COMPARISON OF CONJUNCTIVAL IMPRESSION CYTOLOGY BETWEEN GLAUCOMA PATIENTS TREATED WITH TOPICAL TIMOLOL MALEATE 0.5% AND TOPICAL LATANOPROST 0.005%

BY
DR TAN SOO HOI
MBBS (MAHE)

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF MEDICINE (OPHTHALMOLOGY)

USM

UNIVERSITI SAINS MALAYSIA

SCHOOL OF MEDICAL SCIENCES
UNIVERSITI SAINS MALAYSIA
KELANTAN

2004

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Tajuk Projek: Comparison of conjunctival impression cytology between glaucoma patients treated with topical timolol maleate 0.5% and topical latanoprost 0.005%.

Tajuk Program: short term

Tarikh Mula: August 2002

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This study provides evidence of conjunctival surface morphological changes after treatment with topical timolol maleate 0.5% and topical latanoprost 0.005%. Within three months of therapy, both these drugs caused significant reduction of goblet cells and mucous granules. The conjunctival surface morphological changes might be due to the active drug itself or the preservative or the combined effect of both. The morphological changes of the conjunctiva caused by the topical anti-glaucoma drugs may affect the outcome of filtering surgery. More studies should be carried out to find the cost-effective drugs, with or without preservatives which cause less conjunctival morphological changes.

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TANDATANGAN PENGERUSI
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KELANTAN

2004

DISCLAIMER

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

Dated 15.11.2003

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Tan Soo Hoi

P-UM 0769

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ABSTRAK

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Perbandingan 'conjunctival impression cytology' diantara pesakit-pesakit glaukoma yang dirawati dengan menggunakan ubat titis timolol maleate 0.5% dan pesakit-pesakit glaukoma yang dirawati dengan menggunakan ubat titis latanoprost 0.005%.

Pengenalan:

Ubat titis didapati boleh menyebabkan reaksi konjunktiva, termasuk ubat titis anti-glaukoma. Jangka masa rawatan yang panjang dipercayai telah menyebabkan kesan parut ke atas bleb filtrasi dan mempengaruhi kejayaan pembedahan filtrasi. Rawatan ubat titis timolol maleate 0.5% dan ubat titis latanoprost 0.005% yang berterusan menyebabkan perubahan morfologi pada permukaan mata.

Objektif:

Perbandingan antara kesan ubat titis timolol maleate 0.5% dengan kesan ubat titis latanoprost 0.005% pada morfologi permukaan konjunktiva.

Tatacara:

Pesakit-pesakit glaukoma yang baru didiagnos dan layak dipilih sebagai subjek untuk kajian ini. Mereka telah dibahagikan kepada dua kumpulan. Kumpulan pertama dirawati dengan menggunakan ubat titis timolol maleate 0.5% manakala kumpulan kedua dirawati dengan menggunakan ubat titis latanoprost 0.005%. Sebelum rawatan ubat titis diberi

kepada pesakit, 'conjunctival impression cytology' yang pertama diambil. Kemudian selepas tiga bulan rawatan, 'conjunctival impression cytology' yang kedua diperolehi. Perubahan morfologi pada permukaan konjunktiva sebelum dan selepas rawatan dikaji dalam setiap kumpulan. Perubahan morfologi permukaan konjunktiva diantara kedua-dua kumpulan pesakit juga dibandingkan.

Keputusan:

Seramai 39 pesakit glaukoma yang baru dikesan diambil untuk kajian ini. 20 pesakit dalam kumpulan Timolol manakala 19 pesakit dalam kumpulan Latanoprost. Selepas tiga bulan rawatan, didapati kedua-dua kumpulan pesakit tiada perubahan morfologi sel epithelia konjunktiva. Namum begitu, secara statistic didapati kekurangan bilangan sel goblet dan mucos granule yang signifikan di kedua-dua kumpulan selepas rawatan tiga bulan diberi. Analisis dengan 'Independent T-test' menunjukkan tiada perbezaan bilangan sel goblet dan mucos granule yang signifikan diantara dua kumpulan.

