be due to overexpression of these proteins in pterygia. It may be due to an abnormal phenotype of pterygium fibroblasts that causes fibroblasts to respond energetically to growth factors [4,62]. Fibroblast abnormality might arise from sunlight damage, which causes these to over-express MMPs [20,56]. However, acquired fibroblast damage fails to explain why pterygium occurs in patients whose fibroblasts do not express MMPs[55].

Heparin binding epidermal growth factor-like epidermal growth factor, a fibrogenic growth factor may be available for at least 48h in pterygia after exposure to UV light has stopped[17,63]. Fibrogenic growth factors such as PDGF are not over-expressed in controls [15,62]. The expression of bFGF in some controls may be the same as in cases, which suggests that over-expression of angiogenic growth factors is not the reason for vibrant fibroblast mitosis or for pterygium to occur [15,62]. Rather, the up-regulation of fibrogenic growth factors is most likely to be the reason for fibroblast proliferation and for pterygium to occur [15,62,63]. Nevertheless, angiogenic growth factors such as bFGF and VEGF are up-regulated in pterygia mainly *via* ROS [15,16,18,64]. Reactive oxygen species in addition directly stimulate capillary growth[65]. Bevacizumab, which is anti-VEGF fails to abolish pterygium recurrence after surgery[66]. Since pterygia occur in the presence of fibrogenic growth factors failure of bevacizumab to abolish post-surgical pterygium recurrence

may be due to its lack of inhibition of fibrogenic growth factors [15]. This seems to suggest that pterygium occurs because fibrogenic growth factors are not inhibited, however, there is no literature that lack of inhibition of fibrogenic growth factors occurs in pterygium.

A fibrogenic growth factor binds to its receptor at the fibroblast cell membrane to form a complex which is internalised to form specific endocytic vesicles [67]. Receptor-regulated sma (small) and mad (mothers against decapentaplegic) proteins abbreviated smad, *via* a series of steps including smad1 and 5 activate the receptor thereby translocating the growth factor to the nucleus [67-70]. A signal for the transcription of genes for fibroblast mitosis is initiated [71]. After adequate signals a different type of specific endocytic vesicles is formed [67]. Inhibitory smad proteins (smad7) in these vesicles terminate the signal for genetic transcription [72,73]. Inhibitory smad proteins stimulate smad ubiquitin regulatory factor-1 (smurf-1), which may compete with smurf-2 to deactivate growth factor receptors [74,75]. The action of smad7 is independent of smurf proteins [72]. Inhibitory smads and smurf proteins are genetically determined [76,77]. Growth factors generate ROS at the cell membrane and TGF-β inhibits antioxidant enzymes[78].

DNA damage Ultraviolet light may damage DNA [20,21,27] irrespective of the dose of radiation, race, or age of the individual [21,27,79]. DNA damage might cause localised limbal stem cell deficiency probably due to migration of both the reserve stem cells and transient amplifying cells[56]. Damaged cells perhaps migrate in all directions [80] assisted by MMPs, which may degrade collagen and fragment Bowman's membrane[20,56]. Pterygium occurs maybe as a result of corneal conjunctivalisation [56]. Migration might be promoted by inflammation whereby epithelial mesenchymal transition occurs to the cells which migrate to the stroma in individuals predisposed to a deficiency of discs large factor-5 (Dlg-5)[81-83]. This may cause a fibrotic mass [80]. The wing-like shape of pterygium may be calculated as due to more epithelial cell loss centrally than at the limbus[84]. However, this theory fails to explain pterygium shape peripheral to the limbus. The theory of DNA damage fails to explain why pterygium develops in those having no evidence of DNA damage [27,85]. Moreover, some pterygium patients do not have predisposition to DNA damage[23,24].

Hereditary predisposition Hereditary predisposition to pterygium development has been acknowledged, but, it has been underemphasized [22,86-89]. The mode of inheritance has been reported to be autosomal dominant based on a study of one [87,88] or two families [86] however, a large sample is necessary to increase credibility [90] since alleles may or may not be transmitted. Autosomal recessive mode might also be possible however, because the original report in French is difficult to find it is difficult to ascertain whether pterygium patients were compared with unaffected individuals or, how many pedigrees or patients were considered[87]. Multifactorial mode is likely but, it was determined using self-reported family histories which were not tested for independence of association with pterygium occurrence [89]. Knowledge of the mode of inheritance facilitates determination of the possible mechanism of pterygium development[91,92].

