EFFECT OF PAPAYA INTAKE ON INTRAOCULAR PRESSURE, RETINAL NERVE FIBER LAYER THICKNESS, OPTIC NERVE HEAD PARAMETERS AND SERUM TOTAL ANTIOXIDANT CAPACITY IN PRIMARY OPEN ANGLE GLAUCOMA PATIENTS

DR CHAN HUI TZE

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DISCLAIMER

I hereby certify that the work in this dissertation is my own except for the quotations and summaries which have been duly acknowledged. I declare that I have no financial interest in the instruments in this study.

Dated 15/11/2020 …….....

Dr. Chan Hui Tze

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ABSTRAK

PENGENALAN

Glaukoma sudut terbuka primer (POAG) adalah neuropati optik yang kronik dan progresif, dimana ia menyebabkan kebutaan yang kekal di dunia ini. Terdapat banyak kajian percubaan terkawal rawak mengenai modaliti rawatan pada pelbagai peringkat dan jenis glaukoma. Modaliti-modaliti tersebut adalah bertujuan untuk mengurangkan tekanan intraokular (IOP). Walau bagaimanapun, terdapat banyak pesakit yang menunjukkan tanda perkembangan penyakit walaupun sasaran IOP telah dicapai. Akhir-akhir ini, perubatan komplementer dan alternatif (CAM) termasuk pengubahsuaian gaya hidup telah menarik minat ramai penyelidik. Buah-buahan yang kaya dengan antioksidan seperti betik mempunyai potensi sebagai CAM di kalangan pesakit POAG. Buah betik tidak bermusim, mudah didapati, sesuai untuk orang berusia dan ialah buah tropika yang agak murah di Malaysia.

OBJEKTIF

Untuk menilai kesan pengambilan buah betik kepada ketebalan lapisan saraf sekitar kepala saraf optik (RNFL), struktur kepala saraf optik (ONH) dan jumlah kapasiti antioksidan serum (TAC) di kalangan pesakit glaukoma sudut terbuka primer (POAG).

KAEDAH KAJIAN

Satu kajian percubaan kawalan rawak terkawal yang melibatkan 40 orang pesakit POAG telah dijalankan di Hospital Universiti Sains Malaysia di antara Februari 2018 dan Oktober 2019. Pesakit yang memenuhi kriteria inklusi dan eksklusi akan dipilih dengan kaedah pensampelan rawak mudah. Blok secara rawak dengan menggunakan sampul surat yang berurutan, untuk merawakkan pesakit POAG kepada kumpulan A dan B. Pesakit dalam kumpulan A diarahkan untuk mengambil satu hidangan betik (~ 250 gram), 3 kali seminggu selama 3 bulan. Tiga (3) ml

darah diambil untuk mengukur TAC di dalam serum. Ketebalan RNFL, IOP, ONH dan TAC serum direkod pada ukuran, 1, 2 dan 3 bulan untuk kedua-dua kumpulan. Pengukuran IOP dilakukan dengan menggunakan alat Goldmann Applanation Tonometer (GAT) dalam posisi duduk. Spectral Domain-Cirrus optical coherence tomography diguna untuk mengukur ketebalan dan parameter ONH, dan RNFL. Serum TAC dianalisis dengan menggunakan QuantiChromTM Antioxidant Assay Kit (DTAC-100). Analisis deskriptif, repeated measures ANOVA dilakukan dengan mengguna SPSS.

KEPUTUSAN

Seramai 67 pesakit POAG telah diperiksa tetapi hanya 40 orang pesakit POAG yang direkrut. Pesakit dalam kumpulan A adalah lebih tua dan mempunyai tempoh penyakit yang lebih pendek tetapi tanpa perbezaan yang signifikan banding dengan kumpulan B. Terdapat bilangan hiperlipidemia yang signifikan tinggi dalam kumpulan B ($p = 0.028$). Terdapat sedikit lebih tinggi purata IOP kumpulan A (15.7±1.6mmHg) berbanding kumpulan B (14.9±1.9mmHg). pada ukuran asas. Berdasarkan RM ANOVA terdapat pengurangan yang signifikan pada IOP di kumpulan A berbanding dengan kumpulan B ($p < 0.001$). Terdapat juga peningkatan yang signifikan dalam ketebalan RNFL di kumpulan A berbanding kumpulan B ($p = 0.001$). Walau bagaimanapun, tiada perbezaan yang signifikan dalam parameter ONH antara dua-dua kumpulan selepas 3 bulan. Juga tiada perbezaan yang signifikan dalam serum TAC ($p = 0.210$) di antara kumpulan A dan B. Dan tiada peningkatan serum TAC dalam kumpulan A selepas 3 bulan pengambilan betik.

KESIMPULAN

Pengambilan buah betik secara ketara mempunyai potensi untuk mengurangkan IOP di pesakit POAG secara tambahan. Buah betik mempunyai kesan positif yang berpotensi kepada ketebalan RNFL tapi tidak kepada parameter ONH.

ABSTRACT

INTRODUCTION

Primary open angle glaucoma (POAG) is a chronic progressive optic neuropathy which responsible for many irreversible blindness in the world. There are many large randomized controlled trial studies on treatment modalities on various stages and type of glaucoma. These modalities aimed at reduction of intraocular pressure (IOP). However, there are still many patients who shown sign of progression of the disease despite of achieving target IOP. Lately, complementary and alternative medicine (CAM) including lifestyle modification has caught the interest of many researchers. Fruits enriched with antioxidants such as papaya has the potential role as CAM in POAG patients. Papaya is a non-seasonal, easily available, and palatable to elderly and relatively inexpensive tropical fruits in Malaysia.

OBJECTIVES

To evaluate the effect of papaya intake on IOP, retinal nerve fiber layer (RNFL) thickness, optic nerve head (ONH) parameters and serum total antioxidant capacity (TAC) in primary open angle glaucoma (POAG) patients.

METHODOLOGY

A randomized single blinded controlled trial involving 40 POAG patients was conducted in Hospital Universiti Sains Malaysia between February 2018 and October 2019. Patients who fulfilled inclusion and exclusion criteria were recruited by simple random sampling method. Block randomization using sequentially numbered, opaque sealed envelopes (SNOSE) was used to randomize POAG patients to group A and B equally. Patients in group A were instructed to consume single serving of raw papaya (~250 gram), 3 times a week for 3 months. Venesection

was also conducted to obtain 3 ml of blood to quantify serum TAC level. The IOP, RNFL thickness, ONH parameters and serum TAC were recorded at baseline, 1st, 2nd and 3rd month for both groups. IOP measurement was done using Goldmann applanation tonometer (GAT) in sitting position. Spectral domain Cirrus high definition optical coherence tomography (HD-OCT) was used to measure RNFL thickness and ONH parameters. Serum TAC was analyzed using QuantiChromTM Antioxidant Assay Kit (DTAC-100). Descriptive analysis, repeated measures ANOVA was performed using SPSS.

RESULTS

A total of 67 POAG patients were screened but only 40 POAG patients were recruited after baseline examination. Patients in group A were older but with shorter duration of the disease but without any significant different to group B. However, there was significant higher number of hyperlipidemia in group B ($p=0.028$). There was slightly higher baseline mean IOP of group A (15.7±1.6 mmHg) compared to group B (14.9±1.9 mmHg). Based on RM ANOVA there was significant further reduction of IOP in group A compared to group B ($p<0.001$). There was also significant increase in RNFL thickness in group A compared to group B ($p=0.001$). However, there was no significant different in ONH parameters between the two groups after 3 months of intervention. There was also no significant different in serum TAC ($p=0.210$) between group A and B. In fact there was no further increase in serum TAC in group A after 3 months of regular intake of papaya.

CONCLUSION

Regular consumption of papaya has potential supplementary IOP reduction in POAG patients. Papaya has potential positive effect on RNFL thickness but not on ONH parameters and serum TAC level. Further research with larger number of glaucoma patients and longer follow up is required to determine the potential supplementary effect of papaya in glaucoma management.

Chapter 1
Introduction

1.1 GLAUCOMA

Glaucoma is an optic neuropathy associated with characteristic structural damage to the optic nerve and associated visual dysfunction that is caused by various pathological processes (Foster PJ et al., 2002). Glaucoma can be classified into 2 broad groups based on the angle structures; open and closed angle glaucoma (Glaucoma Research Foundation, 2012). This can be further subdivided into primary and secondary glaucoma (Quigley HA, 1996). Although glaucoma encompasses a diverse group of disease, its hallmark include progressive irreversible damage to the optic nerve head (ONH) and retinal ganglion cells with corresponding visual field loss (Tuulonen A and Airaksinen PJ, 1991).

1.2 PRIMARY OPEN ANGLE GLAUCOMA

Primary glaucoma, of open angle category includes POAG, Juvenile Open Angle Glaucoma (JOAG), Normal Tension Glaucoma (NTG) and congenital glaucoma (Glaucoma Research Foundation, 2012). POAG is characterzied as a chronic, slowly progressive visual field loss and optic nerve cupping, often associated with an elevated intraocular pressure (IOP) with visually open anterior chamber angles by gonioscopy, without underlying secondary ocular disease (European Glaucoma Society, 2014).

The cardinal structural features of open angle glaucoma at the optic disc are a reduction in neuroretinal rim area and enlargement of the optic cup (Garway-Heath et al., 1997). Diagnosis of glaucoma is based on signs of functional vision loss associated with characteristic morphological changes of the ONH and retinal nerve fiber layer (RNFL).

1.3 MANAGEMENT OF GLAUCOMA

As a disease of longevity, glaucoma patients often requires lifelong treatment and carries a risk of serious visual impairment. Despite the emergence of new surgical and medical options, current therapies remain imperfect, and novel treatment options are desired. Disease progression in glaucoma is common and despite treatment, majority of patients still progress (Rossetti L et al., 2010). Furthermore, studies have demonstrated that patients with apparently well-controlled IOP exhibit glaucomatous disease progression, primarily due to the unpredictability of treatment outcomes (CNTGS).

Current management guidelines from the American Academy of Ophthalmology Preferred Practice Pattern recommended lowering the IOP toward a targeted level known as target pressure. Target pressure is a value or range of values of IOP at which the clinician believes that the rate of disease progression will be slowed sufficiently to avoid functional impairment from the disease (American Academy of Ophthalmology: 2010**)**. Large randomized control trials found that all modalities of IOP reduction slow the progression of glaucomatous optic neuropathy (Collaborative Normal-Tension Glaucoma Study Group (CNTGS) 1998, AGIS, OHTS).

1.4 GLAUCOMA MONITORING

Glaucomatous damage can be quantified using either structural (changes in the ONH and RNFL) by optical coherence tomography (OCT) or functional loss (visual field defects) by automated perimetry, or a combination of both (Brusini and Johnson, 2007; Medeiros FA et al, 2012a). Clinically, glaucoma progression can be observed by monitoring ONH changes

such as increased vertical cup to disc ratio (VCDR), disc haemorrhage, peripapillary atrophy, nasalization of blood vessels and neural retinal rim thinning.

Monitoring of disease progression is commonly done using serial evaluation of longitudinal series of visual field (functional) measurements (Kirwan JF et al, 2014; Saunders LJ et al, 2014). Standard automated perimetry (SAP) is one of the most common method for assessing visual field changes in glaucoma and measure the rate of glaucoma progression (Heijl A, 1989).