Kesimpulan:

Ini dapat disimpulkan bahawa selepas tiga bulan rawatan ubat titis timolol maleate 0.5% dan ubat titis latanoprost 0.005% diberi, bilangan sel goblet dan mucos granule didapati berkurangan. Namum begitu, tiada perubahan morfologi sel epithelia yang berlaku dalam kedua-dua kumpulan. Ini membuktikan bahawa ubat titis glaukoma, timolol maleate 0.5% dan latanoprost 0.005% boleh menyebabkan perubahan morfologi permukaan konjunktiva dalam masa tiga bulan. Namum begitu, tiada perbezaan perubahan morfologi permukaan konjunktiva diantara kumpulan Timolol dan kumpulan Latanoprost.

ABSTRACT

Title:

Comparison of conjunctival impression cytology between glaucoma patients treated with topical timolol maleate 0.5% and topical latanoprost 0.005%.

Introduction:

All topical medications are known to cause conjunctival reactions, and topical anti-glaucoma drugs are also no exception. Long term drug induced toxicity to the conjunctiva is postulated to cause filtering bleb scarring and filtration surgery failure. Chronic application of topical timolol maleate 0.5% and topical latanoprost 0.005% had been shown to alter the morphology of the conjunctival ocular surface.

Objective:

To compare conjunctival surface morphological changes with the use of the topical timolol maleate 0.5% and topical latanoprost 0.005%.

Methodology:

Newly diagnosed glaucoma patients who met the selection criteria were randomly divided into two groups. One group treated with topical timolol maleate 0.5%, another group treated with topical latanoprost 0.005%. Before the treatment was started, the first conjunctival impression cytology was taken. After three months of treatment, the second conjunctival impression cytology was obtained. The changes that occurred between the

first and second conjunctival impression cytology in the individual group were analyzed. Conjunctival surface changes that occurred with the use of topical timolol maleate 0.5% were also compared with the conjunctiva surface changes that occurred with the use of topical latanoprost 0.005%.

Results:

There were thirty-nine newly diagnosed glaucoma patients included in this study. Twenty patients were in the Timolol group and nineteen patients in the Latanoprost group. In both groups of patients, there was no change of the conjunctival epithelial cell morphology after three months of anti-glaucoma therapy. However, there was statistically significant reduction of the goblet cell and mucous granule density in both groups of patients after three months of the topical anti-glaucoma therapy (P- value <0.001). By using the independent T-test, there was no significant difference of the goblet cell and mucous granule density between the Timolol group and the Latanoprost group after three months of treatment.

Conclusion:

This concludes that both timolol maeate 0.5% and topical latanoprost 0.005% cause great reduction of conjunctival goblet cells and mucous granules within three months of treatment. However, the conjunctival epithelial cell morphology remained normal after three month of treatment in both groups of patients. This study gives evidence that topical timolol maleate 0.5% and topical latanoprost 0.005% cause morphological changes of the conjunctival surface after short term (three months) therapy. However, there was no

difference in the conjunctival morphological changes between the Timolol group and Latanoprost group.

1. INTRODUCTION

1.1 BACKGROUND

The conjunctiva is a passive semi-permeable barrier that allows drugs to enter the eye. It is also a delicate tissue that can respond to stress with inflammation, scarring, keratinization and neovascularization (James D Brandt, 1991). These conjunctival changes will affect the tear film layer and it may affect the outcome of filtration surgery.

Virtually all topically administered medications are known to cause conjunctival reactions, and the topical anti-glaucoma drugs are also no exception (James D Brandt, 1991). Most patients undergo surgery only after months of topical drug treatment, and long term drug induced toxicity may predispose patients to poor outcome after filtration surgery.

Lavin MJ and associates found that long term use (more than one year duration) of topical anti-glaucoma medications can adversely affect the result of filtration surgery (Lavin MJ et al, 1990). The results of the study done by Sherwood MB and associates suggest that exhaustive medical therapy, before filtration surgery is offered, increases the number of tissue inflammatory cells and decreases the number of epithelial goblet cells (Sherwood MB, 1989).

The expression of HLA-DR on conjunctival epithelial cells in patients taking topical anti-glaucoma eye drops also indicates the increased ability of epithelial cells to induce immune inflammation with subsequent fibrosis (Ihan A et al, 2000). There was also an

increase in myofibroblastic cell proliferation in fistulized rabbit conjunctiva treated with glaucoma medication compared with fistulized conjunctiva that was not treated with topical eye drops (Terri L Young et al, 1990). These tissue cellular changes may enhance the external bleb scarring and filtration surgery failure (Sherwood MB, 1989). Patients on long term multiple topical anti-glaucoma drugs was postulated to have lower trabeculectomy success rate in comparison with patients undergoing primary trabeculectomy (Broadway David, 1993). The number of the conjunctival goblet cells may also relate to intraocular pressure control after trabeculectomy (Gwynn DR, 1993).

