# THE EFFECTS OF TOPICAL ANTIGLAUCOMA DRUGS ON THE CONJUNCTIVAL CELL PROFILE


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I hereby certify that the work in this dissertation is my own except for the quotations and summaries which have been duly acknowledged.

Dated 30.10.2001

Liza Sharmini bt. Ahmad Tajudin

P-UM0352

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dengan kontrol. Sel polimof, sel masts, fibroblast dan sel goblet di dapati berkurangan pada konjunktiva yang terdedah kepada ubat topikal antiglukoma berbanding dengan kontrol. Tiada sebarang perubahan yang signifikan secara statistik terhadap sel profil konjunktiva pada yang terdedah hanya satu jenis ubat topikal antiglaukoma berbanding dengan yang menerima lebih dari satu. Begitu juga tiada perubahan yang signifikan bagi yang menerima rawatan kurang dari 12 bulan berbanding dengan yang melebihi atau sama dengan 12 bulan. Purata jangkamasa rawatan dengan ubat topikal antiglaukoma adalah selama 22.82 ± 16.12 bulan (1.9 tahun). Bilangan sel plasma didapati meningkat secara signifikan pada konjunktiva yang menerima ubat topikal samada timolol sahaja atau berkombinasi dengan pilocarpine berbanding dengan kontrol.

Walaupun dibatasi oleh saiz sampel yang kecil, terdapat bukti kesan awal yang menunjukan kehadiran inflammasi kronik secara subklinikal ke atas konjunktiva yang menerima ubat topikal antiglaukoma selama 1.9 tahun. Rawatan dengan ubat topikal antiglaukoma selama 12 bulan dianggap selamat tanpa menyebabkan sebarang perubahan signifikan kepada sel profil konjunktiva. Ubat topikal timolol sahaja atau berkombinasi dengan pilocarpine hanya menyebabkan perubahan minimal terhadap sel profil konjunktiva.

**ABSTRACT**

Topical antiglaucoma drugs were implicated to cause subclinical chronic inflammation, which was believed to be responsible in inducing excessive scarring of the filtering bleb. Our aim is to investigate the effects of topical antiglaucoma drugs on conjunctival cell profile when compared to eye not expose to the drugs. The effects of duration of treatment, type of drugs and number of topical antiglaucoma drugs on conjunctival cell profile were also evaluated.

Histological analysis were done on twenty-two conjunctiva biopsies, which were obtained from twenty-two eyes and divided into study and control group. The study group comprised of eleven biopsies, which were obtained from glaucomatous eye that was exposed to topical antiglaucoma drugs for at least three months. According to the number of medication, the study group was further divided into single treatment group (Group B) and multiple treatment group (Group C). Based on duration of treatment, the study group was divided into less than 12 months (Group I) and more or equal to 12 months (Group II). Based on type of medication, the study group was further divided into timolol only group and combination timolol and pilocarpine group. The control group (Group A) consists of eleven age-matched biopsies, which were obtained during cataract surgery. Quantitative histological analysis of goblet cells, inflammatory cells, masts cells and fibroblasts was done under light microscope.

There were statistically significant increased in lymphocytes and plasma cells count in conjunctiva exposed to topical antiglaucoma drugs compared to control. Polymorphs, masts cells, fibroblasts and goblet cells count were reduced in the study group compared to control. There were no statistically significant changes in conjunctiva cell profile

between single and multiple treatment groups. Similarly, there were no statistically significant changes in conjunctival cell profile between those exposed to the drugs more or equal to 12 months and those less than 12 months. Mean duration of exposure to the drugs was 22.82 ± 16.12 months (1.9 year). Plasma cells were significantly increased in conjunctiva exposed to either timolol only or in combination with pilocarpine compared to control.

Although hampered by small sample size, there was early evidence of subclinical chronic inflammation in conjunctiva exposed to topical antiglaucoma drugs for at least 1.9 years. The minimum use of topical antiglaucoma drug up to 12 months was considered safe without any significant changes in conjunctival cell profile. Timolol only or in combination with pilocarpine cause minimal changes in conjunctival cell profile.

# 1. INTRODUCTION

Conjunctiva is a delicate thin translucent mucous membrane that covers the posterior surface of the eyelids. It is reflected at the superior and inferior fornices onto the anterior surface of the eye. Conjunctiva acts as a passive semipermeable barrier, allowing the entry of topical antiglaucoma drugs and therefore exposed to the side effect of these drugs.