1.5 OXIDATIVE STRESS AND GLAUCOMA

Oxidative stress is known to contribute to glaucoma etiology and progression (Levin et al. 1996, Green et al. 1995, Bautista et al. 1999). Oxidative stress cause disturbance of the trabecular meshwork (TM) cells by causing cellular loss and overexpression or alteration in the structures of various glycoproteins in the extracellular matrix. These cause TM dysfunction lead to impairment of aqueous humour outflow and subsequently IOP elevation (Wentz-Hunter et al. 2004, Li et al. 2004). The damage increases when total antioxidant status decreases in the serum and aqueous humour of glaucoma patients (Rana et al. 2016).

Survival of RGCs is critically sensitive to the oxidative redox state, and decreasing reactive oxygen species (ROS) generation promotes the survival of RGCs (Geiger et al., 2002). One of the mechanisms proposed for RGC death in glaucomatous eyes is that elevated IOP-induced axonal injury at the ONH results in the blockage of neurotrophin transport to RGC bodies (Anderson and Hendrickson, 1974; Minckler et al., 1976; Pease et al., 2000; Quigley and Addicks, 1980). Apart from mechanical injury and neurotrophin deprivation, vascular insults

at the ONH (Flammer, 1994; Hayreh, 1985; Osborne et al., 2001) have been proposed to be responsible for RGC death in glaucoma.

1.6 DIETARY ANTIOXIDANT AND GLAUCOMA

Antioxidants represent the first line of defense against oxidative stress and are obtained through the diet and produced internally (Raman et al. 2016). Multiple cross-sectional studies were conducted using food frequency questionnaires in an attempt to link certain fruits and vegetables to glaucoma. Giaconi and colleagues concluded that there is a decreased likelihood of glaucoma with a greater intake of fruits and vegetables rich in vitamins A and C, and carotenes (Giaconi et al., 2008). Another study found a decreased glaucoma risk in those who consumed fruits and vegetables rich in vitamin A and carotenes, namely collard greens, kale, carrots, and peaches (Coleman et al., 2012). Cao and colleagues suggested that the plasma antioxidant capacity is increased with intake of a diet rich in sources of antioxidants such as fruit and vegetables (Cao et al. 1998).

In the study by Kang and associates (Kang et al. 2003), the authors did not find an association between the risk of POAG and six food groups, specifically citrus foods, cruciferous vegetables, yellow vegetables, green leafy vegetables, all fruits combined, and all vegetables combined, with the use of a food-frequency questionnaire. Although the strength of the study by Kang and associates was a large 10-year prospective study, the authors used self-report to identify cases of POAG. In their population of nurses and physicians, only 11.0% to 14.8% of subjects who reported glaucoma were considered actually to have glaucoma by the investigators. In addition, the investigators did not evaluate the records of a random sample of subjects who did not report a diagnosis of glaucoma; this could result in an additional

measurement bias because 50% or more of subjects with glaucoma are unaware of the diagnosis (Coleman, 1999; Topouzis et al. 2008).

1.7 NUTRIENTS AND ANTIOXIDANTS CONTENT OF PAPAYA

Papaya (Carica papaya L. cv. Eksotika) is one of the most commonly consumed tropical fruits by Malaysians. Papaya is a perennial plant which is non-seasonal, easily available and affordable by many. Papaya fruit belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a wide variety of diseases (Mello et al. 2008, Munoz et al. 2000). The content of antioxidants in papaya is among the highest compared to other local fruit (Lim Y.Y et al. 2007). Papaya has known to be a rich sources of powerful antioxidant nutrients such as, vitamin C, flavonoids and carotenes (Wall 2006).

Flavonoids comprised of neuroprotective compound with potent antioxidant and free radical scavenging properties. Flavonoids demonstrated high potency in protecting RGCs from oxidative stress–induced death potency with low toxicity (Pamela and Anne, 2005). In addition, flavonoids have been shown to induce neurite outgrowth (Sagara et al. 2004), reduce inflammation (Read, 1995), and inhibit endothelial cell proliferation (Fotsis et al. 1998). Papaya also contains vitamin B, folate and pantothenic acid; minerals such as, potassium, copper, magnesium; and fibre. In addition, papaya contains several unique protein-digesting enzymes including *papain* and *chymopapain*. These enzymes along with its antioxidant nutrients have been shown to reduce inflammation (Owoyele BV et al. 2008).

A single serving of papaya (276 gram) provides 168mg of vitamin C accounting for 224% of daily recommended intake, 2622 IU of vitamin A, 756 mcg beta carotene, 245 mcg of lutein

and zeaxanthin. It provides 119 calories per serving with medium glycemic index that make it suitable for diabetic patients. In comparing papaya with other fruit such as banana, apple, mango, and pineapple, it has been found to be more superior in providing good natural source of macronutrients (carbohydrates and proteins) and micronutrients (vitamin A and vitamin C) (Peterson et al. 1982).

Excessive consumption of papaya (greater than 30 mg a day) can cause carotenemia, which is otherwise harmless (Rock 1997). Papaya only contains about 6% of the level of beta carotene found in carrots (the most common cause of carotenemia) (Aravind et al., 2013). Papain is also a potential allergen, according to Purdue University, people who eat too much papaya and ingest high levels of papain may develop symptoms consistent with hay fever or asthma, including wheezing, breathing difficulties and nasal congestion (Aravind et al., 2013). The high fibre content of papaya can also contribute to unrest of the digestive system.

1.8 RATIONALE OF STUDY

Based on food frequency questionnaire (FFQ), food rich with vitamins and antioxidant are found to reduce the risk of glaucoma (Pamela et al. 2005; Giaconi et al 2012; Coleman et al. 2008). The content of antioxidants in papaya is among the highest compared to other local fruit. Due to easily available, inexpensive and the higher concentration of antioxidants, there is potential role of papaya as the supplement or complementary treatment for glaucoma. However, there is no available study on frequent consumption of fresh papaya as complementary treatment for IOP reduction in glaucoma patients. The potential effect of papaya on structural changes of ONH and RNFL has not been studied previously also.

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Chapter 2

Objective

STUDY OBJECTIVES

2.1 General Objective

To evaluate the effect of papaya intake on intraocular pressure (IOP), retinal nerve fibre layer thickness (RNFL), optic nerve head (ONH) parameters and serum total antioxidant capacity (TAC) in primary open angle glaucoma (POAG) patients.

2.2 Specific Objective

2.2.1 To compare the IOP measurement at baseline, first, second and third month between POAG patients taking papaya and not taking papaya.

2.2.2 To compare retinal nerve fibre layer (RNFL) thickness and ONH parameters at baseline, first, second and third month between POAG patients taking papaya and not taking papaya.

2.2.3 To compare the serum total antioxidant capacity (TAC) level at baseline, first, second and third month between POAG patients taking papaya and not taking papaya.

Chapter 3
Manuscript

Title: A RANDOMIZED CONTROLLED TRIAL ON THE EFFECT OF DIETARY INTAKE OF PAPAYA ON INTRAOCULAR PRESSURE, RETINAL NERVE FIBER LAYER THICKNESS, OPTIC NERVE HEAD PARAMETERS AND SERUM TOTAL ANTIOXIDANT CAPACITY IN PRIMARY OPEN ANGLE GLAUCOMA PATIENTS

Authors:

Author 1
Dr Chan Hui Tze
MBBS (Melaka Manipal Medical College)
Trainee in Ophthalmology

Address 1
Department of Ophthalmology
School of Medical Sciences
Universiti Sains Malaysia
Health Campus
Jalan Raja Perempuan Zainab 2,
16150, Kota Bharu, Kelantan

Address 2
Ophthalmology Clinic
Hospital Universiti Sains Malaysia
Jalan Raja Perempuan Zainab 2,
16150, Kota Bharu, Kelantan
Email: chanhuitze@gmail.com

Author 2
Prof. Dr Hamid Jan bin Jan Mohamed
Dip. Medical Lab Technology (USM); B.Sc. Nutrition & Community Health (UPM); M.HSc. Nutrition (UKM); Ph.D Nutrition (London) ; Grad. Cert. Academic Practice (London)
Department of Nutrition and Dietetics Programme
School of Health Sciences
Universiti Sains Malaysia
Health Campus
Email: hamidjan@usm.my

Author 3
Assoc. Prof. Dr Che Badariah Ab Aziz
MBBS (UM), MSC (USM), PhD (Nottingham)
Department of Physiology
School of Medical Sciences
Universiti Sains Malaysia
Health Campus
Jalan Raja Perempuan Zainab 2,
16150, Kota Bharu, Kelantan
Email: badariah@usm.my

Author 4 (corresponding author) Prof Dr Liza-Sharmini Ahmad Tajudin
MBBS (Malaya), MMed (Ophthal)(USM), PhD (UK), AM (Malaysia)
Professor of Ophthalmology and Senior Consultant Ophthalmologist (Glaucoma)
Department of Ophthalmology
School of Medical Sciences
Universiti Sains Malaysia
Health Campus
Jalan Raja Perempuan Zainab 2,
16150, Kota Bharu, Kelantan
Email: lizziesharmini@gmail.com

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Address for reprints:
Department of Ophthalmology
School of Medical Sciences
Universiti Sains Malaysia Health
Campus
16150 Kota Bharu Kelantan
Tel: +609 767 6353/6363
HP: +6019 9179227
Fax: +609 765 3370
liza@usm.my; sharminiliz@live.com

3.1 ABSTRACT

Purpose: To evaluate the effect of papaya consumption on intraocular pressure (IOP), retinal nerve fiber layer (RNFL) thickness and optic nerve head (ONH) parameters and serum total antioxidant capacity (TAC) in Malaysian residents with primary open angle glaucoma (POAG).

Methods: A randomized single-blinded controlled trial involving 40 patients with POAG was conducted between February 2018 and October 2019. The patients were divided into two groups (n=20): Group A consumed a single serving of papaya (~250 gm) three times a week for 3 months; Group B consumed a normal diet. Each group recorded daily dietary intakes, and the assigned treatment was continued throughout the study. IOP, RNFL, ONH parameters and serum TAC were recorded at baseline and then monthly for three months by a single blinded operator. Data were analyzed by repetitive-measure analysis of variance.

Results: No significant difference was noted in mean age, sex, or race between the groups. The mean duration of diagnosis was 63.2 months (SD: 14.1) for group A and 67.6 months (SD: 13.6) for group B. The mean baseline IOP was slightly higher in group A, at 15.7 mmHg (SD 1.6). Group A showed greater reductions in IOP, recording 1.6 mmHg (SD: 0.4), 2.3 mmHg (SD: 0.3), and 3.2 mmHg (SD: 0.4) at 1, 2, and 3 months, respectively. The difference in IOP between the two groups was significant ($p<0.001$). Group A showed increasing pattern of RNFL thickness with mean thickness of 73.00µm (SD: 11.1) at baseline, 74.20 µm (SD: 10.5) at 1 months, 74.90 µm (SD: 10.3) at 2 months and 76.10 µm (SD: 10.5) at 3 months. There was significant increment of RNFL thickness in group A compared to group B ($p=0.001$). There were no significant association between papaya consumption with serum TAC

(p=0.210), vertical cup disc ratio (p=0.174), and rim area (p=0.415).