There are few classes of anti-glaucoma drugs. Beta-adrenergic receptor blocker- topical timolol maleate 0.5% is usually used as the first line medication in most of the glaucoma cases in the absence of systemic contraindication. However, it has been shown that chronic application of a commercial preparation of topical timolol maleate 0.5% altered the morphology of conjunctiva ocular surface and impairing the quantity and quality of the mucous layer of the tear film (Herreras JM et al, 1992). Recently, there is increasing usage of topical latanoprost 0.005%- a prostaglandin F2α analogue. It has been used as a first line treatment in some medical centers. Chronic application of topical latanoprost 0.005% also has adverse effect on the ocular surface (Fraunfelder et al, 2002).

Slit-lamp examination of the ocular surface with or without flourescein or Rose Bengal staining gives only a rough idea about the changes occurring in the epithelium. However, the histological section, which gives better histological information, can cause scarring to

the conjunctiva. The use of impression cytology had been proven to be a good method for conjunctival surface assessment.

The conjunctival impression cytology can be obtained by the apposition of cellulose acetate filters onto the conjunctiva. This method is less invasive, easy and cheap to be performed. It has been used in identifying conjunctival changes related to long term topical anti-glaucoma drug use (James D Brandt et al, 1991).

In this study, the conjunctival impression cytology done for the patients using topical timolol maleate 0.5% and patients using topical latanoprost 0.005% might reveal valuable information regarding the conjunctival surface morphological changes that developed with the use of the respective topical anti-glaucoma drug.

1.2 CONJUNCTIVA

The conjunctiva is a thin mucous membrane that lines the eyelids. It is reflected at the superior and inferior fornices onto the anterior surface of the eyeball. Histologically, the conjunctiva has an epithelial covering of stratified columnar cells consisting of two to five layers resting on a substantia propria of loose connective tissue. Goblet cells are found scattered along the surface of the conjunctiva and it is most numerous over the infero-nasal part of the bulbar conjunctiva (Kirk RW et al, 1999). Their mucous secretion is important contributing stability to the tear film. The conjunctiva also acts as a semi-

permeable membrane, allowing the entry of topical medications. The side effects of the medications might cause morphological changes of the conjunctiva.

In glaucoma-filtering surgery, a fistula is created between the anterior chamber and the subtenon space. The conjunctival bleb is formed between the bulbar conjunctiva and the subtenon space. Thus the success rate of the glaucoma-filtration surgery depends on many factors, not the least of which is the existence of a healthy conjunctiva. Therefore it is important to identify the conjunctival morphological changes that developed with the use of the topical anti-glaucoma drugs.

1.3 CONJUNCTIVAL IMPRESSION CYTOLOGY

Conjunctival impression cytology is a method of obtaining cytological specimen from the conjunctival surface. It refers to the application of cellulose acetate paper to the ocular surface to remove the superficial layer of conjunctival epithelium. It has high cell pick up rate and detailed cell resolution is achieved after fixation and staining procedure (Divani SN et al, 1997). With this method, the morphology of the conjunctival surface can be studied. This technique is non invasive, easy to perform and causes minimal discomfort to patients (Divani SN et al, 1997).

1.3.1 THE USE OF CONJUNCTIVAL IMPRESSION CYTOLOGY IN OCULAR SURFACE ASSESSMENT

Egbert et al first introduced ocular impression cytology into ophthalmology field in 1977. These authors used cellulose acetate filter paper for the collection of the superficial layer of cells from conjunctiva. This method has subsequently been modified by several authors for investigation of a variety of ocular surface disorders. It has provided insights into a number of ocular surface disorders, including dry eyes (Nelson JD et al, 1983), vernal keratoconjunctivitis, hypovitaminosis A (Polizzi A et al, 1998)(Keenum DG et al, 1990), squamous metaplasia (Tole DM et al, 2001)(Scheffer CG Tseng, 1985), ocular pemphigoid, Stevens Johnson syndrome and also in detecting superficial viral infection of the eye (Thiel MA et al, 1997).

