A PILOT STUDY OF INTRALESIONAL RANIBIZUMAB ON PTERYGIUM VASCULARITY, SIZE AND RECURRENCE RATE

BY
DR. HO HAO CHI
MD (USM)

Dissertation Submitted In Partial Fulfilment Of The Requirements For The Degree Of Master Of Medicine (OPHTHALMOLOGY)

SCHOOL OF MEDICAL SCIENCES
UNIVERSITI SAINS MALAYSIA
2015

DISCLAIMER

I hereby certify that the work in this dissertation is my own except where assistance was specifically acknowledged. The sources of all references have been clearly acknowledged.

Dated: 11/3/2015

_____

Dr. Ho Hao Chi

PUM0335/11

ACKNOWLEDGEMENT

To my supervisor, Assoc. Prof. Dr. Mohtar Ibrahim, I would like to express my gratitude for his guidance and encouragement during the preparation for this dissertation.

A special thanks to my co-supervisor, Dr. Khairy Shamel Sonny Teo, for his guidance throughout the study.

My sincere appreciation to Assoc. Prof. Datin Dr. Zunaina Embung and Assoc. Prof. Dr. Wan Hazabbah Wan Hitam, lecturers in Department of Ophthalmology, School of Medical Sciences, Universiti Sains Malaysia for their kind assistance and invaluable advice.

I would like to thank Prof. Hasnan b. Jaafar, lecturer in Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia and all the staff in pathology laboratory for the help and co-operation.

I would also like to express my gratitude to Assoc. Prof. Dr. Leow Chee Seng, Director of Graduate School IIC University of Technology and for his advice and help in statistical analysis of the data.

To all the lecturers, colleagues and staff of Department of Ophthalmology, my greatest thanks for your support and friendship.

No words can express my gratitude to my family for their patience, love, understanding and inspiration.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

ABSTRAK

Pengenalan:

Pterigium adalah satu penyakit mata yang biasa di Malaysia kerana lokasi negara ini terletak berhampiran dengan khatulistiwa. Kajian terkini telah mendapati bahawa factor pertumbuhan endothelial vascular (VEGF) hadir dalam jumlah yang besar dalam epitelium pterigium terutama di kepalanya berbanding konjunktiva normal yang menunjukkan VEGF terlibat dalam angiogenesis dan pertumbuhan proliferatif fibrovaskular pterigium. Oleh itu, anti-VEGF telah dicadangkan sebagai ubat tambahan tanpa label untuk pembedahan pterigium.

Objektif:

Untuk menilai kevaskularan dan saiz pterigium utama selepas suntikan ranibizumab intralesi dan kadar perulangan selepas pembedahan pterigium tanpa jahitan.

Metodologi:

Pesakit yang memerlukan pembedahan eksisi pterigium primer yang memenuhi kriteria kemasukan dan pengecualian dikenal pasti. Mereka kemudian secara rawak dimasukkan ke dalam kumpulan rawatan dan kawalan. Kumpulan rawatan disuntik dengan ranibizumab intralesi (0.3 mg/0.03 mL) 1 minggu sebelum pembedahan. Gambar segmen depan diambil sebelum dan 1 minggu selepas suntikan. Perubahan dalam kevaskularan pterigium (peratusan kawasan pterigium dilindungi oleh saluran darah) dan saiz (peratusan kawasan kornea dilindungi oleh pterigium) diukur menggunakan perisian analisis imej, Imej J. Satu minggu selepas suntikan ranibizumab, kedua-dua kumpulan rawatan dan kawalan menjalani

pembedahan eksisi pterigium dan lekatan autograf konjunktiva dengan gam fibrin. Rawatan susulan telah dibuat kepada pesakit selama 1 tahun bagi memantau perulangan dan komplikasi.

Keputusan:

36 pesakit (18 setiap kumpulan) telah selesai menjalani kajian. Min perubahan dalam kevaskularan pterigium adalah 1.48 (4.65)% manakala saiz pterigium adalah 0.28 (2.71)%. Kedua-dua pengurangan adalah tidak signifikan secara statistic (masing-masing p = 0.195 dan 0.672). Kadar perulangan dalam kumpulan rawatan adalah 22.2% (n = 4) manakala kumpulan kawalan adalah 16.7% (n = 3). Kadar perulangan antara kumpulan tidak signifikan secara statistik (p > 0.950).