Conclusions: Regular intake of fresh papaya can help to reduce IOP and improve RNFL thickness in patients with POAG. These patients should be encouraged to include fruits, especially papaya, in their diets.

Keywords: primary open angle glaucoma, intraocular pressure, serum total antioxidant capacity, papaya

3.2 INTRODUCTION

Glaucoma is estimated to affect 76.0 million persons aged 40–80 years in 2020 and 111.8 million by 2040. The highest prevalence of primary open angle glaucoma (POAG) is reported in Africa, while Asia has the highest incidence of primary angle closure glaucoma (PACG).[1] Raised intraocular pressure (IOP) is the most significant risk factor for glaucoma development and progression,[2,3] and the basis of glaucoma management remains IOP reduction through different treatment modalities. [4,5] However, some patients continue to suffer from progressive optic neuropathy and visual field loss despite a well-controlled IOP.[6] Consequently, newer therapeutic approaches have been examined in an attempt to slow the progression of glaucoma through alternative mechanisms. Many of these agents currently fall within the definition of complementary and alternative medicine (CAM), which aims to inhibit apoptosis of the retinal ganglion cells, reduce oxidative stress, and optimize perfusion of the optic nerve.[7–10]

The National Center for Complementary and Alternative Medicine defines alternative medicines as treatments and healthcare practices that are not taught widely in medical schools,

not generally used in hospitals, and not usually reimbursed by medical insurance companies.[11] The increasing emphasis on holistic approaches and lifestyle modifications for combating diseases and attaining a healthier body has meant that many people are now receptive to CAM. Up to 5–10% of patients with glaucoma seek these supplementary treatments, and almost 60% use more than one modality.[12-14] The results of an extensive questionnaire survey have indicated that a dietary intake rich in antioxidants is potentially protective against the development and progression of glaucoma.[15-22] Fruits and vegetables have been identified as rich sources of antioxidants.[23,24]

One of the most commonly consumed fruits in Malaysia and other tropical countries is papaya (*Carica papaya*). The papaya tree belongs to the Caricaceae family, and several species of the Caricaceae have been used as remedies against a wide variety of diseases.[25,26] Most studies have reported that papaya fruits and leaves have high antioxidant capacity due to their high contents of vitamin B (in leaves), C, E, flavonoids, and carotenoids (in fruits).[27,28] Flavonoids are low-toxicity neuroprotective compounds with free radical scavenging properties that show high potency in protecting retina ganglion cells (RGCs) from oxidative stress–induced death.[29] Flavonoids have also been shown to induce neurite outgrowth,[30] reduce inflammation,[31] and inhibit endothelial cell proliferation.[32] Oloyede et al.[33] reported that papaya consumption by mice significantly increases the activities of glutathione reductase, a critical molecule for resisting oxidative stress and maintaining the reducing environment of the cell.

The nutrient content, and especially the high content of antioxidants, in papaya makes this fruit a relatively inexpensive and easily available dietary supplement for patients with glaucoma in tropical countries. Glaucoma is a disease of longevity, so the high palatability of papaya makes

it a good CAM treatment for the elderly. Papaya is also a better natural source of macronutrients (carbohydrates and proteins) and micronutrients (vitamin A and vitamin C) than other fruits.[34] Our objective in the present study was to determine the effects of papaya consumption on IOP, RNFL thickness, ONH parameters and serum total antioxidant level in patients with POAG.

3.3 MATERIALS AND METHODS

A randomized single-blinded control trial was conducted involving patients with POAG between February 2018 and October 2019 at Hospital Universiti Sains Malaysia, Malaysia. POAG was defined as optic nerve damage and disc abnormalities (vertical cup disc ratio, VCDR > 97.5th percentile) with typical glaucomatous visual field defects in an eye with no evidence of angle closure on gonioscopy and no identifiable secondary causes[35]. This study received ethical approval from Research and Ethical Committee, School of Medical Sciences, Universiti Sains Malaysia (USM/JEPeM/17100474) and was conducted in accordance with the World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects.

The recruitment of patients with POAG was conducted among glaucoma patients attending the ophthalmology clinic at the Hospital Universiti Sains Malaysia. The inclusion criteria included a confirmed case of POAG, age between 40 and 70 years, good compliance with pressure lowering agents, and an achieved target IOP. Exclusion criteria included (i) active smokers, (ii) irritable bowel disease, (iii) poorly controlled diabetes mellitus with HbA1c of >9.0%, (iv) a history of previous intraocular surgery within the past 6 months, other than uncomplicated cataract treatment (trabeculectomy or glaucoma drainage device implantation), and (v) media opacities that affected the reliability of optical coherence tomography (OCT) measurements.

Patients who were allergic to papain or had a history of taking antioxidant supplements 3 months prior to recruitment were also excluded. If both eyes were eligible, the right eye was selected.

A total of 67 patients were invited to participate in the study. Their demographic data, systemic co-morbidities, and duration and treatment of POAG were obtained from the medical records. After receiving a briefing about the study, two patients claimed they were allergic to papaya and another five patients expressed their dislike of papaya and refused to participate. Seventeen patients refused to be enrolled due to transportation problems (13 patients) or poor family support (4 patients). At the end of the initial briefing, a total of 43 patients agreed to participate in this study (Figure 1).

A thorough ocular examination was conducted, including IOP measurements and optic nerve head (ONH) imaging using a HD Cirrus OCT instrument. Only patients with high quality OCT images and signal strength of more than 6/10 were selected for randomization. Three patients were excluded due to poor OCT images after three attempts with a dilated pupil. The selected patients were then randomized into 2 equal-sized groups (A and B) using sequentially numbered, opaque sealed envelopes.

The patients in group A were assigned to consume raw papaya as a single serving (~250 gram) once per day, three times per week for a three-month duration. They were not allowed to blend, cook, or add any preservative or sweetener to the papaya. The patients in group B were assigned to consume their normal dietary intake.

Fresh papayas bought from a nearby farmers' market were distributed weekly to group A by a research assistant throughout the study period. To ensure compliance, group A patients were reminded weekly to consume their papaya through text messages or phone calls from a research assistant. Patients who consumed papaya less than three times a week were excluded from the study. Each patient in both groups was given a dietary diary to document the consumption of papaya and other foods, including other fruits.

The side effects from papaya consumption, such as carotenemia, hypervitaminosis, asthma-like symptoms, nasal congestion, diarrhea, and symptoms of hyperglycemia in patients with diabetes,[36] were monitored through self-reporting or enquiry by the primary investigator (CHT in weekly phone calls or during the clinic visits. None of the patients in group A developed any side effects of papaya. All current topical pressure-lowering drugs were continued throughout the study, without any changes.

The IOP measurements were conducted using a Goldmann Applanation Tonometer (GAT) (Haag-Streit International, UK) with the patient in a sitting position. The measurements were made between 0900 and 1200 by the primary investigator (CHT), who was blinded to the subject's grouping. An IOP measurement was taken at baseline and then monthly for three months for each patient in both groups. Before the IOP measurement, the subjects were advised to avoid drinking coffee or any caffeinated drink 24 h before and to take only a light meal 2 h before to reduce the effect of hyperglycemia and hydration on IOP. Three IOP readings were taken, and the mean reading was used for analysis.

Venesection was conducted on patients from both groups to obtain 3 mL of blood, which was

stored in a plain tube. The blood sample was centrifuged at 5000 gforce for 10 min to separate the serum, which was stored in a -80 C freezer until analysis. Quantification of total antioxidant capacity (TAC) was performed using a QuantiChromTM Antioxidant Assay Kit (DTAC-100, BioAssay System, USA), according to the manufacturer's instructions. The serum was mixed with the diluted reagent in an Eppendorf tube, transferred to a 96-well plate and measured with an ELISA reader at optical density of 570 nm. A prepared standard curve and a formula provided in the test kit were used to calculate the serum TAC. The concentration of serum TAC was measured in blood samples taken at baseline, 1, 2, and 3 months post recruitment.

All data were rechecked to avoid incorrect data entry and missing data using IBM SPSS Statistic version 24. Descriptive analysis of age, sex, race, and systemic disease were analyzed using a paired-t test and chi-square test. Repeated measures analysis of variance (RM ANOVA) was applied to repeated IOP and serum TAC measurements between the two groups. A p-value of <0.05 was deemed statistically significant.

3.4 RESULTS

3.4.1 Patient characteristics

A total of 40 patients with POAG were recruited and randomized to the two groups (Figure 1). Patients in group A were older than those in group B, but the difference was not statistically significant. Patients in group B had a longer duration of diagnosis with POAG. The number of hyperlipidemic patients was also significantly higher in group B (4 times higher) than in group A (Table 1). Most patients were bilateral POAG, and the right eye (90%) was selected for analysis. Most patients had vision of 6/12 or better. In group A, 11 (55%) patients were pseudophakic and 9 (45%) were phakic, whereas group B had an equal distribution of

pseudophakic and phakic patients.

3.4.2 Effect of papaya intake on intraocular pressure

The mean IOP at baseline was slightly higher in group A than in group B, but the difference was not statistically significant. Subsequently, group A showed a further reduction in IOP after 1, 2, and 3 months of papaya intake (Figure 2a), whereas the mean IOP remained static in group B, without any significant changes throughout the 3 months of follow up. The differences between group A and group B were statistically significant and remained strong even when analyzing intra and intergroup effects using RM ANOVA ($p<0.001$) (Figure 2a). The mean IOP difference was significant in almost all pairings of time of follow-up except for 1–2 months after papaya intake (Table 2).

3.4.3 Effect of dietary papaya intake on retinal nerve fiber layer

Mean RNFL thickness in group A (73 μm) were higher at baseline on comparison with group B (69 μm) but without statistically significance. There was significant thicker RNFL thickness in group A compared to group B at 3 months post study intervention ($p=0.001$) (Figure 2b). Group A showed exponential increased in RNFL thickness during the monthly OCT assessment (Figure 2b). There were minimal changes in RNFL thickness in group B (Figure 2b). The comparison of RNFL thickness according to pairing of visits shown significant difference between baseline-3 months, 1 month-3 months and 2 months-3 months post intervention in group A (Table 3). The mean increase of RNFL thickness was 3.1 μm (95% CI 1.2, 5.0) between baseline and 3 months post consumption of raw papaya (Table 3).

3.4.4 Effect of dietary papaya intake on optic nerve head parameters

The mean vertical cup disc ratio in group B was significantly higher than group A at baseline, second and third month (Figure 2c).However, there was no statistically significant different between the two groups based on RM ANOVA (Figure 2c). The rim area shown see-saw effect in group B and static changes in group A (Figure 2d). There was also no significant difference in mean rim area between group A and B (Figure 2d).

3.4.5 Effect of papaya intake on serum TAC

The mean serum TAC was significantly higher in group A than in group B at baseline. Subsequently, serum TAC remained significantly higher in group A than in group B at 1, 2, and 3 months (Figure 2e). However, no statistically significant difference was evident between the two groups based on RM ANOVA (Figure 2e).

3.5 DISCUSSION

Interest is growing in the role of dietary intake in glaucoma due to the medicinal potential of modification of eating habits as a supplementary treatment.[37] The papaya plant, and especially its leaves, has potential as a CAM source for various diseases, and papaya fruit has been included in various concoctions for CAM.[38-40] In the current study, a significant reduction in IOP and RNFL increment was confirmed following consumption of a serving of papaya three times a week for 3 months.