Similarly, conjunctiva is also the most delicate and precious structure during filtration surgery. The aim of trabeculectomy surgery is to achieve reduction of intraocular pressure by creating a new channel for aqeous outflow between the anterior chamber and the subtenon space. Conjunctiva bleb is created between the subtenon space and bulbar conjunctiva. The success of filtration surgery therefore depends on the state of the conjunctiva, thus implying the importance of the conjunctiva in both medical and surgical treatment.

Histologically, conjunctiva comprises of epithelium and substantia propria. The conjunctiva epithelium varies according to the anatomical site; ranging from stratified squamous non-keratinazing epithelium to stratified columnar epithelium. Goblet cells are found scattered throughout the conjunctiva and most numerous inferonasally. These mucous secreting cells are only found in the epithelium layer. The amount of goblet cells varies according to the site of biopsy. Though they are more numerous in substantia propia layer, neutrophils and lymphocytes are also found in the epithelium.

Immunocompetent cells are usually found crowded against the epithelium, about 0.5mm into the stroma. The stroma or substantia propria layer comprises of connective tissue containing blood vessels, nerves, glands and immunocompetent cells ready to protect

the conjunctiva. Lymphocytes contribute 70% of total immunocompetent cells (100,000/$mm^3$) (Allansmith MR et al, 1978). The average number of plasma cells is 45,000/$mm^3$ in normal conjunctiva. Lymphocytes and plasma cells play an important role in normal defense mechanism. Mast cells (5,000/$mm^3$) and neutrophils (2,000/$mm^3$) are also found in this layer. Macrophages are not found in normal conjunctiva unless there is an acute or chronic inflammation. The number of immunocompetent cells in this protective layer varies with the presence of insult.

Fibroblasts, which act as 'packing' material, are also found abundantly in the stroma. Fibroblasts have the ability to regenerate and multiply in numbers. Stimulation of other inflammatory cells especially macrophages and activated lymphocytes promote proliferation of fibroblast in wound healing process. Proliferation of fibroblast may leads to excessive subconjunctiva scarring. Exaggeration of healing process of the conjunctiva in trabeculectomy surgery is believed to be responsible in failure of filtering bleb.

Topical antiglaucoma drugs are believed to cause subclinical inflammation of conjunctiva (Sherwood MB et al, 1989, Broadway DC et al, 1994). Histological study on conjunctival cell profile showed a significantly increased in numbers of lymphocytes, neutrophils, plasma cells, macrophages, mast cells and fibroblast, and also a reduction of goblet cells in conjunctiva exposed to long-term topical antiglaucoma drugs.