Kesimpulan:

Satu suntikan intralesi ranibizumab (0.3 mg/0.03 mL) tidak mengurangkan kevaskularan pterigium dan saiz secara ketara dalam masa 1 minggu. Suntikan ranibizumab intralesi pra-pembedahan tidak mengurangkan kadar perulangan pterigium.

ABSTRACT

Introduction:

Pterygium is a common eye disorder in Malaysia due to the country's location near to the equator. Recent study has found that vascular endothelial growth factor (VEGF) is present in great amount in pterygium epithelium especially in its head compared to normal conjunctiva suggesting that VEGF is involved in the angiogenesis and proliferative fibrovascular growth of pterygium. Thus, anti-VEGF has been proposed as an off-label adjunct to pterygium surgery.

Objective:

To evaluate the vascularity and size of primary pterygium after intralesional ranibizumab injection and the recurrence rate following sutureless pterygium surgery.

Methodology:

Patients presenting for primary pterygium excision who fulfilled our inclusion and exclusion criteria were identified. They were then randomised into treatment and control groups. Treatment group was injected with intralesional ranibizumab (0.3 mg/0.03 mL) 1 week prior to surgery. Anterior segment photographs were taken before and 1 week after the injection. Changes in pterygium vascularity (percentage of pterygium area covered by vessels) and size (percentage of cornea area covered by pterygium) were measured using image analysis software, Image J. 1 week after ranibizumab injection, both treatment and control groups

underwent pterygium excision and conjunctiva autograft adhesion with fibrin glue. Patients were followed-up for 1 year to monitor for recurrence and complication.

Results:

36 patients (18 each group) completed the study. Mean change in pterygium vascularity was 1.48 (4.65)% while pterygium size was 0.28 (2.71) %. Both reductions were not statistically significant ($p = 0.195$ and 0.672 respectively). Recurrence rate in treatment group was 22.2% ($n = 4$) while controlled group was 16.7% ($n = 3$). Recurrence rate between groups was not statistically significant ($p > 0.950$).

Conclusion:

Single intralesional injection of ranibizumab (0.3 mg/0.03 mL) did not reduce the pterygium vascularity and size significantly in 1 week time. Pre-operative intralesional ranibizumab did not reduce pterygium recurrence rate.

1 INTRODUCTION

1.1 Background

Pterygium appears as a wing-like triangular (with apex towards the corneal centre) fibrovascular growth from bulbar conjunctival over the limbus onto the cornea. Histologically, pterygium is characterised by collagen fibres elostatic degeneration of the conjunctival stroma. The effect of pterygium can range from cosmetic, astigmatism to blinding if visual axis is encroached but mostly it is asymptomatic.

1.2 Epidemiology of pterygium

Pterygium is a common eye disorder in Malaysia due to the country's location near to the equator. Prevalence is as high as 22% in equatorial areas compared to 2% in latitudes above 40° (Dake *et al.*, 1989). Prevalence of pterygium increases in dry, windy environment and increasing age (Goldberg and David, I976). However, recurrence is more frequent in young adults than older individuals (Lewallen, 1989).

1.3 Pathogenesis of pterygium

Pterygium was previously thought to be a degenerative condition. However recent evidence suggests that pterygium is in fact a proliferative condition that is strongly correlated with exposure to ultraviolet radiation (UVR) (Hill and Maske, 1989).

Recent study found that vascular endothelial growth factor (VEGF) is present in great amount in pterygium epithelium especially in its head compared to normal conjunctiva by studies employing immunohistochemistry, suggesting that VEGF plays an important role in pterygium development.(Lee *et al.,* 2001). Thus, it is postulated that VEGF is involved in angiogenesis and it is produced by corneal fibroblasts in response to local conjunctival inflammation or other noxious stimulus caused by environmental UVR, dryness and dust (Blaudschun *et al.,* 2002).

To investigate further the association between VEGF and pterygium formation, Poenaru Sava *et al.* (2014) found that VEGF gene 460C polymorphism is associated with pterygium formation in female patients. Females who carried the allele which the gene is contained have increased risk of developing pterygium at a younger age.