To the best of our knowledge, the present study is the first pilot clinical trial to assess the effect of intake of a single dietary fruit on patients with glaucoma or to examine the effect of papaya consumption on IOP, RNFL, ONH parameters and serum TAC. Fruits and vegetables have been established as potential anti-oxidant dietary sources for treatment of chronic degenerative diseases such as diabetes and cancer.[40-43] Similar findings were found for patients with glaucoma based on many studies using food frequency questionnaires.[37,44] For example, Bonyadi et al.[45] reported that daily oral intake of saffron capsules, an antioxidant supplement, resulted in a statistically significant reduction of IOP in patients with POAG.

Group B contained a significantly higher number of hyperlipidemic patients. Some evidence suggests that statins (a treatment for hyperlipidemia) cause further reductions in IOP through facilitation of aqueous humor outflow.[46] The immunomodulatory properties of statins have also been proposed to protect retinal ganglion cells (RGCs) against glaucomatous damage.[47] The statin effect could explain the lower IOP seen at baseline in group B. However, another possibility is that papaya had a higher pressure-lowering effect than statins in this study. The protocol of the present study required monthly visit and special home visit by the research assistant, which may motivate them to be more adherence and persistence to their present treatment regime which leads to better IOP control as well.

A 3-day-per-week papaya consumption was adopted in the present study. In general, daily consumption of papaya is recommended to achieve adequate serum levels of antioxidants and to maximize the potential effects of antioxidants on patients with glaucoma. However, the repeated consumption of the same food every day may lead to boredom, loss of appetite, and palatability issues, which may affect compliance. Therefore, we decided to adopt a regime of

3 servings of papaya per week. This interval also decreased the risk of hypervitaminosis, carotenemia, and hyperglycemia, especially in diabetic patients.[48,49]

The increased in RNFL thickness, one could postulate this is due to edema as there were no significant association seen with VCDR and rim area. There is possibility of edema due to the inflammation or reaction towards sudden influx of nutrient in the RNFL. On the other hand, papaya also contains papain, an enzyme to reduce inflammation[50]. Papain has been used for treatment of burn in pediatric patients[51]. Papaya also contains other endopeptidase which are used to treat edema[52]. Therefore, edema of RNFL causing wrong interpretation of thicker RNFL is less likely. There is no significant ONH parameter changes may be due to short follow-up period. Structural changes may need longer follow-up to occur compare to biochemical changes.

No significant elevation of serum TAC was observed in group A. In fact, the level of serum TAC was difficult to interpret because it dropped at 2 months. This decline could be due to many confounding factors, which are unavoidable in a study involving food intake. Although dietary diaries were maintained and electronic reminders were given, the patients' compliance with papaya intake was not guaranteed. Errors in dietary reporting could also have resulted in biases. Physical activities and emotional instabilities could also affect serum TAC level.[53,54] Frequent visits to the hospital and changes in dietary consumption (papaya intake) based on this protocol may also have caused unwanted stress to the patients.

The antioxidant content of papaya is affected by the maturity of the fruit upon consumption. In this study, papaya was delivered to each patient's home on a weekly basis and kept in the

refrigerator, so the freshness of the papaya was not standardized. The papayas were slightly ripened at the beginning of the week and fully ripened at the end of the week. Significant differences have been reported on the antioxidant activities of papaya fruit based on the ripening stage[55,56]. The scavenging activity for free radicals, the total flavonoid content, and the ferric reducing antioxidant power of papaya are also linearly associated with the ripening stage of the fruit.[57] This could explain the erratic concentrations of serum TAC observed in our study.

This study had a prospective design and was conducted in a single center without any dropouts. However, it had some limitations. One was its sample size, which was rather small (40 patients with POAG). A multiple-center randomized controlled trial should perhaps be conducted in the future to increase the sample size. Another limitation was that the target IOP was also not personalized in this study. We used a cutoff point of less than 18 mmHg[57] as a definition of good IOP control. Further limitations in this study include residual confounding by unmeasured variables, such as visual field defects, family history of glaucoma, and certain lifestyle factors.

In conclusion, papaya has potential clinical use as a CAM for IOP reduction. A larger sample size in prospectively designed trials is required to confirm the initial findings presented in this report. Patients with POAG should be advised to consume more fruits, and particularly papaya, as this may help in IOP reduction.

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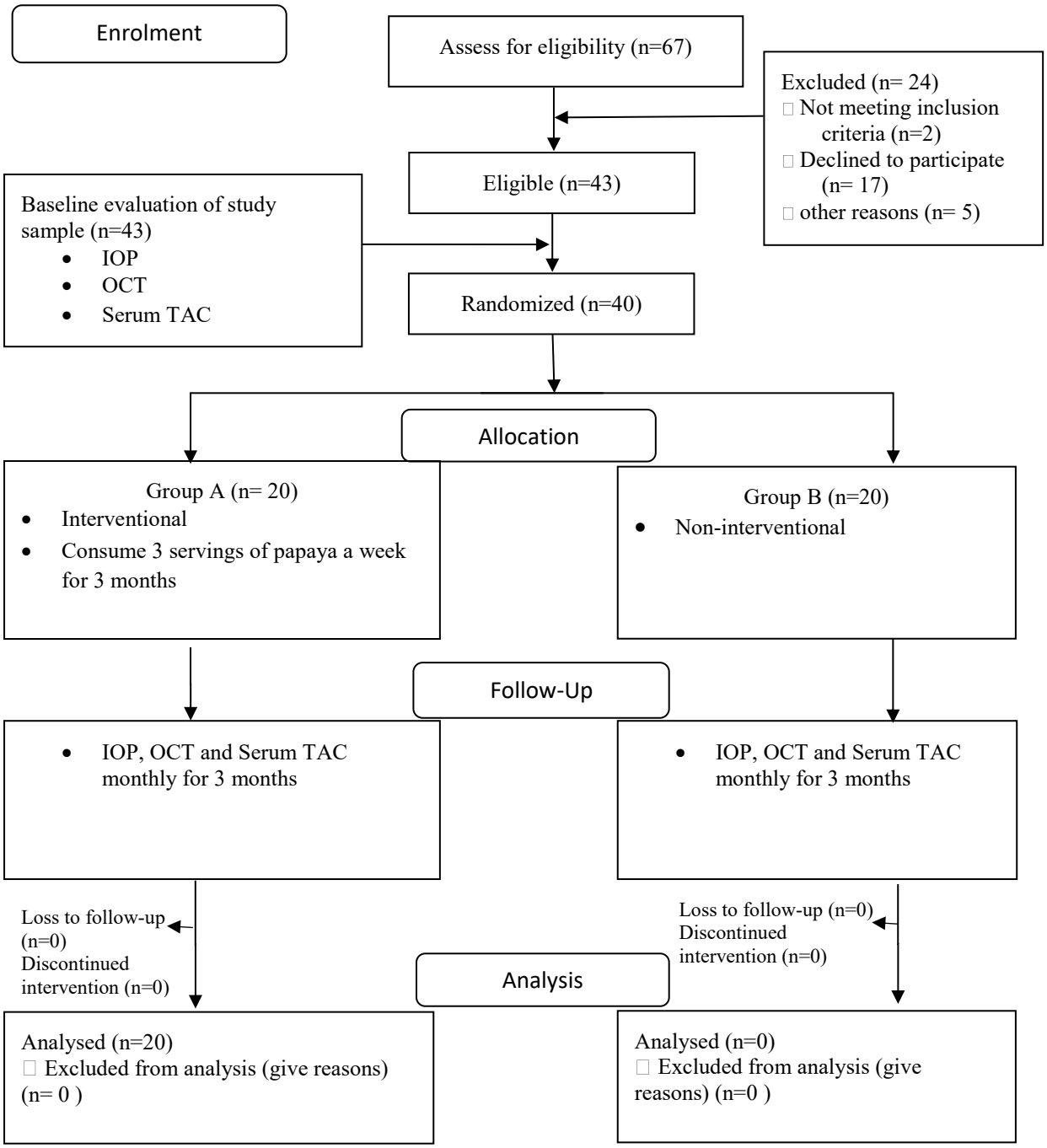
3.7 Tables and figures