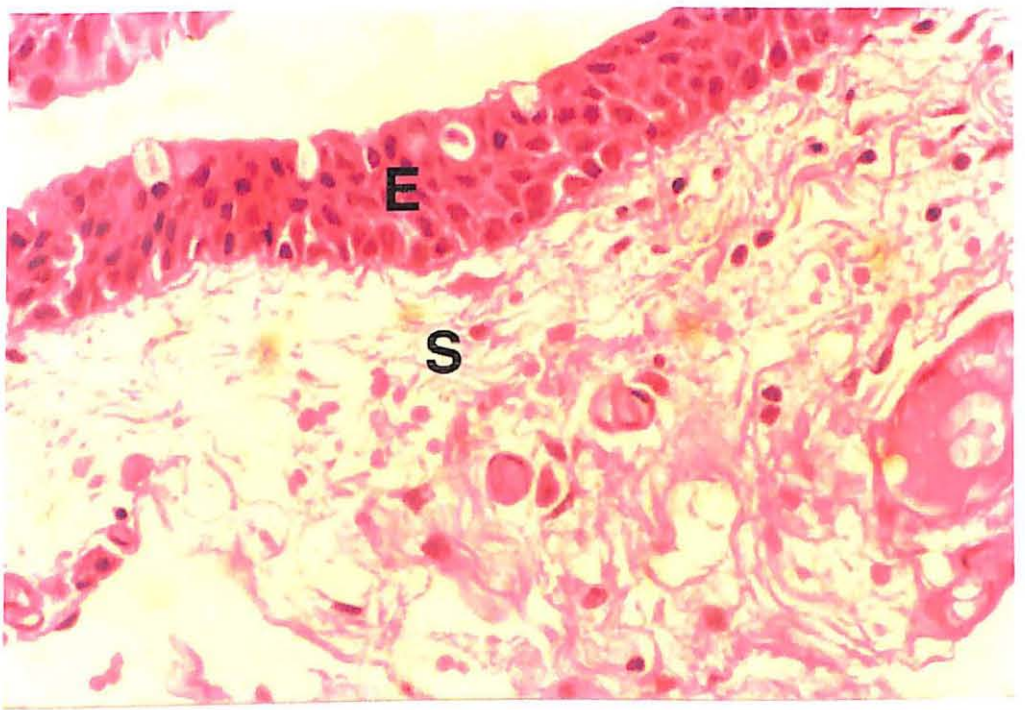


Figure 1: The conjunctiva showing both the epithelium (E) and substantia propria (S).

(400X; Haematoxylin and eosin staining)

## 1.1. OBJECTIVES

### 1.1.1 General objectives

To investigate the effects of topical antiglaucoma drugs on conjunctival cell profile when compared to eye not expose to topical antiglaucoma treatment.

### 1.1.2 Specific objectives

i. To compare the difference in conjunctival cell profile between eye exposed to topical antiglaucoma and eyes not exposed to the topical antiglaucoma drug.

ii. To study the difference in conjunctival cell profile between eyes exposed to single topical antiglaucoma drug to those exposed to multiple topical antiglaucoma drugs.

iii. To study the difference in conjunctival cell profile between eyes exposed to topical antiglaucoma drugs for less than 12 months to those exposed for more than 12 months.

iv. To compare the difference in conjunctival cell profile according to type of topical antiglaucoma drugs.

# 2. BACKGROUND

Glaucoma is defined as an optic neuropathy characterized by a specific pattern of optic nerve head damage and visual field defect, which represents a final common pathway resulting from a number of different conditions, which can affects the eye (Ritch R, 1998). An elevated intraocular pressure (IOP) is the most important risk factor for the development or the progression of the glaucomatous damage; it is still only a risk factor but not a disease itself. Approximately 1-2 % of the population was affected and estimated 50 millions people worldwide have glaucoma (Shields MB et al, 1996). Quigley HA (1996) estimated by the year 2000, 66.8 million people were diagnosed with primary glaucoma. Glaucoma is the second leading cause of vision loss in the world. In Malaysia, glaucoma is the fourth major cause of blindness (Selvarajah S, 1992), following cataract, trauma and infection.

The management of glaucoma presents a great challenge to the ophthalmologist. The main aim is to improve the quality of life and to retard the progress of optic nerve damage. Even though medical treatment with antiglaucoma drugs remains the first choice of treatment modality, early surgical intervention has been a subject of interest for years (Smith RJH, 1972), (Migdal C and Hitchings R, 1986). Jay JL and Murray SB (1988) found that there was higher risk in delaying surgical intervention in primary open angle glaucoma. They found that early surgical intervention provides better control of intraocular pressure though there were no significant different in visual acuity outcome.

Medical treatment comprised of topical, oral and intravenous medications. Topical medications were able to provide intraocular pressure control directly to the eye, minimizing other systemic complications compared to oral or intravenous medications.

Topical antiglaucoma medications such as β-antagonist, miotics, carbonic anhydrase inhibitor, adrenergic and the latest prostaglandin F2α analogue, Xalatan, were not only costly in a long run but also accompanied with side effects. Both systemic and local side effects may later affect patient's compliance (Sherwood MB and Spaeth GI, 1990). Poor compliance may leads to poor control and other modalities of treatment might be needed.

Current postulation (Sherwood MB et al, 1989) (Broadway DC et al, 1994 a) (Ariturk N et al, 1995) believed that long-term treatment with topical antiglaucoma drug induced some degree of changes in conjunctiva cell profile due to subclinical inflammation. There was also evidence of increased proliferation of fibroblast, which may stimulate exaggerated healing respond. The success of filtrating surgery in general and trabeculectomy in particular depends on incomplete wound healing in order to maintain the outflow of aqueous through subtenon space.