UVR affects the expression of various cytokines, and growth factors other than VEGF such as basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF) and suppressed level of pigment epithelium-derived factor (PEDF) which also play a role in pterygium formation (Di Girolamo *et al.,* 2004; Kria *et al.,* 1996).

Other proposed pathogenesis are expression of P53 oncogene, viral infection, immunological, environmental, genetic and hereditary factors (Cilova-Atanasova, 1971; Hill and Maske, 1989; Pinkerton *et al.,* 1984; Solomon, 1985; Tan *et al.,* 1997b; Tsironi *et al.,* 2002).

1.4 Surgical treatment of pterygium

The definitive treatment is by surgical removal of the lesion. The first reported surgical treatment of pterygium was more than 3000 years ago. Since then, many variant of this procedure have been published. The most common operative technique at present is conjunctival autograft closure due to its low recurrence of 2.6%-39% (Lewallen, 1989). The transplantation of limbal stem cells from the graft helps regenerate new corneal epithelial cells in addition to inhibiting conjunctival epithelial invasion of the cornea (Tseng, 1989).

The use of fibrin adhesive glue to secure the conjunctival autograft further reduces the recurrence rate, surgical time, and postoperative pain when compared with sutures (Ratnalingam *et al.,* 2010).

1.5 Adjunct to surgical treatment of pterygium

The current treatment of pterygium focuses on prevention of its recurrence (Lekhanont *et al.,* 2012). The main concern for pterygium treatment is the recurrence which can be aggressive with more exuberant fibrovascular growth response (Lekhanont *et al.,* 2012). Many adjunctive modalities have been introduced to prevent recurrence. These include beta irradiation and cytotoxic ankylating agent such as thiotepa and mitomycin C. However, none comes without serious complications and thus not used routinely nowadays (Hosseini *et al.,* 2007).

Based on the high expression of VEGF in pterygium tissue and its role in pterygium angiogenesis, anti-VEGF has been proposed as an adjunct to pterygium surgery.

1.6 Literature review

Hu *et al.* (2014) recently conducted a meta-analysis on randomised controlled trials using bevacizumab in treatment of pterygium. They concluded that topical or subconjunctival bevacizumab is relatively safe and well tolerated in the treatment of pterygium but has no statistically significant effect in pterygium recurrence.

Appendix A presents the recent studies reporting on the use of ranibizumab on pterygium, while appendix B presents the recent studies reporting on the use of bevacizumab on pterygium.

1.7 Rationale of the study

To our knowledge, there have only been 3 published studies on the use of ranibizumab on pterygium (Galor *et al.*, 2010; Hurmeric *et al.*, 2013; Mandalos *et al.*, 2010). However, none of the 3 studies address on the pterygium recurrence rate. This study addressed the gap in the literatures. The outcome will determine the potential use of ranibizumab as an adjunctive modality in pterygium surgery to preventing recurrence. The results will also serve as a platform for further study of ranibizumab uses in pterygium.

2 OBJECTIVES

2.1 General objective

To compare the pterygium vascularity and size before and after intralesional ranibizumab injection and the recurrence rate following sutureless pterygium surgery.

2.2 Specific objectives

1. To compare the vascularity of primary pterygium before and 1 week after intralesional ranibizumab (0.3 mg/0.03 mL) injection.

2. To compare the size of primary pterygium before and 1 week after intralesional ranibizumab (0.3 mg/0.03 mL) injection.

3. To compare the recurrence rate at 1 year after sutureless pterygium surgery between the group with and without intralesional ranibizumab injection.

3 METHODOLOGY

3.1 Study design

Prospective randomised controlled trial.

3.2 Population, time and setting

Study population

All patients who attended eye clinic in HUSM and for primary pterygium excision.

Study duration

September 2012 - September 2014

Study location

Eye clinic, Hospital Universiti Sains Malaysia (HUSM)

3.3 Sampling procedure

Simple random sampling procedure was applied in this study. The study population who met with the inclusion and the exclusion criteria were labelled with a serial number. The study subjects were then selected randomly using a random digit table.

Based on the randomly selected numbers, the patients were then divided into two groups:

- Treatment group (odd numbers): patients with intralesional ranibizumab
- Control group (even numbers): patients without intralesional ranibizumab

3.4 Sample size

Sample size calculation was done by using PS (Power and Sample Size) software. Power was set at 0.8 for each objective.