An immunocytological study was done by using conjunctival impression cytology to investigate the expression of two inflammatory markers, class II MHC antigens HLA DR and the receptor to IgE (CD23) in normal and diseased conjunctiva (Christophe Baudouin, 1992). Thiel et al have described the use of a new Biopore membrane device for obtaining conjunctival cells for the diagnosis of superficial viral infection. Immunocytological staining of these samples can provide virological results within one to four hours (Thiel MA et al, 1997). Tole et al also having success with impression cytology in the diagnosis of ocular surface squamous neoplasia using Biopore membrane (Tole DM et al, 2001). However, the cellulose acetate papers offer greater sampling flexibility. Scheffer CG Tseng (1985) had differentiated the process of squamous

metaplasia of conjunctival epithelium into six stages by impression cytology using cellulose acetate paper. Electron microscopy of impression-acquired conjunctival epithelial cells also has potential for the study of sub-cellular, cellular and intercellular morphology in diseases of the ocular surface (Steven L Maskin et al, 1986).

1.3.2 METHODS OF FIXATION AND STAINING OF IMPRESSION CYTOLOGY SPECIMENS

The conjunctival impression cytology samples were fixed with a mixture of 70% alcohol, 37% folmaldehyde and glacial acetic acid (Figure 6). There are a few staining methods of the conjunctival impression cytology specimens, like haematoxylin and periodic acid Schiff stain (PAS), Toluidine blue staining, Papanicolaou staining, peroxidase staining and immunoflourescence staining. The commonest stain used was haematoxylin and periodic acid Schiff stain. It provides good staining and visualization of conjunctival epithelium and goblet cells.

Periodic acid Schiff stain is a special stain for demonstration of mucin, reticulum and glycogen. The reaction is based on the fact that certain tissue elements are oxidized by the periodic acid, one of the reaction products being aldehyde. The Periodic acid cleaves the carbon-carbon bonds where those carbon atoms have adjacent hydroxyl group (-OH), or adjacent hydroxyl and amino groups ($-NH_2$). The Schiff reagent (Figure 9) is used to demonstrate the aldehyde produced after hydrolysis with hydrochloric acid (Culling CFA, 1983).

Sodium metabilsulfite is used as a buffer for the removal of excessive Schiff reagent. Counterstaining of the nucleus is by the haematoxylin stain (Figure 10) whereas dehydration is done with the alcohol (Figure 11). Xylene solution acts as a clearing agent. The tissue become clearer as the alcohol is replaced owing to the difference in the refractive index.

The haematoxylin and periodic acid Schiff's staining procedure was as follows: (1) rinse with distilled water for 2 minutes; (2) immerse in 0.05% periodic acid for 2 minutes (Figure 7); (3) rinse with distilled water; (4) immerse in Schiff reagent diluted 1:1 with water for 8 minutes (Figure 9); (5) rinse with tap water for 2 minutes; (6) immerse in 0.05% sodium metabilsulfite for 2 minutes; (7) rinse with tap water for 2 minutes; (8) stain with haematoxylin for 30 seconds (Figure 10); (9) rinse with tap water for 2 minutes; (10) decolorized for 2 minutes using 95% alcohol (Figure 11); (11) decolorized with absolute alcohol for another 2 minutes (Figure 11); (12) transfer to xylene solution for 10 minutes; (13) allow the slide to dry; (14) the specimen is ready for mounting (Figure 12).

1.3.3 STANDARDIZATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY

Martinz et al (1995) found that the conjunctival cell pick up rate was dependant on the pore size of the filter paper and the pressure applied during the apposition of the filter paper to the conjunctival surface. The maximum cell acquisition was achieved by filter paper with medium pore size (0.22μm) and a pressure of 60g during the sampling

procedure. In this study, Millipore GSWP01300 filter papers with the pore size of 0.22μm were used for the conjunctival impression cytology.