Figure 1: Consort figures of participants



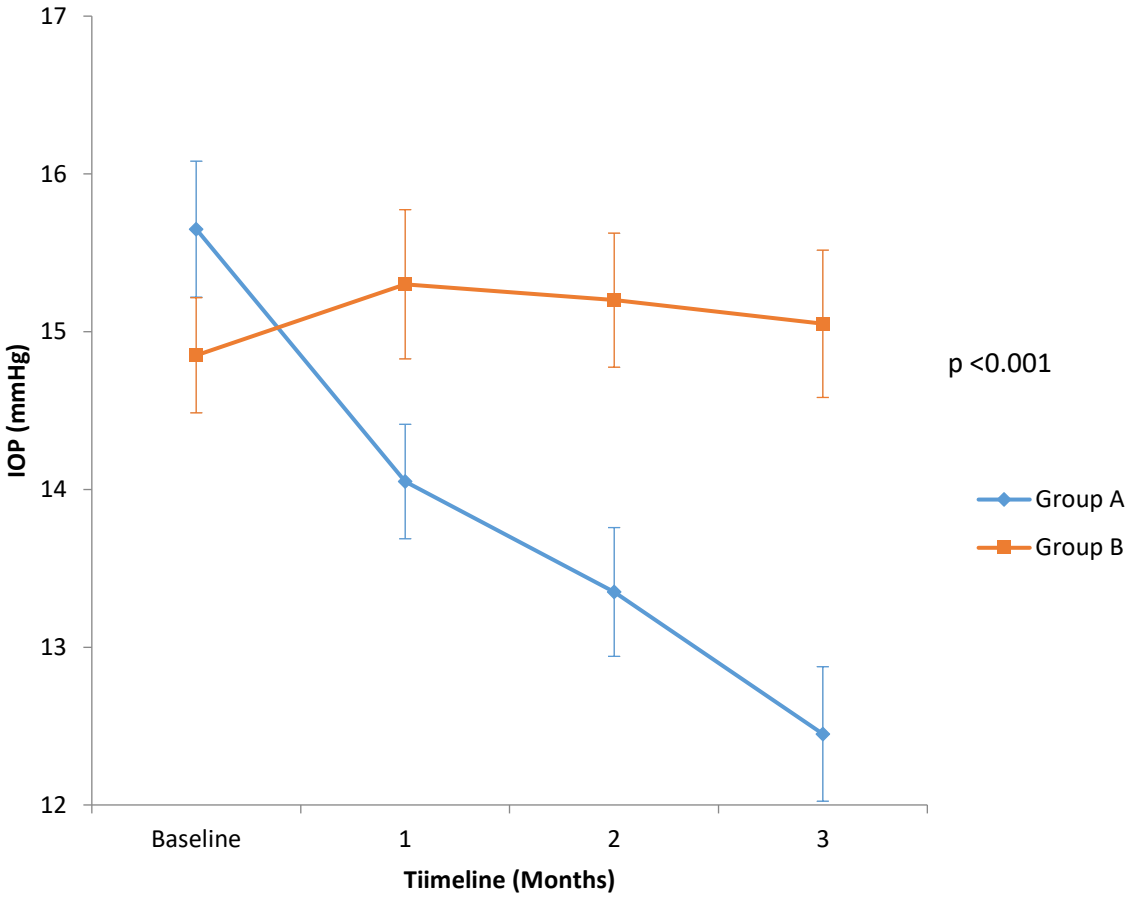


Figure 2a: Effect of dietary papaya on IOP

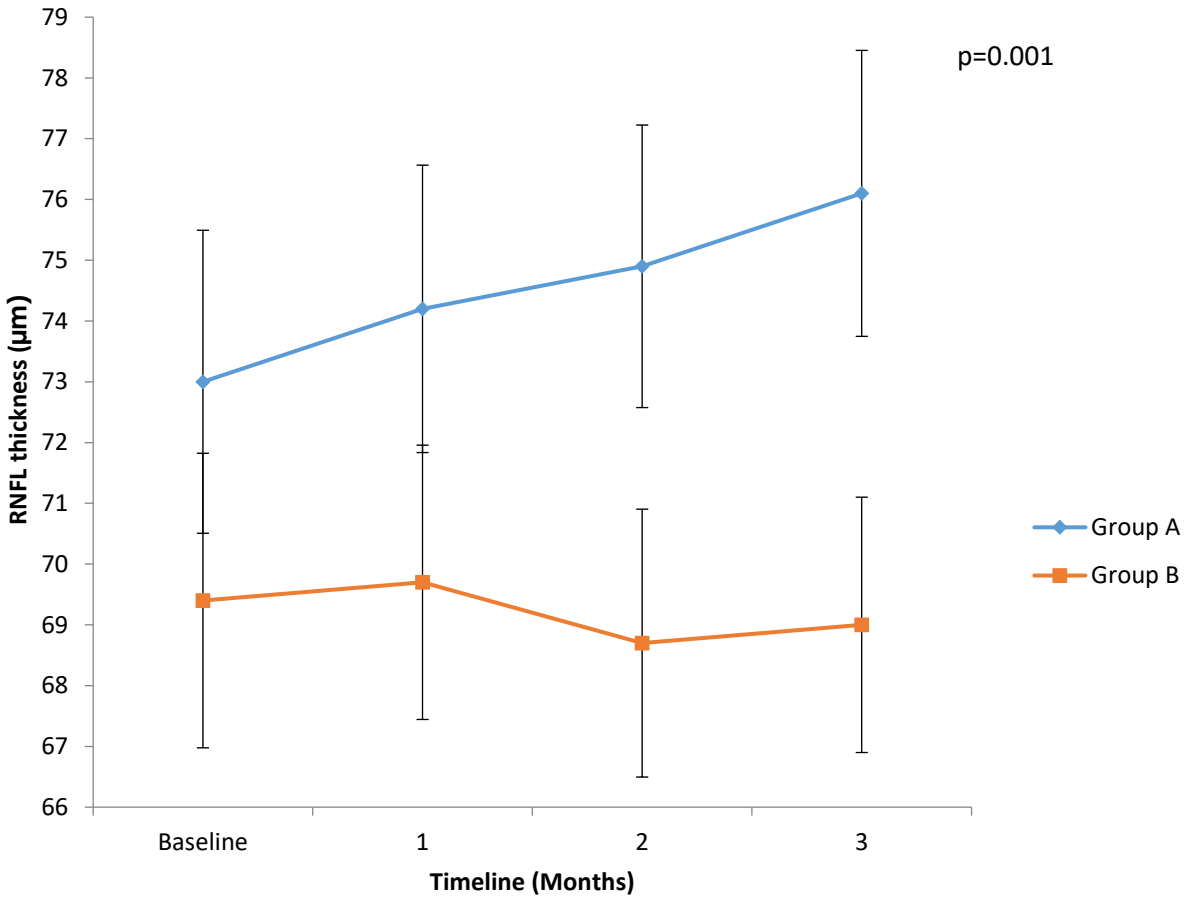


Figure 2b: Effect of dietary papaya intake on RNFL thickness

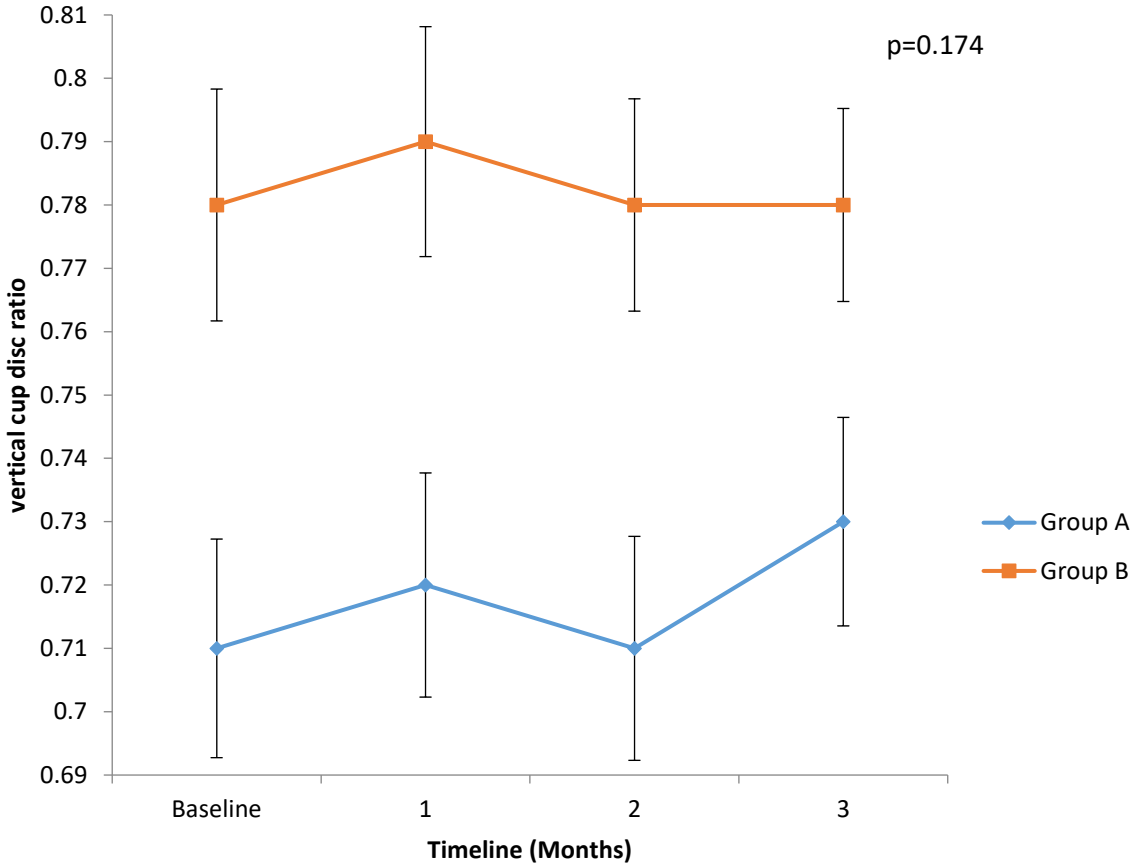


Figure 2c: Effect of dietary papaya intake on vertical cup disc ratio

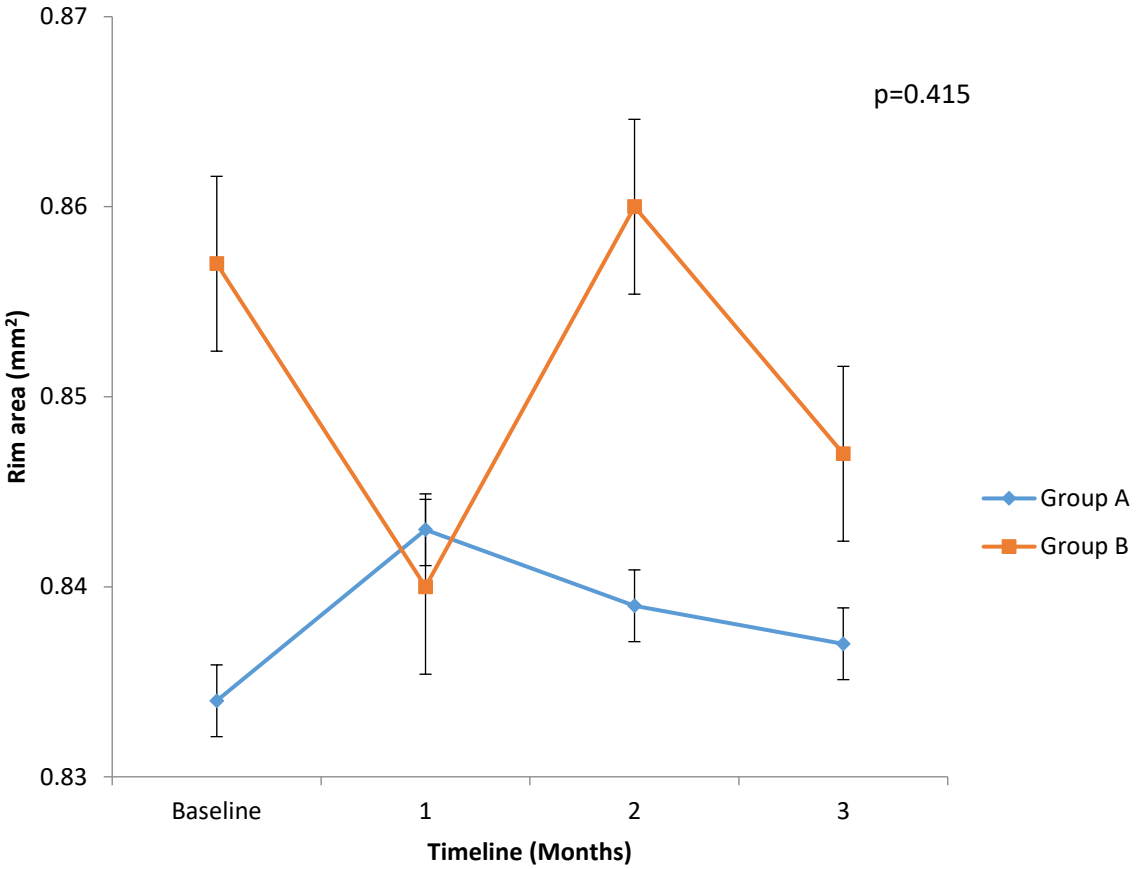


Figure 2d: Effect of dietary papaya intake on rim area

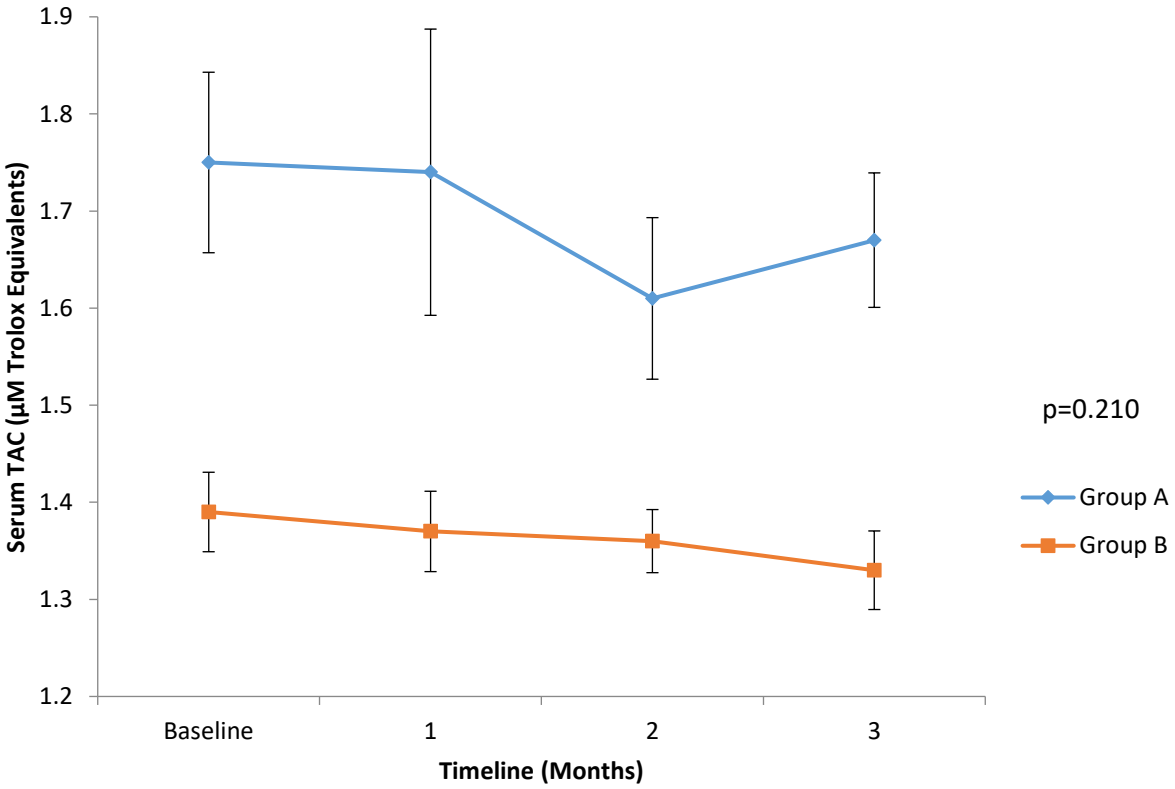


Figure 2e : Effect of dietary papaya intake on serum TAC

Table 1: Distribution of demographic data among study group

Characteristic	Group A (n=20)	Group B (n= 20)	P value
Age (years)	62.2 ± 7.7	59.6 ± 5.3	0.205#
Sex (n)			
Male	9 (45%)	10 (50%)	0.752*
Female	11 (55%)	10 (50%)	
Race (n)			
Malay	16 (80%)	18 (90%)	0.376*
Chinese	4 (20%)	2 (10%)	
Systemic disease (n)			
DM	10 (50%)	13 (65%)	0.337*
HTN	9 (45%)	15 (75%)	0.053*
HPL	2 (10%)	8 (40%)	0.028*
IHD	1 (5%)	1 (5%)	1.000*
CKD	0	2 (10%)	0.147*
Duration of diagnosis (months)	63.2 ± 14.1	67.6 ± 13.6	0.321*

DM-Diabetes mellitus, HTN-Hypertension, HPL-Hyperlipidemia, IHD-Ischemic heart disease, CKD-Chronic kidney disease

Statistical analyses were performed using the

independent t-test

* chi-square test

$p<0.05$ is significan

Table 2: Monthly comparison of mean IOP difference for Group A and B

	Group A				Group B			
Time Difference *	Mean IOP difference (SD) (mmHg)	95% CI of the difference		p-value	Mean IOP difference (SD) (mmHg)	95% CI of the difference		p-value
		Lower Bound	Upper Bound			Lower Bound	Upper Bound	
Vo-V1	1.6 (0.4)	0.366	2.834	**0.007**	-0.4 (1.1)	-0.964	0.064	0.083
Vo-V2	2.3 (0.3)	1.128	3.472	**<0.001**	-0.3 (1.0)	-0.860	0.160	0.167
Vo-V3	3.2 (0.4)	1.926	4.474	**<0.001**	-0.2 (1.0)	-0.694	0.294	0.408
V1-V2	0.7 (0.2)	-0.130	1.530	0.135	0.1 (0.7)	-0.269	0.469	0.577
V1-V3	1.6 (0.3)	0.635	2.565	**0.001**	0.2 (0.9)	-0.202	0.702	0.262
V2-V3	0.9 (0.2)	0.104	1.696	**0.021**	0.1 (0.9)	-0.312	0.612	0.505

*Vo- Baseline visit, V1-month 1 visit, V2-month 2 visit, V3-month 3 visit

A one-way repeated measures analysis of variance (RM ANOVA) with Bonferroni correction $p<0.05$ is significant

Table 3: Monthly comparison of mean RNFL difference for Group A and B

	Group A				Group B			
Time Differ-ence*	Mean RNFL difference (SD) (µm)	95% CI of the difference		p-value	Mean RNFL difference (SD) (µm)	95% CI of the difference		p-value
		Lower Bound	Upper Bound			Lower Bound	Upper Bound	
Vo-V1	-1.20 (0.6)	-3.093	.693	0.466	-0.30 (3.7)	-2.052	1.450	0.724
Vo-V2	-1.90 (0.7)	-4.149	.349	0.134	0.65 (4.8)	-1.614	2.910	0.555
Vo-V3	-3.10 (0.6)	-4.997	-1.203	**0.001**	0.35 (2.3)	-0.769	1.490	0.520
V1-V2	-0.70 (0.5)	-2.256	.856	1.000	0.95 (3.3)	-0.617	2.517	0.220
V1-V3	-1.90 (0.4)	-3.215	-.585	**0.003**	0.65 (3.1)	-0.793	2.093	0.358
V2-V3	-1.20 (0.4)	-2.281	-.119	**0.024**	-0.30 (4.4)	-2.360	1.760	0.764

*Vo- Baseline visit, V1-month 1 visit, V2-month 2 visit, V3-month 3 visit

A one-way repeated measures analysis of variance (RM ANOVA) with Bonferroni correction $p<0.05$ is significant

3.8 Letter to Editor of Journal of Complementary Therapies in Medicine

26th October 2020
Kathi Kemper
Editor-in-Chief
Complementary Therapies in Medicine

Dear Sir,

Please find the attached manuscript entitled **"Effect of Dietary Papaya Intake on Intraocular Pressure, Retinal Nerve Fiber Layer Thickness, Optic Nerve Head Parameters and Serum Total Antioxidant Capacity in POAG patients"** for your consideration for publication as original article in American Academy of Ophthalmology Journal.

The use of complementary and alternative medicine, targeting both IOP-dependent and non-IOP-dependent mechanisms in glaucoma has received much interest from ophthalmologists and glaucoma patients. We evaluated the effect of nutritional antioxidant in POAG patients in our population. There was statistical significant reduction of IOP and RNFL increment with regular dietary papaya consumption.

Based on our knowledge, this is the only available study looking into the effect of a single dietary fruits in POAG patients.

Without doubt, our manuscript will provide invaluable knowledge to the scientific community especially among ophthalmologists and public health. This manuscript is not been considered or submitted in any other journal.

I look forward to a favorable reply from you and I thank you in anticipation.

Yours sincerely,

Professor Dr Liza-Sharmini Ahmad Tajudin
MBBS (Malaya), M.Med (Ophthal) (USM), PhD (UK)
Senior Consultant Ophthalmologist Department of Ophthalmology
School of Medical Sciences
Universiti Sains Malaysia
Health Campus
16150 Kota Bharu
Kelantan
MALAYSIA
Email : liza@usm.my
sharminiliz@live.com
Tel no : 609 767 6353