Sample size calculation for objective 1

Objective 1: To compare the vascularity of primary pterygium before and 1 week after intralesional ranibizumab (0.3 mg/0.03 mL) injection.

Sample size was calculated based on paired t-test. The mean change in corneal vascularisation after 2 bevacizumab injections was -0.1% and standard deviation was 0.19 (Bahar *et al.*, 2008).

Design = paired t-test

Input: $\alpha = 0.05$, power = 0.8, $\delta = 0.1$, $\sigma = 0.19$

Sample size = 30

Sample size calculation for objective 2

Objective 2: To compare the size of primary pterygium before and 1 week after intralesional ranibizumab (0.3 mg/0.03 mL) injection.

Sample size was calculated based on the mean difference in size of vascularised conjunctival bed before and after subconjunctival bevacizumab injection was 8.08 ± 5.5 % (Bayar *et al.*, 2014).

Design = paired t-test

Input: $\alpha = 0.05$, power = 0.8, $\delta = 0.0808$, $\sigma = 0.055$

Sample size = 6

Sample size calculation for objective 3

Objective 3: To compare the recurrence rate at 1 year after sutureless pterygium surgery between the group with and without intralesional ranibizumab injection

Sample size was calculated based on no recurrence in the group treated with subconjunctival bevacizumab versus 9% recurrence rate in the control group (Ozgurhan *et al.*, 2013).

Design = independent, prospective, two proportions, uncorrected Chi-square test

Input: $\alpha = 0.05$, power = 0.8, $p_0 = 0.09$, $p_1 = 0.005$ (estimated recurrence in treatment group), m = 1

Sample size = 97 per arm

Due to the high cost of ranibizumab and our limited financial resources, it is not feasible to recruit such large sample size. Therefore, we were only able to recruited sample size of 18 patients on each arm. With 18 subjects per arm, the post-hoc power for objective 1, 2 and 3 are 55%, 100% and 22% respectively.

For a pilot study, the sample size should be 10% of sample projected for the larger parent study (Connelly, 2008). 18 subjects per arm had met this criterion for all the 3 objectives.

Furthermore, for a study of medical filed, Julious (2005) and Van Belle (2011) suggested 12 subjects.

3.5 Selection criteria

3.5.1 Inclusion criteria

- All patients presenting with primary nasal pterygium who were indicated and agreed for pterygium excision surgery.
- Grade T3 (fleshy) pterygium.

3.5.2 Exclusion criteria

- Patients with temporal or both nasal and temporal pterygium.
- Patients with other ocular surface disorders.
- Patients with glaucoma.
- Previous ocular surgery or trauma.
- History of systemic thromboembolic event.
- Pregnant women and lactating mother.

3.6 Protection of confidentiality

To protect confidentiality of the patients, all photographs were assigned with a serial number. Data analysis was referred to the serial number. Photographs were kept under password protected storage by the main researcher.

3.7 Funding

This research received no grant from any funding agency in the public, commercial or non-profit sector.

3.8 Ethical approval

This study was approved by Human Research Ethics Committee of USM on 31st July 2013 [USM/JEPeM/269.3.(1)].

This study adhered to the tenets of the Declaration of Helsinki.

Informed consent was obtained from all patients after the nature and possible consequences of the study had been explained. All patient recruited were treated with intension to treat. Any care that requires urgent attention or referral was managed accordingly.

3.9 Definition of terminologies

3.9.1 Primary pterygium

Primary pterygium was diagnosed clinically as fibrovascular growth from bulbar conjunctival extending beyond the limbus onto the cornea. It had never been excised previously. Clinical diagnosis was later confirmed with histological examination of the excised tissue.

Pterygium was graded according to simple grading system by Tan *et al.* (1997a).

Grade T1: Atophic pterygium, episcleral vessels unobscured and the least likely to recur.

Grade T2: Intermediate pterygium with episcleral vessels partially obscured.

Grade T3: Fleshy pterygium, episcleral vessels totally obscured and the most likely to recur.

Only grade T3 nasal pterygium was selected for this study.

3.9.2 Ranibizumab

Ranibizumab (Lucentis, Genetech.Inc) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment produced in Escherichia coli cells by recombinant DNA technology.