Lavin MJ et al (1990) studied the influence of the topical antiglaucoma medications on the success of trabeculectomy surgery. He found that earlier and higher number of failure in patient receiving topical antiglaucoma medications compared to primary surgically treated group. Although he did not eliminate several confounding factors such as the initial intraocular pressure and visual field, duration of the disease and surgeon factor, his finding provide the link between the effect of topical antiglaucoma medication and the success rate of trabeculectomy.

Broadway DC et al (1994) correlated his histological finding of the conjunctiva biopsies with the success rate of surgery either primary trabeculectomy or triple procedure.

Although he managed to obtain 124 conjunctiva biopsies, only 106 patients were available for follow-up up to a minimum period of 6 months. The success of surgical treatment was defined as intraocular pressure of 21 mmHg or less without any supplement medication. Lower success rate (45%) was observed in those patients exposed to more number of medications. The duration of treatment was also noted to be longer in those who received greater number of topical medication. Trabeculectomy success rate was similar between patients who received minimal treatment and those treated with topical β-blocker alone. Linking to his previous study on conjunctiva cell profile, he concluded that the preoperative subclinical inflammation induced by previous topical medication was identified as a risk factor for failure of trabeculectomy.

However, Johnson DH et al (1994) in a retrospective study found that preoperative chronic topical antiglaucoma medication did not influence the outcome of filtration surgery. He studied the filtrating surgery success in three eras without β-blocker topical medication, β-blocker era and Argon laser trabeculoplasty. There was no statistically significant difference in term of control of intraocular pressure in those three eras. The success of filtrating surgery was defined as intraocular pressure less than 21 mmHg or 20% reduction from preoperative pressure.

The effect of topical antiglaucoma drugs on conjunctiva can manifest clinically or only with histological changes without any clinical sign. Broadway DC et al (1993) divided the effect to a spectrum of conjunctiva reaction, ranging from total tolerance to severe clinical disease. Drug induced pseudopemphigoid is at the end of the spectrum with obvious clinical sign and symptoms. This severe conjunctiva reaction was reported in long term used of adrenaline, pilocarpine, echothiopate iodide and recently β-locker

(Fiore PM et al, 1987). Allergic reaction is another example, which usually due to the preservative rather than the drug itself. Incidence of follicular conjunctivitis has also been reported.

Severe reaction was usually an exception rather than the rule. Subtle clinical changes, without meticulous examination usually passed unknowingly. Wright P (1997) had described subtle squamous metaplasia of the conjunctiva with clinical evidence of reduction in epithelial wetting and increased conjunctiva keratinization. Histologically there was reduction of goblet cells, epithelial keratinization, and keratohyaline granule formation, subepithelial infiltration with lymphocytes and plasma cells and increased number of fibroblasts with some degree of fibrosis. This reaction may help to explain the pathology of inferior forniceal shortening observed in prolonged sympathomimetics treatment. Schwab IR et al (1992) observed similar shortening of the inferior fornix on exposure to any topical antiglaucoma drugs for at least three years. The incidence increased with increasing age.

Sherwood MB et al (1989) found that there was increased number of macrophages, lymphocytes, mast cells and fibroblasts in both conjunctiva and Tenon's capsule and significant decreased of epithelial goblet cells in conjunctiva biopsies exposed to long term multiple topical antiglaucoma treatments. His finding illustrated the presence of subclinical inflammation that may be responsible in subconjunctival fibrosis. This finding gave an important evidence of effect of topical antiglaucoma drug, which was only a postulation earlier. However his study only involved 40 biopsies and he did not specify the medication involved.

Broadway DC et al (1994) did a similar study on a larger sample involving 124 biopsies. He found similar evidence of subclinical inflammation in the conjunctiva exposed to topical antiglaucoma drugs for more than 3 years regardless of the drug types. He also found that the duration of treatment was longer in those exposed to multiple treatments. He also studied on the accumulative effect of β-blocker, miotics and sympathomimetics on conjunctival cell profile. Topical β-blockers alone appeared to have minimal effect on the cell profile. Triple therapy, combination of β-blocker, miotics and sympathomimetics had shown the greatest effect. By indirect comparison, sympathomimetics alone would probably exert the greatest adverse effect on conjunctiva.