3.9 Journal Format for Journal of Complementary Therapies in Medicine

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It is the author's responsibility to ensure that a patient's anonymity be carefully protected and to verify that any experimental investigation with human subjects reported in the manuscript was performed with informed consent and following all the guidelines for experimental investigation with human subjects required by the institution(s) with which all the authors are affiliated. Authors should mask patients' eyes and remove patients' names from figures unless they obtain written consent from the patients and submit written consent with the manuscript.

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The Journal of Glaucoma is now accepting submissions for Cover Art. Images may be cropped or resized to fit into the allotted cover space. A minimum size of 3" to 5" is required. Acceptance is contingent on the artist's signing the journal's copyright release (see Authorship Verification Process). Authors must also submit a related Cover Art description of 50 to 200 words and is subject to editing. High resolution images of 300 dpi is important, preferably .JPG or .EPS files. Permissions for reproductions must be uploaded with the submission is applicable. Submissions will be accepted through Editorial Manager. Please choose Cover Art as the article type.

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All Original Articles must include a précis of 35 words or fewer summarizing the main finding/outcome of the study. The précis should not duplicate the abstract conclusion. The précis should also be included in your TEXT before your abstract.

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The TEXT portion of your manuscript must be submitted in WORD (.DOC or .DOCX format). Organize the manuscript into four main headings: Introduction, Materials and Methods, Results, and Discussion. Define abbreviations at first mention in text and in each table and figure. If a brand name is cited, supply the manufacturer's name and address (city and state/country). Acknowledge all forms of support, including pharmaceutical and industry support, in an Acknowledgments paragraph. Be sure your text is carefully reviewed for proper grammar and syntax prior to submitting. Non-native English-speaking authors may consider the aid of editing services. Should you choose to employ professional editing services, please be sure the company you choose has knowledge of ophthalmology terminology and its proper usage. Please see the Language and Editing Services section listed above.

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For a list of standard abbreviations, consult the Council of Biology Editors Style Guide (available from the Council of Science Editors, 9650 Rockville Pike, Bethesda, MD 20814) or other standard sources. Write out the full term for each abbreviation at its first use unless it is a standard unit of measure. Keep abbreviations to the minimum. Do not use abbreviations in the abstract.

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The authors are responsible for the accuracy of the references. Key the references (double-spaced) at the end of the manuscript. List the references in the order of their citation in the manuscript. Cite abstracts and unpublished data, such as papers submitted but not yet accepted for publication or personal communications, in parentheses in the text. If there are more than three authors, name only the first three authors and then use et al. Refer to the List

of Journals Indexed in Index Medicus for abbreviations of journal names, or access the list at http://www.nlm.nih.gov/tsd/serials/lji.html. Sample references are given below:

Journal article
1. Budenz DL, Chen PP, Weaver YK. Conjunctival advancement for late-onset filtering bleb leaks: indications and outcomes. *Arch Ophthalmol* 1999;117:1014-1019.

Book chapter
2. Skuta GL, Morgan RK. Corticosteroid-induced glaucoma. In: Ritch R, Shields MB, Krupin T, eds. *The Glaucomas*. St. Louis: Mosby; 1996:1177-1188.

Entire book
3. Gelatt KN, ed. Veterinary Ophthalmology. Philadelphia: Lippincott Williams & Wilkins; 1999

World Wide Web
7. Gostin LO. Drug use and HIV/AIDS [*JAMA* HIV/AIDS web site]. June 1, 1996. Available at: http://www.ama-assn.org/special/hiv/ethics. Accessed June 26, 1997.

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Legends must be submitted for all figures. They should be brief and specific, and they should appear on a separate manuscript page after the references. This is the last page of your TEXT. Use scale markers in the image for electron micrographs and indicate the type of stain used. Do no embed images or tables. This is TEXT only.