1.4 TOPICAL ANTI-GLAUCOMA DRUGS

There are a few classes of topical anti-glaucoma drugs:

1. Beta adrenergic receptor blockers: Timolol, Levobunolol, Betaxolol, Carteolol, Metipranolol. They lower the intraocular pressure by decreasing the aqueous production.
2. Prostaglandin agonist: latanoprost. It lowers the intraocular pressure by increasing the uveoscleral outflow.
3. Cholinergics (Direct acting): pilocarpine, carbachol, aceclidine. They increase the aqueous drainage through the trabecular meshwork by opening up the anterior chamber angle.
4. Cholinergics: Cholinesterase inhibitors (Indirect acting): physostigmine, demecarium, edrophonium, ecothiophate, isoflorophate. Their mechanism of action is same as the cholinergic eye drops.
5. Adrenergic agonists: epinephrine, dipivefrin, apraclonidine, brimonidine. They reduce aqueous production by alpha 2 effect and increase aqueous drainage by beta 2 effect.
6. Carbonic anhydrase inhibitors: dorzolamide hydrochloride. They decrease aqueous production by inhibiting the carbonic anhydrase.

1.4.1 TOPICAL TIMOLOL MALEATE O.5%

Topical timolol maleate 0.5% is commonly used in our clinical practice. It is a nonselective beta1 and beta 2 adrenergic antagonist. The composition of the drug is timolol maleate (0.5g/100ml), monosodium crystalline phosphate 1 H2O (0.37g/100ml), benzalkonium chloride (0.01g/100ml), crystalline sodium chloride (0.22g/100ml), disodium crystalline phosphate 1 H2O (2.60g/100ml), and purified water (100ml). It is useful in treatment of all types of glaucoma, irrespective of the state of the angle. In the absence of systemic contraindications, it is frequently the first choice medication. It antagonizes the effects of beta agonists at beta-receptors by competing with catecholamines and it reduces the intraocular pressure by decreasing aqueous secretion.

The side effect of topical timolol maleate can be divided into systemic side effects and ocular side effects. The main systemic side effects are bronchial constriction and cardiovascular side effects such as bradycardia, decreased cardiac contractility and prolongation of atrio-ventricular conduction. Ocular allergic and toxic reactions with timolol therapy have been reported. Burning and conjunctival hyperaemia may occasionally occur and are frequently associated with superficial punctate keratopathy and corneal anaesthesia. Chronic therapy with timolol has been shown to damage the ocular surface and impair the quantity and quality of the mucus layer of the tear film (Herreras et al, 1992) (James D Brant et al, 1991) (Christophe Baudouin et al, 1999).

1.4.2 TOPICAL LATANOPROST 0.005%

Latanoprost 0.005% is a new prostaglandin F2-alpha analogue with intraocular pressure lowering effect at least equal to timolol 0.5% (Peter Watson et al, 1996) or better than timolol 0.5% (Carl B Camras et al, 1996). Each ml of latanoprost 0.005% contains 50 mcg latanoprost and benzalkonium chloride 0.02g/100ml. Other ingredients include sodium chloride, sodium dihydrogen phosphate monohydrate and disodium phosphate anhydrous water. It enhances uveoscleral outflow and its pressure lowering effect is therefore additive to most other medications.

It has little systemic side effect. Local side effects consisting mainly of ocular irritation and mild conjunctival hyperaemia occur in about 10% of patients. It is contraindicated in uveitis as it exacerbates the inflammation. It may also cause increased pigmentation of the iris and hypertricosis. Cystoid macular edema had been reported with the use of latanaprost in the aphakic and pseudophakic eyes. Treatment with latanoprost also produced conjunctival morphological changes (Robert J Noecker, 2003).

1.4.3 COMPARISON BETWEEN TOPICAL TIMOLOL MALEATE 0.5% AND TOPICAL LATANOPROST 0.005%

Topical timolol maleate 0.5% is the first line topical anti-glaucoma drug that we commonly use. Topical latanoprost 0.005% is a newer topical anti-glaucoma drug. Latanoprost 0.005% admininistered once daily reduced intraocular pressure as well as

timolol 0.5% administered twice daily (Peter Watson et al, 1996). The study done by Carl B Camras et al revealed that the intraocular pressure reduction achieved with latanoprost 0.005% is greater than that produced with timolol 0.5% (Carl B Camras et al, 1996). The aim of this study is to compare the ocular surface changes caused by the topical latanoprost 0.005% and the topical timolol maleate 0.5%.

Christophe Baudouin and associates (1999) found that Benzalkonium Chloride induces histopathological and inflammatory changes in the ocular surface and in deeper ocular structures treated by anti-glaucoma drugs. Pisella PJ et al (2002) also found that the ocular symptoms and signs of irritation are less prevalent when preservative-free drops are used. The topical timolol maleate 0.5% contains 0.01g/100ml benzalkonium chloride whereas topical latanoprost 0.005% contains 0.02g/100ml benzalkonium chloride. However, the topical timolol maleate 0.5% is used twice daily whereas topical latanoprost 0.005% is used once daily. Therefore the patients treated with topical timolol maleate 0.5% and the patients treated with topical latanoprost 0.005% will receive equal amount of benzalkonium chloride daily.