Ariturk N et al (1996) also supported the evidence of subclinical inflammation of the conjunctiva induced by chronic topical antiglaucoma medications though in a smaller scale. However Baun O et al (1995) disagreed with the findings and attributed his finding to less traumatic technique in obtaining the biopsies. He used a special forceps and a tweezers in obtaining the biopsies. He postulated that trauma induced during cutting and transporting the conjunctiva might cause an increased in the inflammatory cells. However his study was limited to a small sample and the duration of treatment was shorter. Gywnn DR et al (1993) in her study found that there were no significant changes in conjunctival cell profile between eyes with failed trabeculectomies and those that were not.

However, there were suggestions that only certain drug was responsible in inducing the effect. Feldman RM et al (1989) found that preoperative treatment of sympathomimetics before trabeculectomy had increased risk of developing Tenon's capsule cyst. The development of Tenon's capsule cyst was not always accompanied with failure of trabeculectomy. However higher intraocular pressure was noted two weeks postoperatively in patients with Tenon's capsule cyst. Histologically the functional bleb showed loosely arranged subepithelial connective tissue with histological clear space (Addicks EM et al, 1983). Broadway DC et al (1994) also implicate sympathomimetics to cause the reaction.

Increased expression of inflammatory markers such as HLA-DR antigen and IgE CD23 receptor were also observed on conjunctiva exposed to topical antiglaucoma drugs (Boudouin C et al, 1994) (Ihan A and Cvenkel B, 2000). The immunocytologic test was conducted on impression cytology specimen. Impression cytology was non-invasive procedure and easy to perform. The only drawback was the impression cytology only detected changes in conjunctiva surface without involvement of the fibroblast in the deeper layer. Exaggerated proliferation of fibroblast was believed to be the most common cause of filtering bleb failure.

Benzalkonium chloride, the commonest and the most studied preservative was also postulated to actually cause the toxic effect rather than the drug itself. This evidence was derived from animal studies (Becquet F et al, 1998) (Baudouin C et al, 1999) and in vitro model of culture conjunctiva cells (De Saints Jean M et al, 2000). Baudouin C et al (1999) did a complementary study on the effect of the preservative in both human and animal conjunctiva. Both conjunctivas demonstrate the similar exaggerated expression

of inflammatory and fibroblasts markers. The similarity helps to abolish the differences between human and animal conjunctiva reaction towards the preservatives. However, he did not standardize the duration of treatment in both groups; human conjunctiva was exposed longer to the drug compared to the animal conjunctiva.

Generally, in vitro experimentation study was not the representative of natural in vivo state. The exposure of the human culture conjunctiva to the drugs was longer and natural replacement of damaged tissue during the intervals between drug instillation was not observed in the in vitro study. Although there were many pitfalls in the experimental studies nonetheless it still provides ethically acceptable and useful information regarding the effect of preservative and the topical drug itself.

Takahashi N (1983) and William DE et al (1992) studied on the effect β-blocker with and without preservative (benzalkonium chloride) on culture of human conjunctiva. Takahashi N (1983) found that benzalkonium chloride was cytotoxic at concentration above 0.005% on culture of human conjunctiva. Timolol (0.5%) and Pilocarpine (1%) alone were not cytotoxic. However his studies confined to the changes of culture human Tenon's capsule fibroblast. The preservative was found to be responsible in stimulation of fibroblast proliferation compared to the drug itself.

There were evidences suggesting changes in conjunctival cell profile exposed to topical antiglaucoma drug caused by both drugs and preservative. However, there was no definitive evidence, which were more responsible. The accumulation effects of multiple drugs and longer duration of exposure might create greater amount of subclinical inflammation and higher risk of trabeculectomy failure.

Reversal of topical antiglaucoma prior to filtration surgery (Broadway DC et al, 1996) was found to increase the success rate of surgery. However, the study involved cessation of sympathomimetics alone in those receiving either sympathomimetics alone or in combination with pilocarpine and β-blocker. Another component was the commencement of topical 1% fluorometholone. There was marked reduction of lymphocytes and pale cells observed from conjunctival cell profile after reversal therapy. However, it was impossible to determine, which component actually responsible in the conjunctiva cell profile changes.

Lately, the used of antimetabolites such as mitomycin C and 5-Fluoroucacil intraoperatively and postoperatively was found to increase and maintain the success of trabeculetomy (Bell RW et al, 1997) (Yaldo MK and Stamper RL, 1993). Late failure was observed after long-term follow-up of initial successful trabeculectomy (Wilensky JT and Chen TC, 1996) had initiated the used of antimetabolites. Fifteen years post trabeculectomy the success rate dropped down to 42% compared to 83% in the first five years. The antimetabolites basically retard the wound healing process and successfully maintain the function of filtering bleb longer.