Modes of Inheritance These may be Mendelian or non Mendelian. Mendelian inheritance may be autosomal dominant, autosomal recessive or sex linked. Non Mendelian inheritance may be multifactorial or mitochondrial. Mendelian and multifactorial modes of inheritance involve genes that are located in the nucleus.

The phenotype in autosomal dominant inheritance is determined by a single defective allele, which is dominant[91,93]. According to Mendelian principles, whether one or both parents are affected the offspring have a 50% chance of being affected as individuals who are homozygous defective are so severely affected that they perish before birth or early in life [91]. Incomplete penetrance sometimes occurs thereby causing a skipped generation[90].

Inheritance in autosomal recessive mode occurs due to two ineffective alleles, which are recessive [40,94]. It may be homozygous recessive [40] or double heterozygous whereby two heterozygous recessive genes that code for the same phenotype are located in different loci [94]. The risk of the offspring becoming affected is 100% if both parents are homozygous recessive while it is 50% if one parent is homozygous and the partner is heterozygous recessive and it is 25% if both parents are heterozygous[40]. There is no risk of having an affected offspring if one parent has normal paired alleles [40]. According to Mendelian principles [91,93] if both parents are double heterozygotes the unaffected to the affected ratio of the offspring is 5:11.

Mendelian principles require that sex linked conditions are always recessive [95] because males lack one pair of alleles in the Y chromosome, which causes inheritance of a defective dominant allele in the Y chromosome to be lethal [91]. Males may become affected when they inherit a recessive allele from an affected or heterozygous mother and the affected fathers may transmit their recessive gene only to their daughters who may become affected only if the mother is homozygous or heterozygous recessive[95].

In multifactorial inheritance genes may interact with environment [96], or, two or more genes coding for different proteins and in different loci may modify one another's effect [97,98] to produce a phenotype. Genes are not defective[91,93] however, genes have to be activated before transcription to messenger RNA, after which protein synthesis may occur[67,71,99]. One active allele is sufficient for the gene to be effective[97], which allows a protein to be synthesized [40]. The risk of transmission to subsequent offspring, which can be predicted

using Mendelian principles [91,93] depends on the genotypes of the mating partners. Affected individuals tend to cluster in families[100].

The polygenic model specifies that the more inactive (determinant) alleles interacting the severer the phenotype[92] and the higher the risk of transmission to subsequent generations [100]. The threshold model requires that the contributions by genes and environment reach a threshold that leads to phenotype whereby sometimes, genes are the main contributor and other times, the environment is the main contributor to the threshold[96].

Recent Studies on Factors in Pterygium Occurrence

These were undertaken in Mankweng Hospital, a tertiary referral centre in Limpopo Province of South Africa, which is mainly rural and it is bisected by the tropic of Capricorn (23.5^0 south of the Equator). The Province is sunny and dry[101] and it is inhabited mainly by the Pedi, Tsonga, Venda, and Tswana. These groups of Bantu people have been reported to practice first degree cousin marriage [102], in which reproduction may promote the occurrence of hereditary conditions [103]. The population of Limpopo in 2009 when these studies begun was estimated at 5227200, of which 2436400 (46.6%) was 20-64y old, and 87% of the population was rural [104]. The methods and results of these studies have been reported[105-108].

The pterygium cases and control patients whose conjunctivas were investigated by immunohistochemistry were selected from the Eye Clinic, and the unaffected individuals matched with pterygium cases were selected from the refraction Clinic. Two hundred and thirty cases and 150 controls matched for age and sex with the first 150 cases, as well as seven unmatched controls whose eyes had been irreparably injured were interviewed and their eyes examined. Data from 150 case-control pairs were analyzed as pre-calculated to give a 20% difference in family history at a power of 80%, assuming a base rate of 10% in controls and P-value of 5%. Of the 300 participants whose data were analyzed the age range was 22-65y, modal range was 40-49y; the females were 3.5 times more frequent than males [106]. Pterygium surgery was done on 200 cases, which had indications for surgery. The indications included corneal astigmatism, pterygium obstructing or threatening to obstruct the visual axis, frequent pterygium inflammation, and disfigurement by pterygium [108]. Interviews were conducted, a full eye examination done, and the pterygia in patients having indications for surgery were excised. The 59 pterygium specimens and 7 control nasal conjunctivas were investigated by immunohistochemistry [107]. The control specimens were obtained from males who were aged 23-51y old. Follow-up family visits were conducted on selected cases and controls[106]. Because alleles may or may not be transmitted a large sample that is necessary for the calculation of a credible mode of inheritance was obtained by combining the relatives of cases and controls into 2 separate families [90,108]. There were 382 combined relatives of pterygium probands and 394, of unaffected probands; their age range was 10-86y, and 275 of 382 relatives of cases (71.9%) were ≥40y old compared with 284 of 394 relatives of controls (72%)[106]. The ratio between the unaffected and pterygium-affected relatives in the combined families of cases was calculated, which was used to determine the likely mode of inheritance based on Mendelian principles[40,91,93,94]. As that ratio was not Mendelian the equivalent ratio was computed and estimated because decimals of individuals do not exist. The estimated ratio predicted the likely genotypes of a mating couple whose offspring are expected to be in the proportion of the estimated ratio. Mendelian principles[93,94] were applied to that mating to depict genotypes of the offspring. One pedigree was used to demonstrate the likely mode of inheritance[87,88].