2. OBJECTIVES

2.1. GENERAL OBJECTIVE

To compare conjunctival surface morphological changes with the use of topical timolol maleate 0.5% and topical latanoprost 0.005%.

2.2. SPECIFIC OBJECTIVE

1. To evaluate and compare the conjunctival epithelial cell morphology between the Timolol group and Latanoprost group.
2. To evaluate and compare the conjunctival goblet cell density between the Timolol group and Latanoprost group.
3. To evaluate and compare the conjunctival mucous granule density between the Timolol group and Latanoprost group.

3. MATERIALS AND METHODS

3.1 RESEARCH STRATEGY

Randomized clinical trial.

3.2 POPULATION, SETTING AND TIME

Study populations: Group 1: Newly diagnosed glaucoma patients treated with topical timolol maleate 0.5% twice daily.

Group 2: Newly diagnosed glaucoma patients treated with topical latanoprost 0.005% once at night.

Period of study: August 2002 till November 2003.

Places of sampling: 1. Ophthalmology Clinic, Department of Ophthalmology, School of Medical Science, Universiti Sains Malaysia.

2. Ophthalmology Clinic, Hospital Kota Bahru.

3.3 SAMPLING, CASE ALLOCATION AND SAMPLE SIZE

3.3.1 SAMPLING METHOD

Randomized sampling was done. Newly diagnosed glaucoma patients were randomly divided into two groups, by closed envelope method. One group was put on topical timolol maleate 0.5% twice daily, and another group on topical latanaprost 0.005% once at night. If both eyes of a single patient were glaucomatous and met the inclusion criteria, one of the eyes would be chosen randomly for the study by closed envelope method. The investigator was blinded from the grouping of the patients. The first conjunctival impression cytology was taken before the treatment was started. The second conjunctival impression cytololgy was obtained after three months of treatment. The conjunctival surface changes were evaluated in each group of patients. The effects of the topical timolol maleate 0.5% and topical latanoprost 0.005% to the morphology of the conjunctival surface were also compared.

3.3.2 SAMPLE SIZE CALCULATION

The calculation of the sample size was based on the previous studies and was calculated using two means formula programmed in the computer. The calculation was made based on these assumptions: confidence interval of 95%, power of study 80%, the different mean of goblet cell density (Δ) between the patients using topical timolol maleate 0.5%

and patients using topical latanaprost 0.005% was 25±27 cells/mm^2, the constant value was zα:1.96 and zβ:o.84 (study power).

Two mean formula:

$$\eta = \frac{2\sigma^2 (Z\alpha + Z\beta)^2}{\Delta^2}$$

$$= \frac{2\times27\times27\ (1.96+0.84)^2}{25\times2}$$

$$= \frac{1458\ (2.8)^2}{625}$$

$$= 2.3328 \times 7.84$$

$$= 18.289$$

There were 2 groups of eyes:

Group 1: included at least 19 newly diagnosed glaucomatous eyes, treated with topical timolol maleate 0.5% twice daily.

Group 2: included at least 19 newly diagnosed glaucomatous eyes, treated with topical latanoprost 0.005% once daily.

3.4 SELECTION CRITERIA

3.4.1 INCLUSION CRITERIA

Newly diagnosed primary open angle glaucoma patients and primary chronic angle closure glaucoma patients without acute and sub-acute attack, age ranged from 40 to 80 years.

3.4.2 EXCLUSION CRITERIA

1. Previous history of ocular surgery and ocular trauma.
2. Chronic use of any topical eye drops for more than 1 month.
3. Patient with history and/ or clinical findings of acute and sub-acute attack of angle closure glaucoma.
4. Secondary glaucoma.
5. Inflammatory eye diseases.
6. Contact lens wear.
7. Evidence of severe ocular surface disorder on slit-lamp examination (eg. severe dry eye).
8. Patients who need more than one type of anti-glaucoma eye drops during the study period (First 3 months of treatment).
9. Patients who did not compliance to the treatment of topical anti-glaucoma drugs.
10. Diabetes mellitus patients with diabetic retinopathy or poor diabetic control.