Apart from the effect of topical antiglaucoma drugs, there were other factors identified to be associated with lower success rate. Younger age group (less than 50 years old), higher preoperative intraocular pressure, previous ocular surgery, previous failed trabeculectomy surgery and secondary glaucoma especially neovascular, uveitic and aphakic glaucoma were found to reduce the success rate of surgery (Borisuth NS et al, 1999). Racial factor was also important to determine the success of trabeculectomy.

Although inconclusive, the success rate of trabeculectomy among Blacks especially American Blacks (Miller RD and Barber JC, 1981) found to be lower compared to Caucasian.

Primary open angle glaucoma in Black patients was found to be more prevalent, more aggressive, earlier in onset and more resistance to medical and laser therapy (Cowan CL et al, 1988). Earlier onset of glaucoma among Blacks commonly necessitated the needs of more than one topical antiglaucoma drug in longer duration before surgical intervention. Trabeculectomy usually performed in patient with high preoperative pressure and severe optic nerve damaged, which may contribute to the poorer outcomes. Furthermore the Tenon's capsule in Blacks was thicker (Mc Millan TA et al, 1992) and higher incidence of keloid formation in skin wound healing indicate innate exaggerated wound healing process.

Recently, there were attempts to establish the difference in conjunctiva cell profile between Blacks and Caucasian. The results were still inconclusive. Broadway DC et al (1994) postulated that the higher number of macrophages in the conjunctiva of Black patient was responsible for triggering an excessive wound healing process. However McMillan TA et al (1992) totally disagreed with the finding. He found that there was increased number of mast cells and neutrophils count in White patients. He concluded that there was no significant different in conjunctival cell profile between Blacks and White.

The success rate of primary trabeculectomy among Asian population mainly Chinese was believed to be between the success rate of Caucasian and Blacks (Wong JS et al,

1998). He reported 36.4% success rate without antimetabolites and 65.8% with antimetabolites at 3 years post trabeculectomy. Miller RD and Barber JC (1980) reported lower success rate of trabeculectomy (31.9%) among Black population after up to 8 years follow up. While success rate in Caucasian was reported up to 98% (Migdal CS and Hitchings RA, 1991). Tan C et al (1996) report an overall success rate trabeculectomy of 43.1% and 48.7% in primary glaucoma. However there was no local study done to correlate the effects of topical antiglaucoma medications with the success rate of trabeculectomy. Similarly there was no local evidence of conjunctiva changes related to topical antiglaucoma drug, which may help to explain the lower success rate compared to Caucasian.

# 3. MATERIALS AND METHODS

## 3.1 RESEARCH STRATEGY

Type of study: Clinical case study

## 3.2 POPULATIONS, SETTING AND TIME

Study population: Patients seen in Ophthalmology clinic HUSM and listed for cataract operation, triple procedure or primary trabeculectomy surgery.

Period of study : NOVEMBER 1998 to SEPTEMBER 2000

Place of study : Ophthalmology Clinic, Hospital Universiti Sains Malaysia

Histopathology Laboratory, Hospital Universiti Sains Malaysia.

## 3.3 ETHICAL APPROVAL

The study received approval from the Ethical Board of School of Medical Science, Universiti Sains Malaysia (Reference number: USM/PPSP/P9/d/95.jld. V1).

## 3.4 SAMPLING AND SAMPLE SIZE

### 3.4.1 SAMPLING PROCEDURE

Stratified sampling

### 3.4.2 SAMPLE SIZE

Sample size was estimated by single proportion formula $\eta = (1.96/\Delta)^2 \rho (1-\rho)$

$\Delta = 0.1$ (confidence interval 90 %)

$\rho = 0.1$(percentage of glaucoma patient on medical treatment)

| | |
|---|---|
| Biopsies from cataract patients (control group) | 11 |
| Biopsies from glaucoma patients (study group) | 11 |
| Total numbers of conjunctiva biopsies | 22 |
| Total numbers of eyes | 22 |
| Minimum estimated sample size | 35 |

3.4.3 PLANS FOR MINIMISING ERROR

i. Patients were selected according to the prescribed selection criteria.

ii. A trial period of one month was given to determine the minimum adequate size of biopsy and proper technique of obtaining the biopsy. Two conjunctiva biopsies were obtained during cataract operation that was later excluded from the study.

iii. A short training period of one month under pathologist guidance to ensure the accuracy in identifying inflammatory cells and other abnormal cells in the conjunctiva especially mast cells, macrophages and fibroblast.

iv. The histological analysis of all the biopsies were repeated by the pathologist in randomize pattern.