Heredity is fundamental for pterygium to occur Family history, which implicates heredity [22,89] was associated with pterygium occurrence [105,106] independent of the use of traditional eye medication and of the unstable tear film[106]. Having diagnosed pterygium-affected relatives, which implicates heredity [8,86-88] was associated with pterygium occurrence hence confirming familial occurrence of pterygium [106]. Pterygium patients with the unaffected individuals had similar exposure to sunlight, which suggests that familial occurrence was due to heredity rather than environment[106].

Traditional eye medication was the only environmental factor associated with pterygium however, the controls also had used this medication in a similar way, obtained from the same practitioners, in the same period, which suggests that traditional eye medicine was not a direct cause of pterygium [106]. Individuals who use African traditional medication are likely to follow certain traditions [109] such as first degree cousin marriage [102]. Reproduction between cousins increases the risk of occurrence of hereditary conditions present in an extended family[103]. The following of the tradition of first degree cousin marriage [102] is the reason for the association of traditional eye treatment with pterygium and so, the use of traditional medication may implicate heredity in pterygium occurrence[106].

The ratio between the unaffected and pterygium-affected individuals in the combined family of pterygium probands was 9:7[106], which suggests digenic inheritance (the simplest form of multifactorial inheritance [97]). Table 1 shows the depicted genotypes of affected and unaffected offspring. A and B indicate active alleles whereas a and b indicate inactive alleles. Bold font indicates predicted genotypes of a pterygium patient. The appearance of only two letters represents predicted alleles in the gametes of the parents. The genotypes of the two parents are AaBb and AaBb. Each letter

Table 1 Depicted genotypes of affected and unaffected offspring

Genotypes	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AAbB	AAbb	AabB	Aabb
aB	aABB	aABb	aaBB	aaBb
ab	aAbB	aAbb	aabB	aabb

whether capital or small represents one type of gene. This table suggests that pterygium cases have at least one type of gene having both alleles inactive (determinant gene). The signals for fibroblast mitosis, generated by fibrogenic growth factors are regulated by inhibitory smad proteins [72,73] and smurf proteins [74,75]. These proteins are genetically determined[76,77] and so it is possible that pterygium fibroblasts undergo vibrant mitosis [62] because of genetic lack of inhibitory smads or smurf proteins.

Figure 1 depicts a pedigree of a pterygium proband. Oval empty drawings illustrate unaffected females and the shaded oval drawings illustrate affected females. Rectangular empty drawings illustrate unaffected males and the shaded rectangular drawing indicates an affected male. The arrow points at the proband. As 2 generations were affected autosomal dominant with incomplete penetrance in the first generation is likely [87]. As only the second and third generations had pterygium patients it is possible that this was due to autosomal recessive mode of inheritance whereby the first generation and the spouse of the proband were carriers[40]. It is also possible that sex linked inheritance was the mode of inheritance since the proband, a male, might have inherited a recessive gene from his carrier mother and he transmitted this gene to his daughter whose mother was a carrier [95]. Because this pedigree does not show a consistent Mendelian pattern Mendelian inheritance is unlikely in pterygium occurrence. Rather, the most likely mode of inheritance is multifactorial because it was determined from a large sample [90,110]. The proband had a short recurrence time (Less than 3mo after surgery [108]) and his daughter was 16 years old. These observations indicate that multifactorial mode of inheritance follows the polygenic model [92]. A short recurrence time and an early onset are signs of severe pterygium [8]. The skipping of the first generation and only one individual in the third generation being affected might suggest the presence of few genes[92]. However, the small size of a pedigree[90] most likely caused it to appear that all siblings in the second generation and only one in the third generation were pterygium patients because alleles may or may not be transmitted. All pterygium patients are predisposed and they may have unaffected relatives [106]. The proportion of the pterygium-affected relatives seems to depend on the proportion of determinant genes (polygenic model [100]). These findings suggest that predisposition to pterygium is unlikely to be the deficiency of Dlg-5[82] because more than one gene seems to be involved in