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Chapter 4

Study Protocol

DISSERTATION PROTOCOL

Effect of Papaya Intake on Intraocular Pressure, Retinal Nerve Fiber Layer Thickness, Optic Nerve Head Parameters And Serum Total Antioxidant Capacity in Primary Open Angle Glaucoma Patients

Dr. Chan HuiTze
P-UM0138/16
Master of Medicine (Ophthalmology)
Department Of Ophthalmology
School Of Medical Sciences
Universiti Sains Malaysia

MAIN SUPERVISOR
Prof. Dr. Liza Sharmini Ahmad Tajudin
Medical Lecturer and Senior Consultant Ophthalmologist (Glaucoma)
Department Of Ophthalmology
School Of Medical Sciences
Universiti Sains Malaysia

CO-SUPERVISOR

Prof. Dr. Hamid Jan bin Jan Mohamed
Nutrition and Dietetics Programme
School of Health Sciences
Universiti Sains Malaysia

TABLE OF CONTENTS

1.0 INTRODUCTION

1.1 Management of Glaucoma

Glaucoma is an optic neuropathy associated with characteristic structural damage to the optic nerve and associated visual dysfunction that is caused by various pathological processes (Foster PJ et al. 2002). It is estimated to affect more than 70 million people worldwide with approximately 10% being bilaterally blind, making it the leading cause of irreversible blindness in the world (Quigley et al. 2006).

Clinically, glaucoma progression can be observed by monitoring optic nerve head (ONH) changes such as increased vertical cup to disc ratio (VCDR), disc haemorrhage, peripapillary atrophy, nasalization of blood vessels and neural retinal rim thinning. Imaging using optical coherence tomography (OCT) has emerged as a modality that can provide quantitative structural features of the optic nerve such as disc size, VCDR, and peripapillary nerve fibre layer thickness. OCT shows promise to reliably demonstrate disease progression prior to manifestation of functional changes; visual field defect (Wessel et al. 2013, Leung et al. 2012). Retinal ganglion cells (RGCs) loss in glaucoma causes specific visual field defects that conform to the topography of the nerve fibre layer bundles.

Current management guidelines from the American Academy of Ophthalmology Preferred Practice Pattern recommended lowering the intraocular pressure (IOP) toward a targeted level known as target pressure. IOP control has been identified as an important factor in preventing visual field loss (The Advanced Glaucoma Intervention Study (AGIS), 2000). Target

pressure is a value or range of values of IOP at which the clinician believes that the rate of disease progression will be slowed sufficiently to avoid functional impairment from the disease (American Academy of Ophthalmology: 2010**)**. In general, the initial target aims for a 20% to 50% reduction in pressure. Large randomized control trials found that all modalities of IOP reduction slow the progression of glaucomatous optic neuropathy (Collaborative Normal-Tension Glaucoma Study Group 1998, AGIS 2000, The Ocular Hypertension Treatment Study 2002).

Laser trabeculoplasty lowers IOP by inducing biological changes in the trabecular meshwork resulting in increased aqueous outflow. The procedure has an excellent safety profile and is performed during an office visit. Although substantial IOP reductions can be achieved in the majority of patients, the effect decreases gradually over time with a failure rate of about 10% per year (Odberg et al. 1999, Shingleton et al. 1993, Shingleton et al. 1987).

In trabeculectomy surgery, aqueous humor egress from the eye is facilitated via a partial thickness sclerostomy. Clinical trials from the Moorfields Eye Hospital indicated surgical trabeculectomy was the most effective IOP-lowering treatment. The Collaborative Initial Glaucoma Treatment Study (CIGTS) found successful lowering of IOP, where surgical group was 2–3 mm Hg lower than that for the medical group. However, visual field and visual acuity outcomes were similar in both groups. Surgical intervention induced formation of cataract significantly compared to medical treatment (CIGTS). On secondary analysis, patients with moderate disease had less visual field loss when treated with surgery first (Musch et al. 2011).

Glaucoma drainage devices (GDD) provide external reservoirs in drainage of aqueous humor are effective in lowering IOP (Gedde et al. 2012). Several alternatives to these procedures have

been proposed and investigated. These include minimally invasive glaucoma surgeries (MIGS) potentially incur less risk of sight-threatening complications (Ayyala et al. 2011**).** However, the success of MIGS remain elusive with less effective in providing IOP reduction and higher risk of complications compared to trabeculectomy (Rulli et al. 2013).

Although IOP lowering treatment can provide neuroprotection and retard the disease progression in many glaucoma patients, it is not always sufficient to fully prevent disease progression (Gűlgű et al. 2006). Oxidative stress is known to contribute to glaucoma etiology and progression (Levin et al. 1996, Green et al. 1995, Bautista et al. 1999). Antioxidants represent the first line of defence against oxidative stress and are obtained through the diet and produced internally (Raman et al. 2016). Dietary antioxidant intake has shown to be protective towards glaucoma (Andrea et al. 2017). Dietary antioxidant is more cost-effective and promising potential as primary prevention for primary open-angle glaucoma (POAG).

1.2 Antioxidant and Glaucoma

There are growing evidences supporting the involvement of oxidative stress as a common component of glaucomatous neurodegeneration at different subcellular compartments of RGCs (Gűlgű et al. 2006). Oxidative processes, occurs under a condition of high energy consumption, light exposure, or age-dependent decline of coping capacity to deal with free radicals, may trigger and mediate the apoptotic death of RGCs in glaucoma (Lipton et al. 1999).

Although RGCs exhibit unique characteristics of antioxidant defence mechanisms (Kortuem et al., 2000), survival of RGCs is critically sensitive to the oxidative redox state, and decreasing reactive oxygen species (ROS) generation promotes the survival of RGCs (Geiger et al., 2002).

ROS are generated in the retina not only in retinal ischemia induced by acute IOP elevation but also in moderate and chronic elevation of IOP (Bonne et al., 1998; Muller et al., 1997). Disturbance of the TM cell status by oxidative stress may lead to cellular loss and an overexpression or alteration in the structures of various glycoproteins in the extracellular matrix, which interfere with the TM function, and lead to impaired aqueous humour outflow and thereby an increase in IOP (Wentz-Hunter et al. 2004, Li et al. 2004). It has been demonstrated that oxidative DNA damage increases while total antioxidant status decreases in the serum and aqueous humour of glaucoma patients (Rana et al. 2016). In addition, increased IOP and visual field damage are significantly related to the amount of oxidative DNA damage affecting TM cells (Izzotti et al. 2006, Saccà et al. 2007).

One of the mechanisms proposed for RGC death in glaucomatous eyes is that elevated IOP-induced axonal injury at the ONH results in the blockage of neurotrophin transport to RGC bodies (Anderson and Hendrickson, 1974; Minckler et al., 1976; Pease et al., 2000; Quigley and Addicks, 1980). Not only mechanical injury and neurotrophin deprivation, but also vascular insults at the ONH (Flammer, 1994; Hayreh, 1985; Osborne et al., 2001) have been proposed to lead to RGC death in glaucoma. Oxidative neuronal injury in glaucoma may also be associated with excitotoxicity of amino acids, including glutamate. Excitoxicity is known to induce ROS generation and oxidative stress which causes glutamate neurotoxicity (Atlante et al., 2001). In addition, retinal glutamate damage has been shown to be mediated in part through nitric oxide, a highly reactive oxidant (Nucci et al., 2005). Endogenous oxidation reduction agents, such as glutathione, have been found to be protective against glutamate-induced toxicity (Levy et al. 1991). More recently, it has also been proposed that light entering the eye interacts with mitochondria, thereby leading to increase in ROS generation of RGCs and their axons. These neurons when in an energetically low state, their antioxidant capacity is

exceeded and compromising their survival (Osborne et al. 2006).

Another series of findings supporting an oxidative component of the neurodegenerative process in glaucoma come from demonstrating oxidative injury through the involvement of tumour necrosis factor (TNF) death receptor signalling. TNF-a is upregulated in the glaucomatous ONH and retina (Tezel et al., 2001b; Yan et al., 2000; Yuan and Neufeld, 2000). TNF-a has recently been identified to be a mediator of RGC death after induction by various stimuli (Tezel and Wax, 2000a; Tezel and Yang, 2004; Tezel et al., 2004b). There is evidence to suggest an increase of glial production of TNF-a, which facilitates RGC death in vitro as a response to glaucomatous stress (Tezel and Wax, 2000a). TNF-a can also mediates RGC death following optic nerve injury (Tezel et al., 2004b) or IOP elevation (Banerjee et al., 2005) in vivo.

Dietary antioxidants have a key role in oxidative stress reduction by inhibiting oxidative reactions and removing free radical intermediates (Pham-Huy, 2008). Cao and colleagues suggested that the plasma antioxidant capacity is increased with intake of a diet rich in sources of antioxidants, such as fruit and vegetables (Cao et al. 1998). Dietary antioxidant may enhance TM function and protect the optic nerve (Levin et al. 1996, Wilson et al. 1997, Schwartz et al. 1996, Schumer et al. 1994). Ascorbic acid, the major antioxidant in the eye, is found in high concentrations in various areas that include the cornea, central corneal epithelium, lacrimal film, vitreous humour and aqueous humour, suggesting an important role in antioxidant protection in ocular health (Raman et al. 2016). Ascorbic acid neutralizes oxygen radicals and singlet oxygen and is a reductant of oxidized vitamin E (Varma, 1991). In addition, ascorbic acid, which is 15 times higher in concentration in the aqueous humor than in plasma (Becker, 1957), may reduce IOP by the depolymerization of the TM hyaluronic acid component (Linner, 1966).

Flavonoids, comprised of a large family of plant-derived polyphenolic compounds, is widely distributed in fruits and vegetables and regularly consumed in the human diet (Middleton et al. 2000, Heim et al. 2002, Ross and Kasum 2002). Flavonoids comprised of neuroprotective compound with potent antioxidant and free radical scavenging properties. Flavonoids demonstrated high potency in protecting RGCs from oxidative stress–induced death potency with low toxicity (Pamela and Anne, 2005). Flavonoids have the ability induce the activity and expression of phase 2 detoxification proteins (Myhrstad et al. 2002, Hou et al. 2001, Valerio et al. 2001) which include enzymes associated with glutathione (GSH) biosynthesis and metabolism and redox-sensitive proteins such as heme oxygenase 1 (HO-1). In addition, flavonoids have been shown to induce neurite outgrowth (Sagara et al. 2004), reduce inflammation (Read, 1995), and inhibit endothelial cell proliferation (Fotsis et al. 1998).

1.3 Nutrients and Antioxidants Content of Papaya

Papaya (Carica papaya L. cv. Eksotika) is one of the most commonly consumed tropical fruits by Malaysians. Papaya is a perennial plant which is non-seasonal, easily available and affordable by many. Papaya fruit belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a wide variety of diseases (Mello et al. 2008, Munoz et al. 2000).

Papaya is rich sources of powerful antioxidant nutrients such as, vitamin C, flavonoids and carotenes (Y.Y.Lim et al. 2007). Papaya also contains vitamin B, folate and pantothenic acid; minerals such as, potassium, copper, magnesium; and fibre. In addition, papaya contains several unique protein-digesting enzymes including *papain* and *chymopapain*. These enzymes along with its antioxidant nutrients have been shown to reduce inflammation

(Bamidele V. Owoyele et al. 2008).