## 3.5 SELECTION CRITERIA

### 3.5.1 CONTROL GROUP (GROUP A)

#### 3.5.1.1 Inclusion criteria

i. Patients who were planned for cataract surgery.

ii. Age-matched with the study group.

iii. Patients must be more than 40 years old and less or equal to 80 years old.

iv. Consented patient. A written consent must be obtained from the patient.

3.5.1.2 Exclusion criteria

i. Patients with ocular problem other than cataract.

ii. Patients with systemic problem associated with ocular manifestation involving conjunctiva. For example pemphigoid, sarcoidosis, systemic lupus etc.

iii. Patients with previous history of administration of topical eye drops for more than 6 months duration.

iv. Patients with previous ocular surgery in the same eye.

3.5.2 STUDY GROUP (GROUP B and C)

3.5.2.1 Inclusion criteria

i. A confirmed case of glaucoma based on clinical examination, gonioscopic findings, visual field changes and elevated intraocular pressure.

ii. Glaucomatous patient receiving topical antiglaucoma drugs for a minimum period of 3 months.

iii. Patient who was planned for primary trabeculectomy or triple procedure.

iv. Patient must be more than 40 years old but less or equal to 80 years old.

v. Consented patient. A written consent must be obtained from the patient.

3.5.2.2 Exclusion criteria

i. Glaucomatous patient with history of acute attack of glaucoma. Patients with history of pain either eye pain or headache and eye redness associated with reduction of vision in chronic angle closure were excluded. Patient with clinical sign such as iris atrophy that may suggest an acute attack was also excluded.

ii. Neovascular glaucoma, uveitic glaucoma, aphakic glaucoma and secondary glaucoma associated with systemic disease.

iii. Glaucomatous patient who was not expose to any topical antiglaucoma drug.

iv. Previous ocular surgery on the same eye.

3.5.2.3 THE NUMBER OF TOPICAL ANTIGLAUCOMA DRUGS

i. Patients who were subjected to only one topical antiglaucoma drug for minimum three months duration were considered under single treatment group (GROUP B).

ii. Patients who were subjected to more than one topical antiglaucoma drug for at least three months were considered under multitreatment group (GROUP C).

iii. An additional topical antiglaucoma drug must be applied for more than three months to be considered under multitreatment group.

iv. Any change in type of topical antiglaucoma drug within the same group with similar action is considered as a single treatment. For example, Betoptic (cardioselective β-blocker) replaced Timolol (β-blocker) in cardiovascular problem.

3.5.2.4 THE DURATION OF TOPICAL ANTIGLAUCOMA DRUGS TREATMENT

i. The duration of treatment was counted from the first month of treatment till the month of surgery.

ii. If the duration of treatment was less than 12 months, it was considered to be in-group I.

iii. If the duration of treatment was equal or more than 12 months, it was considered to be in group II.

## 3.6 INSTRUMENT

### 3.6.1. Light microscope

Light microscope Leica DMR under 40X objective lens was used in histological analysis.

### 3.6.2. Computer and CCD camera

The light microscope was connected to the computer PC Leica Q 500IW. A special program, Leica Q Win was installed for the histological analysis. A camera, Leica MPS 60 was also attached to the microscope and connected to the computer.