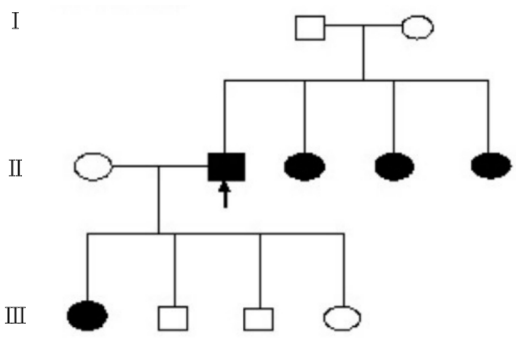


Figure 1 Pterygium pedigree.

pterygium development whereas Dlg-5 deficiency involves only one gene.

Age and small pterygium extent seem to be associated with pterygium recurrence after surgery perhaps due to the patients' selection criteria [108]. Also, large pterygium extent seems to be associated with recurrence perhaps due to inadequate treatment[111]. It is possible that pterygium size has no relationship with post-surgical recurrence. Pterygium fleshiness appears to be associated with pterygium recurrence after excision probably because excision was not followed by adjunctive treatment [112] otherwise, fleshiness has no relationship with post-surgical recurrence[108]. Since it is likely that pterygium occurrence is due to dormant genes post-surgical recurrence (pterygium progression [108]) can be explained by continued genetic inactivity [113]. The patient's age, pterygium size, and its fleshiness most likely depend on pterygium progression to be associated with post-surgical recurrence. However, genes controlling pterygium occurrence have yet to be established.

Sunlight exposure is only a trigger for pterygium to occur All the participants had been exposed to sunlight and excessive exposure had no relationship with pterygium occurrence [106], which is similar to recent reports [32,34]. Since sunlight damage may induce chronic inflammatory cell infiltration in the conjunctival stroma [26] the presence of chronic inflammatory cells in all pterygium samples, and the inhibition of MMPs in all pterygia and controls[107] support the finding that all pterygium patients as well as controls had been exposed to sunlight [106]. As the inflammatory cell infiltrate varied in pterygia that had collagen degeneration (sign of prolonged UV radiation[54]) this shows that the degree of infiltration was not related to the level of exposure to the sun. Sunlight irrespective of its degree of exposure may be only a trigger for pterygium occurrence in those predisposed to pterygium formation [106]. This may occur by inducing oxidative stress at the ocular surface[28]. Also, sunlight may be only a trigger for pterygium recurrence after excision[108].

Chronic inflammation is only a promoter of pterygium enlargement Inflammatory cell infiltration in pterygium samples is a sign of inflammation [46]. Although the inflammatory cell count was correlated with pterygium size,

which suggests that inflammation may contribute to pterygium enlargement inflammation is unlikely to be a determinant of enlargement because pterygia irrespective of their size tended to have a low count [107]. The degree of the inflammatory cell infiltration may indicate the severity of inflammation rather than pterygium size [107]. Inflammation irrespective of its severity may be just a promoter of pterygium enlargement. Because pterygia tended to be mildly inflamed this suggests that epithelial mesenchymal transition is unlikely to be the mechanism for pterygium to occur as epithelial mesenchymal transition requires that inflammation be severe for it to occur[81].

Inhibition of MMPs in the fibroblasts and stroma of all pterygium samples and controls is most likely to be due to inflammation [107]. Inflammation activates TGFβ [47,48], which stimulates the fibroblasts to synthesize collagen [49,50]. In addition, TGFβ inhibits MMPs [51,52]. The synthesized collagen is deposited randomly, which causes previously transparent tissues to become opaque [53]. Collagen is the reason that pterygia are fleshy and it is the reason for the cap. Inhibition of MMPs minimizes collagenolysis [12] and it suggests that the collagen degeneration which was present in most of the pterygia and controls was not due to MMPs. This contradicts previous studies [20,56] perhaps because the participants in the present study had not used spectacles[107]. Transforming growth factor-beta up-regulates VEGF [59], which stimulates neovascularisation[60] hence, inflammation in addition promotes pterygium neovascularisation.