It is designed for intraocular used in wet age-related macular degeneration, macular oedema following retinal vein occlusion and diabetic macular oedema.

It acts by binding to receptor binding site of active forms of VEGF-A, including the biologically active, cleaved form of this molecules, $VEGF_{100}$.

Its use in pterygium is off-labelled and there is no census on the correct dosage for its injection. Dosage of 0.3 mg/0.03 mL was used in this study.

No previous documentation of half-life of ranibuzumab in pterygium was found.

3.9.3 Job location

Job location was divided into outdoor and indoor. Outdoor job location was defined as 4 hours or more per day during daylight of outdoor job activity.

3.9.4 Corneal pterygium area

Corneal pterygium area is defined as the percentage of pixels occupied by the pterygium within the cornea (Enkvetchakul *et al.*, 2011). Aim is to quantify the pterygium size as a mean to monitoring regression post-ranibizumab injection. The final results were derived from the average results from the both principle researcher (Dr. Ho Hao Chi) who was not masked and co-supervisor (Dr. Khairy Shamel Sonny Teo) who was masked of the treatment choice.

$$\text{Percentage of corneal pterygium area} = \frac{\text{Pterygium surface area (in pixels)}}{\text{Cornea surface area (in pixels)}} \times 100\%$$

3.9.5 Pterygium vessel area

Pterygium vessel area is defined as the percentage of pixels occupied by the vessels within the pterygium (Mandalos *et al.*, 2010). Aim is to quantify the pterygium vascularity as a mean to monitor vessels regression post-ranibizumab injection. The final results were derived from the average results from both the principle researcher who was not masked and the co-supervisor who was masked of the treatment choice.

$$\text{Percentage of pterygium vessels area} = \frac{\text{Pterygium surface area covered by vessels (in pixels)}}{\text{Pterygium surface area (in pixels)}} \times 100\%$$

3.9.6 Recurrent pterygium

Recurrence was observed clinically after 1 year from pterygium excision surgery.

Grading of recurrent pterygium by Prabhasawat *et al.* (1997):

Grade 1: Normal appearance of operative site.

Grade 2: Some fine episcleral vessels, but without any fibrous tissue in the excised area extending up to but not beyond the limbus.

Grade 3: Presence of additional fibrous tissues in the excised area without invading the cornea.

Grade 4: True recurrence with a fibrovascular tissue invading the cornea.

Recurrence in our study is defined as true recurrence or grade 4 lesion. Recurrence was confirmed with agreement by both the principle researcher who was not masked and the supervisor (Assoc. Prof. Dr Mohtar Ibrahim) who was masked of the treatment

3.10 Study procedure

3.10.1 Subjects

All patients attended Ophthalmology Clinic, Hospital Universiti Sains Malaysia from September, 2012 to September, 2014 who fulfilled the selection criteria were offered to participate in this study. Only those who gave written informed consent were recruited. They

were then randomised and divided into 2 groups, the treatment group and the control group. The treatment group received intralesional ranibizumab injection and the control group which did not receive ranibizumab injection. Patients were not masked of their treatment choices because those who did not received intralesional ranibizumab injection were not subjected to injection of placebo.

3.10.2 Image acquisition

Anterior segment photographs were taken using Kowa VX-10 fundus camera adapted with Nikon digital camera D70s. The patients' eyes focused at primary position during image acquisition.

For the treatment group, first anterior segment photographs were taken immediately before injection of intralesional ranibizumab. Second photographs were taken 1 week after the injection, and coinciding with immediately before pterygium excision surgery. Third photographs were taken 1 year after pterygium excision.

For the control group, first anterior segment photographs were taken immediately before pterygium excision surgery. Second photographs were taken 1 year after pterygium excision.

3.10.3 Image analysis

Bock *et al.* (2008) and Tatham *et al.* (2011) had shown that semiautomated threshold analysis of corneal vessels is more accurate and reproducible then manually outlining each corneal vessels.

Most studies on pterygium and cornea vascularity used NIH (National Institutes of Health) image software, Image J (Bahar *et al.*, 2008; Bayar *et al.*, 2014; Fallah *et al.*, 2010; Fallah Tafti *et al.*, 2011). We used Image J 1.48v. It is available for free online download in rsb.info.nih.gov/nih-image/. It consist of a 6-step algorithm improvised from methods previously described: contrast enhancement, cornea and pterygium boundary estimation, grey value image, background subtraction, auto threshold, automatic calculation (Benayoun *et al.*, 2012; Bock *et al.*, 2008; Enkvetchakul *et al.*, 2011).