3.5 ETHICAL APPROVAL

This study was approved by the Research and Ethics Committee, School of Medical Sciences, Universiti Sains Malaysia on 21 August 2002. This was supported by the USM short term grant (Reference number: 304/PPSP/6131263).

3.6 DEFINITION OF TERMS

1. Primary open angle glaucoma (POAG)

It is a multi-factorial syndrome in which acquired progressive optic nerve damage is related, at least in part, to intraocular pressure higher than the nerve fibers can tolerate. POAG consists of a variety of variably overlapping entities. Although the etiological factors of the individual entities, or the broader group of conditions called POAG, are unknown, POAG is secondary to as-yet-unrecognized forces. The term *primary* itself suggests an unknown cause.

POAG is a group of chronic, bilateral conditions that are almost always symmetrical. It is characterized by the following features: evidence of glaucomatous optic nerve damage; adult onset; normal-appearing, open anterior chamber angles; and absence of other known causes of open-angle glaucoma. In the case of glaucomatous optic nerve damage, the characteristic appearance of the optic disc and retinal nerve fiber layer includes thinning or notching of the tissue of the neuroretinal rim of the disc, a progressive increase in cupping of the optic nerve, retinal nerve fiber layer defects, and flame-shaped haemorrhage crossing the outer edge of the disc. The presence of damage to the visual field is evidenced by arcuate defect, nasal step, para-central scotoma and generalized depression in the absence of other causes or explanations for the field defect.

POAG can be classified solely based on the intraocular pressure level, into two categories: the high-tension type, in which intraocular pressure elevates above the statistically normal range, which is above 21mmHg, and the low –tension type (low-tension glaucoma or normal-tension glaucoma, in which the intraocular pressure is consistently in the statistically normal range. The two subgroups of POAG overlap and the clinical difference between the two subgroups are not always clear cut (Tarek M Eid and George L Spaeth, 2000).

2. Pseudoexfoliation glaucoma: It is a form of primary open angle glaucoma. In pseudoexfoliation glaucoma, a grey-white, fibrillogranular material is deposited in and around the anterior segment of the eye. The origin of the fibrillogranular material is multifocal. It is from the abnormal basement membrane produced by aging epithelial cells. Exfoliation materials were demonstrated in the conjunctival stroma of pseudoexfoliation glaucoma patients. However, there was no evidence of exfoliation materials near basement membrane of conjunctival epithelium and blood vessels. The basement membrane of conjunctival epithelium showed no remarkable abnormalities (Young Bae Roh et al, 1987).

3. Primary chronic angle closure glaucoma (Chronic PACG)

It is appositional or synechial closure of the anterior chamber angle caused by the pupillary block in the absence of other causes of angle closure. Slowly progressive closure of the angle may lead to sustained elevation of intraocular pressure. Symptoms

usually are absent until visual field loss becomes advanced. Patients with chronic PACG have no evidence of a previous acute attack of pupillary block by history or physical examination. In early cases, the glaucoma is due to appositional angle closure without peripheral anterior synechiae formation. Later on, progressive peripheral anterior synechiae formation develops (synechial angle closure). This chronic form of angle closure may be complicated by a superimposed acute episode, presumably triggered by other mechanisms of action (eg. cataract). A rare variant of angle closure, creeping angle closure, may occur, especially in patient with dark brown irises. In this form, the closure begins anterior to the ciliary body, near the Schwalbe's line, such that the posterior trabecular meshwork may remain open, bridged by the synechiae. Eventually, there may be an acute attack, or the peripheral anterior synechiae may permanently occlude the angle, leading to elevated intraocular pressure and glaucomatous damage.

On examination of the chronic PACG, the intraocular pressure is elevated, anterior chamber is quiet and usually deeper than in patients who have acute attacks, there is no corneal oedema, and the angle is operatively closed, with or without peripheral anterior synechiae. Presence of Glaucomflecken and/or sector atrophy of the iris indicates a previous acute attack. The optic disc and visual field show glaucomatous damage similar to that seen with POAG ((Tarek M Eid and George L Spaeth, 2000).

4. Impression cytology

It refers to the technique by which the superficial layers of the ocular surface are removed with the application of cellulose acetate filter materials.