Limbal stem cell damage was not associated with pterygium[107]. This suggests that DNA damage[19,29] is unlikely to be a factor in pterygium development. The predisposition to DNA damage [23,24] is unlikely to be the predisposition to pterygium occurrence.

Proposed Model of Pterygium Development Figure 2 shows the proposed model of pterygium development, which is a flow chart showing that pterygium development is influenced by heredity in conjunction with sunlight exposure. Sunlight exposure, *via* oxidative stress induces growth factor production, angiogenesis, chronic inflammation, and collagenolysis. Bold black font indicates determinant factors, bold black font in italics indicates promoting factors, and bold black arrows show the determinant pathway. Normal black font in italics indicates a trigger and bold yellow arrows show the triggering pathway. Heredity[105,106] influences growth factors to cause vibrant fibroblast mitosis[63]. Sunlight induced oxidative stress triggers growth factors [28,61] and it triggers angiogenesis by directly stimulating capillaries to grow [65]. Oxidative stress triggers inflammation[18,41] in sunlight exposed conjunctivas [106], and it causes collagenolysis also [41,107]. Vibrant mitosis produces many fibroblasts [62], which are stimulated by inflammation [47] to collectively synthesize collagen [49] exuberantly. Excessive collagen is deposited in

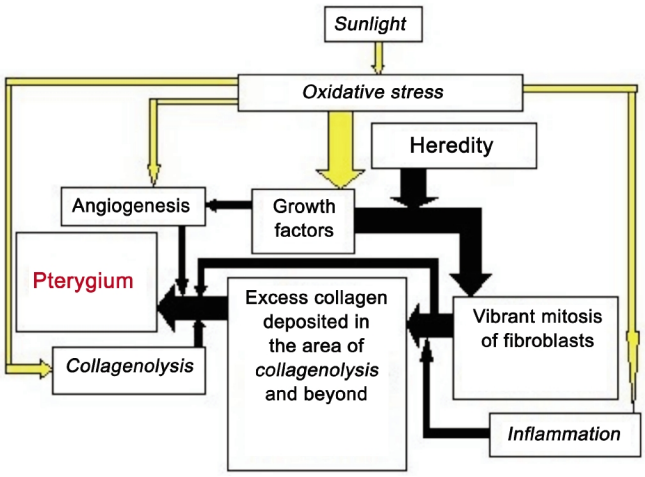


Figure 2 Proposed model of pterygium development.

the damaged area and beyond the margins of the damaged matrix to develop the pterygium cap. The excessive collagen is invaded by fibroblasts, and new blood vessels stimulated by growth factors and ROS to develop pterygium. Cap collagenolysis [41] facilitates the fibroblasts and new blood vessels to invade the stroma. Bowman's membrane probably gets fragmented due to the location of the cap in it [4,6]. Pterygium onset is at the surgical limbus probably because of the numerous endothelial cells [10], which generate abundant ROS after sunlight exposure[28].

Figure 3 depicts sub-model 1, which is a flow chart showing that heredity sustains pterygium development *via* oxidative stress generated by proliferating fibroblasts and endothelial cells. Bold font indicates determinant factors, bold font in italics shows promoting factors, and orange arrows indicate pathways involving ROS, and a plain arrow indicates a subsidiary pathway for growth factor production. After sunlight has triggered pterygium onset the proliferating fibroblasts generate ROS, through which production of fibrogenic and angiogenic growth factors is sustained [61]. Through ROS the proliferating fibroblasts sustain inflammation [18,39,41,42]. Matrix damage [41,107] and angiogenesis (by directly stimulating capillary growth [65]) are also sustained. The replicating endothelial cells generate oxidative stress thereby stimulating endothelial cells and fibroblasts to produce fibrogenic and angiogenic growth factors [61]. Also, capillary growth is directly stimulated [65]. Angiogenic growth factors are also induced by inflammation [57,58]. It seems that sustenance of pterygium development can be terminated if hereditary predisposition is halted, perhaps by activation of previously dormant genes[99,108].