1. **Contrast enhancement**

Contrast enhancement was made to the image to sharpen image contrast making visible structure more distinguishable. It is done by clicking on "process" then, "enhance contrast". "saturated pixels" value was set at 0.4 as per default. "normalize" *and* "equalize histogram" are not checked so as not to alter the pixel of the images.

2. **Cornea and pterygium boundary estimation**

Using contrast enhanced images, the corneal surface area was manually outlined by "oval selection" tool according to the innermost vessels of the limbal arcade (Bock *et al.*, 2008).

The image was cropped by clicking on “edit” followed by “clear outside”. The cornea with its background removed became the region of interest.

The cropped images of cornea with its “oval selection” tool still present, click “analyse” then click “measure”. The number displayed under “area” is the cornea surface area in pixels. The number was then recorded.

The pterygium border was drawn manually using “freehand selection” tool. Then, click “analyze” then click “measure”. The number displayed under “area” was recorded as the pterygium area in pixels. The number was recorded. The image of outlined pterygium was then cropped by clicking “edit”, then “clear outside”.

3. Grey value image

The contrast enhanced images that has yet to be cropped were converted to 8-bits grey value images by clicking “image”, then “type”, then “8-bit”. This marked the darkest and the lightest pixel into black and white relatively and other pixels linearly in between. Each pixel can have a value of 0 (black) to 255 (white). The vessels will stand out as being darker than their background.

4. Background subtraction

Using the grey value images, background filtering was done by clicking “process”, then “subtract background”. “Rolling ball radius” was set at 50.0 pixels and the “light background” option was ticked as per default. The outcome was an image that has background filtered to reduce false positive and false negative.

5. Auto threshold

Thresholding of images were done to highlight vessels automatically. It was done on 8-bits greyscaled images which background had been lightened. Only greyscaled images can be thresholded. The algorithm to thresholding the images were to click on “image”, “adjust”, and then “auto threshold”. “Default” method was chosen.

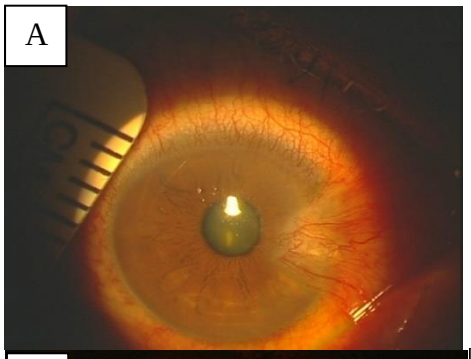
6. Automatic calculation

To select the area of pterygium vessels, open both the cropped pterygium image and the thesholded images were opened. Then, “process” followed by “image calculator” were clicked. Both images were selected as “image 1” and “image 2”. The “operation: OR” were selected, then “OK” was clicked.

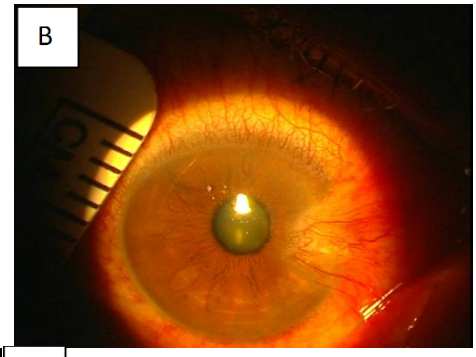
Because structure in the grey value images cannot be selected automatically, the images were converted to RGB images again by clicking “image”, “type”, and “RGB color”.

With the RGB coloured images, threshold visible vessels were selected automatically by first clicking on “image”, “adjust”, then “threshold”. Secondly, choose “threshold color: red”, then adjust the threshold bar until all the vessels turn colour from grey to red. Thirdly, “select” button was clicked followed by “analyze”, and finally “measure”. The number displayed under “area” is the pterygium vessel area in pixels.