5. Conjunctiva

It is a thin mucous membrane that lines the eyelids and is reflected at the superior and inferior fornices onto the anterior surface of the eyeball. It is divided into bulbar conjunctiva and palpebral conjunctiva. In this study, the impression cytology was taken from the superior bulbar conjunctiva.

6. Topical timolol maleate 0.5%

It is a type of topical anti-glaucoma drug. It is a non-selective beta 1 and beta 2 adrenergic antagonist. It reduces the intraocular pressure by decreasing aqueous secretion.

7. Topical latanoprost 0.005%

It is a prostaglandin F2α analogue. It enhances uveo-scleral outflow thereby lowering the intraocular pressure.

3.7 EQUIPMENT

1. Topical anesthesia (4% novesin) (Figure 2)
2. Millipore filter paper (GSWP01300) (Figure 5)
3. Glass slide (Figure 1)
4. Adhesive tape
5. 70% alcohol (Figure 11)
6. 30% formaldehyde
7. Glacial acetic acid
8. Periodic acid Schiff stain and Haematoxylin stain (Figure 9) (Figure 10)
9. Sodium metabilsulfite
10. 95% acid alcohol (Figure 11)
11. Absolute alcohol (Figure 11)
12. Xylene solution
13. Light microscope (Leica DMR) (Figure 14)
14. Leica Qwin Software
15. Leica Q 5001w (CPU) computer (Figure 14)

3.8 COLLECTION OF DATA

3.8.1 CONSENT

Informed written consent was taken from all the patients.

3.8.2 HISTORY AND CLINICAL EXAMINATION

Patients were randomly divided into 2 groups as mentioned earlier. The person who was going to do the conjunctival impression cytology and examination of the specimen was masked from the grouping of the patients. Patient's demographic data were recorded in the data collection form. Detailed history was asked from every patient: any history of allergic conjunctivitis, usage of any topical medications, contact lens wearing, history of ocular and conjunctival surgery, ocular trauma, any symptoms of ocular surface disorders, past medical history etc. Only those patients who fulfilled the selection criteria would be included in the study. Patients with history of acute and sub-acute attack of angle closure glaucoma were excluded from the study.

All the eyes were examined under slit-lamp to identify any ocular surface abnormalities. Patients with any evidence of external eye disease or inflammatory eye diseases were excluded. For both groups of newly diagnosed glaucoma patients, the first conjunctival impression cytology was taken just before commencing the topical anti-glaucoma treatment. The second conjunctiva impression cytology was taken after 3 months of the treatment.

3.8.3 METHOD OF SPECIMEN COLLECTION

Patients were explained about the detail of the procedure and the written consent was taken. He/she was then asked to lie supine on an examination couch. Topical anaesthesia

Novesin 0.4% (Figure 2) was instilled into the eye before the procedure. One piece of round filter paper (Millipore GSWP01300) was cut equally into two pieces. The patient was instructed to look down towards the chest. One piece of the filter paper was held using a forceps and placed against the superior bulbar conjunctiva 2 mm away from the superior limbus (Figure 3). The straight cutting edge of the filter paper was directed towards the limbus. The filter paper was pressed gently for 3 seconds with the blunt end of the forceps, to ensure close contact of the filter paper with the ocular surface. After gentle pressure for 3 seconds, the filter paper was removed with a peeling movement (Figure 4). The reflection of the filter paper would become faded if the specimen had adhered to it. The filter paper was then adhered to the glass slide by adhesive tape with the specimen facing up (Figure 5).

Figure 1: Fixation solution, slides and forceps used in specimen collection during conjunctival impression cytology.

Figure 2: Novesin 0.4%, the local anaesthetic solution used during conjunctival impression cytology.

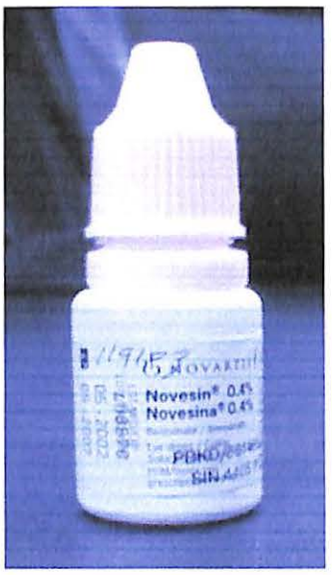

Figure 3: Filter paper placed on the superior bulbar conjunctiva.