Because the conjunctiva and Tenon's fascia are loosely attached before the surgical limbus, thereafter the two, fused, are firmly attached to the episclera [9], and the limbus and cornea are compact[11], it is most likely that there is increasing centripetal resistance to the expanding fibrovascular mass, which causes it to be shaped like a wing. The role of

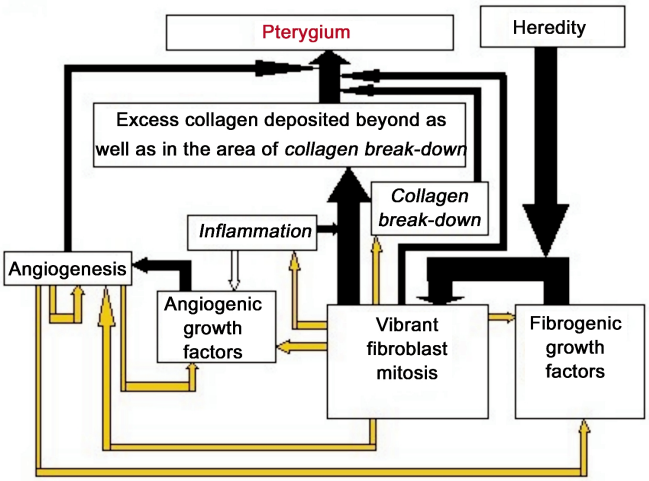


Figure 3 Sub-model 1.

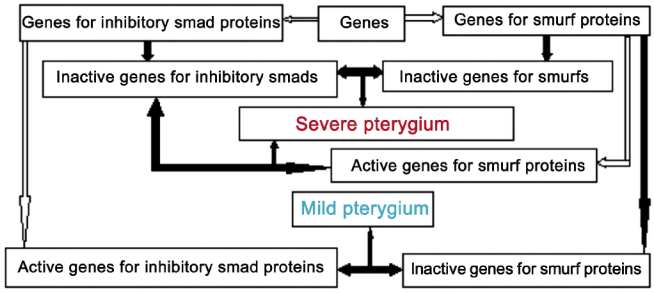


Figure 4 sub-model 2.

pterygium inflammation is to promote pterygium enlargement [107] and fleshiness by stimulating collagen synthesis[49,50] and its conservation[107].

Figure 4 depicts sub-model 2, which is a flow chart showing that heredity determines pterygium severity. Bold font indicates determinant factors. Double arrows indicate interaction. Normal arrows show normal outcome and bold single arrows show an abnormal outcome. The genes involved in pterygium occurrence may be those for inhibitory smad proteins [76] or for smurf proteins [77]. Because the inhibition of signals for mitosis, generated by growth factors is independent of receptor inhibition [72] it may be that interaction between inactive genes for inhibitory smads and inactive genes for smurfs or, between inactive genes for inhibitory smads and active genes for smurf proteins causes severe pterygium to occur. Because degradation of growth factor type 1 receptors depends on smad 7 [74] it may be that a mild pterygium occurs if active genes for smad 7 proteins interact with inactive genes for smurf proteins.

Since numerous fibrogenic growth factors are present in pterygium[15-17] it is possible that pterygium size is determined by the proportion of growth factors lacking inhibitory smads or smurf proteins (polygenic model[100]). Severity in large pterygia may be determined by the proportion of growth factors lacking inhibitory smad proteins (polygenic model[92]).

CONCLUSION

Hereditary predisposition is fundamental for the onset and sustenance of pterygium. Pterygium size and severity are most likely to be determined by hereditary factors. Predisposition to pterygium occurrence most likely follows multifactorial mode of inheritance, which is of the polygenic model. It is possible that two types of genes, one for inhibitory smad proteins and the second for smurf proteins are inactive thereby predisposing to pterygium occurrence. It seems that fibrogenic growth factors are crucial for pterygium to develop, and it seems that pterygium angiogenesis follows fibroblast proliferation, collagen synthesis, and collagenolysis.

Sunlight is only a trigger for pterygium to occur, perhaps *via* reactive oxygen species. It appears that inflammation and collagen damage, which are most likely to be due to oxidative stress only promote pterygium enlargement.

Recommendations Genetic counselling to advise family members regarding risks for pterygium development seems far off at present although it might play a role with further investigation in high risk communities. Studies to determine the molecular nature of predisposition are recommended.

Control of sunlight exposure by use of spectacles/sunglasses in predisposed individuals is encouraged. Control of collagen synthesis seems to be an attractive option to minimize enlargement of the fibrovascular mass in those predisposed.

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