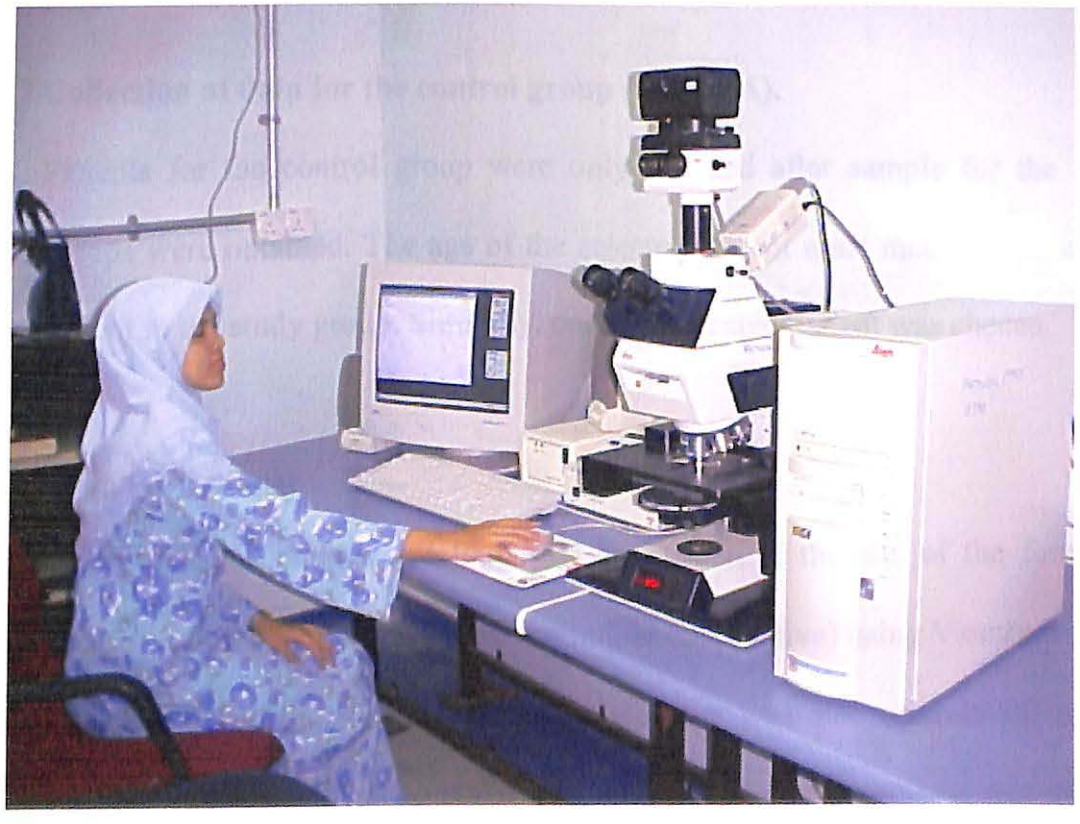

Figure 2: The light microscope is attached to the computer and CCD camera.

## 3.7 METHODS

### 3.7.1 Data collection

#### 3.7.1.1 Collection of data for the study group (Group B and C).

Patients were chosen during preoperative assessment. A thorough ocular and systemic examination was done on patient who fulfilled the selection criteria. Ocular examination comprised of slitlamp examination, funduscopic and gonioscopic examination as well as visual field evaluation. Patients with chronic angle closure glaucoma were only chosen if by direct questioning and based on clinical record not suggestive of any previous acute attack. Clinical sign suggestive of previous acute attack such as iris atrophy was also important to rule out the possibility of the acute attack. Demographic data of the selected patient was recorded in Form B. The data basically obtained from the clinical record. A written consent (Form A) was obtained from the patient, a day prior to the operation.

#### 3.7.1.2 Collection of data for the control group (Group A).

Patients for the control group were only selected after sample for the study groups were obtained. The age of the selected patient must matched the age of patient in the study group. Similarly, only a consented patient was chosen.

### 3.7.2 Conjunctiva biopsy

A wedge shaped conjunctiva biopsy was obtained at the site of the fornix or limbal-based conjunctiva flap (superior bulbar conjunctiva) using Vannas scissors as illustrated in Figure 3. A conjunctiva forceps was also used to lift up the conjunctiva at the beginning of the surgery and conjunctiva scissor was used to

dissect the Tenon's capsule. In order to minimize the damage and crumpling effect, Weck's cell was passed through the dissected Tenon's capsule before cutting the specimen. Conjunctiva biopsy was performed while forming the conjunctival flap at the initial step of the surgery after superior bridle suture was done. The size varies from 2 to 5mm X 2 mm, according to the availability of the conjunctiva and to avoid compromising the surgery. Site and size of specimen was not marked before obtaining the biopsy. The measurement was purely by gross estimation. The same surgeon who performed the surgery performed the biopsy. The specimen was promptly fixated with 10% formalin, which contains 10 ml of 40% formaldehyde and 90 ml of distilled water.