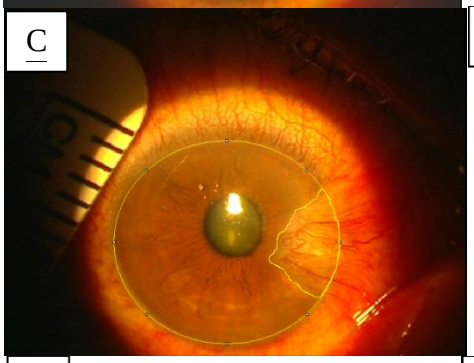
A



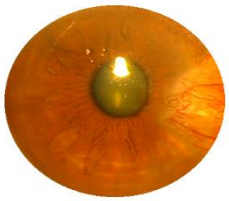
B



C

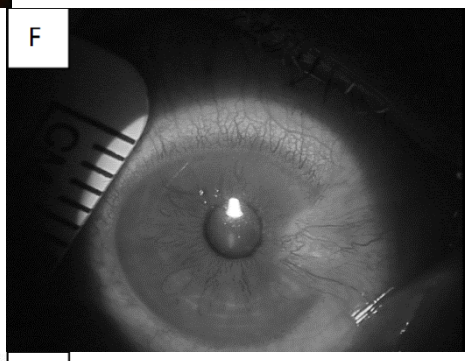


D

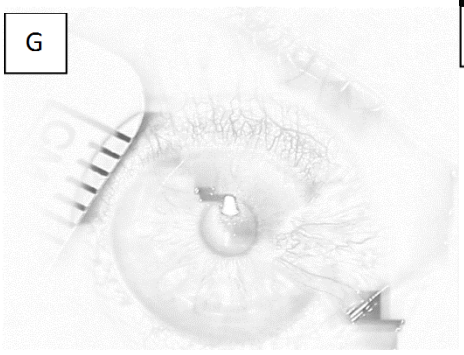


E

F



G



H

I

J

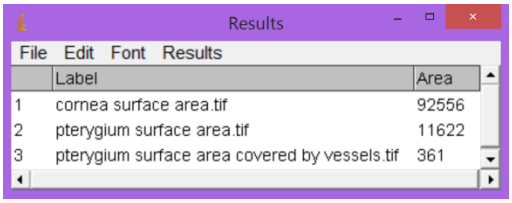

Results
File Edit Font Results
Label Area
1 cornea surface area.tif 92556
2 pterygium surface area.tif 11622
3 pterygium surface area covered by vessels.tif 361

Figure 3.1 Sequential image analysis. (A) Original anterior segment image. (B) Contrast enhanced image; (C) cornea limbus and pterygium outlined; (D) cornea with background removed; (E) pterygium with background removed; (F) 8-bits grey value image; (G) background subtracted image; (H) threshold image; (I) calculated image of cropped pterygium image and threshold image; (J) automated calculation of cornea, pterygium and pterygium surface vessels area in pixels.

3.10.4 Intralesional ranibizumab injection

Patients who were under intralesional ranibizumab group were treated as follows.

Patients' eyes were anaesthetised with topical proparacaine 0.5%. The eyes were then clean, draped and speculum applied. Topical povidone 5% was then applied to the cul-de-sac for 3 minutes and flushed away. Intralesional ranibizumab 0.3 mg/0.03 mL was then injected into the body of the pterygium along the limbus.

Patients were allowed home with topical chloramphenicol 0.5% 6 hourly for 1 week. Patients were then scheduled for pterygium excision in 1 week.

3.10.5 Surgical procedure

The pterygium excision surgery was done under topical anaesthesia using proparacaine 0.5%. Topical phenylephrine 2.5% was also instilled to reduce conjunctival bleeding.

Surgical area was then cleaned, draped and lid speculum inserted. Topical povidone 5% applied to the cul-de-sac for 3 minutes and then flushed away. Intralesional lignocaine 1% was injected into the body of pterygium.

The pterygium was then excised from the apex to the base using Took knife and Wescott scissors. The demarcation line of the pterygium intended to be excised was crushed using artery forceps to reduce bleeding. Once the pterygium had been excised, residual

fibrovascular tissue was scrapped off using Took knife. Haemostasis was achieved by pressure applied to bleeding site using cotton tip bud.

The bare sclera was then covered with free conjunctival autograft that was harvested from superior bulbar conjunctiva after carefully separating it from underlying Tenon capsule. Size of the graft overlaps the edges of defect by 1mm horizontally and vertically.

Conjunctival graft was then placed onto the bare sclera with fibrin adhesive glue (Tisseel VH; Baxter Healthcare, Corp). Care was taken to maintain proper orientation of limbus edge towards limbus.

The excised pterygium from both groups was labelled and the specimens were transported to pathology laboratory in 10% normal buffered formalin for examination under light microscopy for histological assessment.

3.10.6 Post-operative follow-up

Patients of both groups received both topical chloramphenicol 0.5% and topical prednisolone forte 1% each 2 hourly for 1 week then tapered down to 6 hourly for 1 month, then the medications were taken off thereafter.

Patients of both groups were followed-up for 1 year postoperatively. Anterior segment photographs were taken at 1 year postoperation. On follow-ups, patients were assessed for

recurrence and complications such as graft dehiscence, conjunctival cyst and tenon granuloma.

3.11 Method to minimize errors

Study subjects were randomised and selected according to inclusion and exclusion criteria. Image acquisition, ranibizumab injection and pterygium excision were carried out by the principle investigator. Image analysis was performed by 2 independent observers.

3.12 Statistical analysis

The acquired data was entered into data collection sheet and subsequent analysis performed using latest IBM SPSS Statistics version 22. A p-value of <0.05 was considered to be statistically significant.

All numerical data was checked for normality by using Shapiro-Wilk test and histogram. Normally distributed data was analysed with parametric test. Independent t-test was used to compare numerical data between 2 groups of independent samples and paired sample t-test to compare numerical data pre and post-intervention of the same sample. Skewed data was analysed using non-parametric test (Mann-Whitney U).

To compare categorical data between the 2 groups, Pearson Chi-square was used. Fisher's Exact test was used when equal or more than 20% of the cells has expected frequency of less than 5.

4 RESULTS

4.1 Demographic data

Table 4.1 Demographic characteristics of patients between the treatment and control group.

	Treatment Group (n = 18)	**Control Group (n = 18)**	**p value**
Age (year)			
Median (IQR)	55.0 (22.3)	57.5 (19.8)	0.350*
Age group, n (%)			
≤55	10 (55.6)	7 (38.9)	0.317#
>55	8 (44.4)	11 (61.1)	
Sex, n (%)			
Male	13 (72.2)	11 (61.1)	0.480#
Female	5 (27.8)	7 (38.9)	
Race, n (%)			
Malay	16 (88.9)	16 (88.9)	> 0.950##
Chinese	2 (11.1)	2 (11.1)	
Job location, n (%)			
Outdoor	6 (33.3)	6 (33.3)	> 0.950#
Indoor	12 (66.7)	12 (66.7)	

* Mann-Whitney U
# Pearson Chi-square test
Fisher's Exact Test
IQR = interquartile range
p value < 0.05 is considered significant

This study was conducted on 36 eyes of 36 patients, 18 on each group. There was no statistically significant difference between the groups in term of age, age group, sex, race and

job location (p = 0.350, 0.317, 0.480, > 0.950 and > 0.950 respectively). Mann-Whitney U, Chi-square and Fisher's exact test were used to analyse the numerical and categorical data.

The median age of patients in this study was 57.00 years with interquartile range of 23.50. Their age ranged from 40 to 77-year-old.

4.2 Pterygium vessel area and corneal pterygium area before and after intralesional ranibizumab injection

Table 4.2 Comparing pterygium vessel area and corneal pterygium area before and 1 week after intralesional ranibizumab injection in the treatment group.

	Before	**After**	**Difference**	**p value**
Pterygium vessel area (%) Mean (SD)	15.33 (4.92)	13.85 (5.79)	1.48 (4.65)	0.195*
Corneal pterygium area (%) Mean (SD)	17.39 (14.16)	17.12 (13.61)	0.28 (2.71)	0.672*

* Paired sample t-test
SD = standard deviation
p value < 0.05 is considered significant

The mean pterygium vessel area before and after ranibizumab injection were 15.33 $\pm$ 4.92% and 13.85 $\pm$ 5.79%, respectively The reduction in pterygium vessel area after ranibizumab injection was 1.48 $\pm$ 4.65%. The difference was statistically insignificant (p = 0.195).