A single serving of papaya (276 gram) provides 168 mg of vitamin C accounting for 224% of daily recommended intake, 2622 IU of vitamin A, 756 mcg beta carotene, 245 mcg of lutein and zeaxanthin. It provides 119 calories per serving with medium glycemic index that make it suitable for diabetic patients. In comparing papaya with other fruit such as banana, apple, mango, and pineapple, it has been found to be more superior in providing good natural source of macronutrients (carbohydrates and proteins) and micronutrients (vitamin A and vitamin C) (Peterson et al. 1982). Moreover, papaya is easily consumed due to its soft consistency especially in elderly. Majority of POAG patients are elderly. Based on the nutrient content especially high content of antioxidant in papaya, relatively inexpensive and availability of papaya in local market, papaya is the best choice to study the effect of dietary intake of antioxidant on glaucoma patients.

2.0 RATIONALE OF STUDY

Based on food frequency questionnaire (FFQ), food rich with vitamins and antioxidant are found to reduce the risk of glaucoma (Pamela et al. 2005). Fruits such as local orange and watermelon are found to reduce the risk of glaucoma progression by 4 folds (95% CI, 2.41-6.52) and 2.3 folds (95% CI, 1.36-3.98) respectively; whereas mango and Sunkist orange are also found to have significant effect on cup to disc ratio [Noor-Asma MN, 2017. Effect of dietary intake on ONH parameters and glaucoma progression in Malays. Submitted thesis for Master of Clinical Ophthalmology, Universiti Sains Malaysia]. However, the content of antioxidant in orange, watermelon and mango is not as high as expected (Ministry of Health Malaysia Guideline, 2016).

Papaya is a non-seasonal fruits which is easily available and relatively inexpensive. It is an excellent source of powerful antioxidants vitamin C and vitamin A, and also rich in vitamin B, fibre, potassium and magnesium. The content of antioxidants in papaya is among the highest compared to other local fruit (Y.Y.Lim et al. 2007). Dietary papaya is chosen over supplement for this study as raw fruits in natural state provide better nutrients as compared to chemically processed supplement. Enzymes and nutrients are easily destroyed during the process. Thus so far, comparison between the beneficial effect of raw fruit and supplement are inconclusive. Therefore, dietary papaya has high potential as complementary treatment for glaucoma. Perhaps in the future papaya can be an important supplementary therapy for glaucoma patients.

3.0 OBJECTIVES

3.1 General Objective

To evaluate the effect of papaya intake on intraocular pressure (IOP), retinal nerve fibre layer thickness (RNFL), optic nerve head (ONH) parameters and serum total antioxidant capacity (TAC) in primary open angle glaucoma (POAG).

3.2 Specific Objective

1. To compare the IOP measurement at baseline, first, second and third month between POAG patients taking papaya and not taking papaya.
2. To compare RNFL thickness and ONH parameters at baseline, first, second and third month between POAG patients taking papaya and not taking papaya.
3. To compare the serum TAC level at baseline, first, second and third month between POAG patients taking papaya and not taking papaya.

3.3 Research Hypothesis

1. There is significant difference in IOP measurement at baseline, first, second and third month between POAG patients taking papaya and not taking papaya.
2. There is significant difference in RNFL thickness and ONH parameters at baseline, first, second and third month for POAG patients taking papaya and not taking papaya.
3. There is significant difference in serum TAC at baseline, first, second and third month between POAG patients taking papaya and not taking papaya.

4.0 METHODOLOGY

4.1 Research Design

This is a randomized controlled trial study.

4.2 Study Location

Ophthalmology Clinic of Hospital Universiti Sains Malaysia (USM).

4.3 Study Duration

February 2018 – October 2019.

4.4 Study Reference Population

POAG patients.

4.5 Study Source Population

POAG patients attending eye clinic Hospital USM.

4.6 Sampling Frame

Patients diagnosed with POAG who attended eye clinic at Hospital USM from February 2018 – October 2019.

4.7 Sampling Method

Simple random sampling method will be applied.

4.8 Study Participants

POAG patients who attended eye clinic Hospital USM selected according to inclusion and exclusion criteria.

4.9 Selection Criteria

4.9.1 Subject and control groups

4.9.1 Inclusion Criteria

4.9.1.1 Confirmed cases of POAG

4.9.1.2 Age between 40 and 70 years old

4.9.1.3 Patients on medical treatment and achieved target IOP

4.9.1.4 Good compliance to pressure lowering agents

4.9.1.5 Patients who are willing to self-sustain intake of papaya

4.9.2 Exclusion Criteria

4.9.2.1 Active smoker

4.9.2.2 History of previous intraocular surgery other than uncomplicated cataract within past 6 months for example trabeculectomy, glaucoma drainage device implantation and etc.

4.9.2.3 Patients with media opacities that affect the reliability of OCT measurements

4.9.2.4 History of allergic to papain

4.9.2.5 Poorly controlled diabetes mellitus with HbA1c of > 9.0%

4.9.2.6 History of taking antioxidant supplement 3 months prior to recruitment, and not taking antioxidant supplement during the study period.

4.9.2.7 Known case of irritable bowel disease

4.9.2.8 Patient in Group B (non-interventional) who has been taking papaya ≥ 3 servings/week or 12 servings/month during study period.

4.10 Randomization

Block randomization method using sequentially numbered, opaque sealed envelopes (SNOSE).

4.11 Blinding

Single blinded study where OCT operator and personnel taking IOP measurement will be blinded.

4.12 Sample Size

Sample size determination for IOP, RNFL thickness and serum TAC are done by using G-Power 3.1.7.

The statistical test is repeated measures ANOVA (RM ANOVA), within factors, between factors and within-between factors

The input parameters are

- Effect size : 0.25
- P-value : 0.05
- Power : 0.8
- No. of groups : 2

- No. of measurements : 3
- Corr among rep measures : 0.5

The final total sample size is 40 POAG patients including 30% dropout with 20 patients in group A (interventional) and 20 patients in group B (non-interventional).

4.13 Definition of Terms

4.13.1 Primary Open Angle glaucoma (POAG)

Defined as optic nerve damage in which the disc abnormalities (VCDR > 97.5th percentile) with typical glaucomatous visual fields defects in an eye which does not have evidence of angle closure glaucoma on gonioscopy and there is no identifiable secondary causes (Foster et al, 2002).

4.13.2 Intraocular Pressure (IOP)

The IOP of the eye is determined by the balance between the amount of aqueous humor - that the eye makes and the ease with which it leaves the eye (American Academy of Ophthalmology, 2008)

4.13.3 RNFL thickness and ONH parameters

OCT is used to measure the RNFL thickness and ONH parameters. The RNFL is formed by the expansion of the fibers of the optic nerve, glaucoma will cause thinning of the RNFL. OCT also give us information of ONH parameter, such as volume of the disc, disc rim, cup depth, and the cup-to-disc ratio which are important to monitor the progress of the disease (Anjum et al. 2015).

4.13.4 Withdrawal Criteria

Withdrawal is considered when subject is unable to comply with the protocol. If the subject in group A consumes papaya 2 times or less in a week or any subject in group A and B consumes antioxidant supplement during study period will be withdrawn from the study. If the study subject developed gastrointestinal discomfort upon consumption of papaya, he/she will be withdrawn from the study. Subjects who were withdrawn from the study will be followed up in accordance to their clinical condition. Subject can withdraw from the study whenever they wish to do so.

4.14 Research Tools

4.14.1 Instruments

a. Snellen Visual Acuity Chart for distance (Reichert, NY, USA)
b. Pinhole occluder
c. Slit lamp biomicroscopy (HAAG-STREIT International, UK)
d. Goldmann applanation tonometer (GAT) AT-900 (HAAG-STREIT International, UK)
e. Condensing lens of 90D and 78D lenses (VOLK, USA)
f. Optical Coherence Tomography (Carl Zeiss, Meditec, USA)
g. 5ml Syringe, Needle, Plain tube
h. Pipette, centrifuge tubes, clear flat-bottom uncoated 96-well plates
i. Plate reader
j. Diary

4.14.2 Medication/Reagent

a. Tropicamide 1% eye drop (dilating agent)
b. Proparacaine Hydrochloride (Alcaine) 0.5% eye drop (topical local anesthesia)
c. Phenylephrine Hydrochloride (Mydfrin) 2.5% eye drop (dilating agent)
d. Serum TAC test kit (QuantiChrom^{TM} Antioxidant Assay Kit (DTAC-100), USA)

4.15 Methods of Data Collection

This study will be conducted after obtaining approval from the Universiti Sains Malaysia Ethical Committee (JPeM) and will be conducted in accordance to World Medical Association

Declaration of Helsinki ethical principles for medical research involving human subjects.

4.15.1 Patient Recruitment

POAG patients attending ophthalmology clinic at Hospital Universiti Sains Malaysia will undergo eye examination. Subjects who fulfil the inclusion and exclusion criteria for the study will be selected. An informed and written consent will be taken and information form given to patients. The selected patients will be randomized into 2 groups (A and B) using sequentially numbered, opaque sealed envelopes (SNOSE). Group A patient will be consuming papaya and group B patient will not consume papaya.

4.15.2 Interventional Protocol

Patients in Group A will have to consume single serving of papaya (~250 g) a day, 3 times a week for 3 months. They are only allow to consume the papaya raw, and not to process or cook it in any other way. The papaya will be distributed by research assistant weekly throughout the study period. Patient will be reminded weekly using text messages or phone call by a research assistant for papaya consumption. Patients in group A and group B will be given a dietary diary throughout the study period, where the patient will mark the intake of fruits listed. The purpose of the diary is also to monitor patient intake of papaya and other fruits as well.

4.15.3 IOP measurement

IOP measurement will be done using Goldmann Applanation Tonometer (GAT) in sitting position by the primary investigator (Chan Hui Tze) who will be blinded. GAT will be calibrated daily before use to measure the IOP. Measurement of IOP will be done only at 8am – 10am (to prevent diurnal variation). Before IOP measurement, subjects

are advised to avoid drinking coffee or any caffeinated drink 24 hours before and only to take light meal 2 hours before (to reduce the effect of hyperglycemia and hydration on IOP). Only one eye will be measured, if both eyes are eligible, only right eye will be selected regardless of severity of glaucoma. Three readings of IOP will be measured and the mean reading will be taken as result. IOP measurement will be taken at baseline, first, second and third month post recruitment for group A and B.

4.15.4 OCT evaluation on RNFL thickness and ONH parameters

Spectral domain Cirrus HD-OCT will be used to measure RNFL thickness and ONH parameters by a trained personnel who is blinded. Patient will sit in front of the OCT machine and rest his/her head on a support to keep it motionless, the OCT will then scan patient's eye without touching it. Scans with signal strength less than 6, eye movement or blinking artifacts within the 1.73-mm radius around the ONH, or with segmentation errors will be repeated. If there is unsatisfactory measurement after 3 attempts, the patient will be excluded from the study. In patient without media opacities but with poor signal strength, the eye will be dilated, and if after dilation still unable to get satisfactory reading after 3 attempts, the patient will be excluded from the study. OCT will be conducted at baseline, first, second and third month post recruitment for group A and B.

4.15.5 Serum total antioxidant capacity

Serum TAC will be measured using QuantiChromTM Antioxidant Assay Kit (DTAC-100). Three (3) ml of patient's blood will be obtained using syringe and needle and will be stored in a plain tube. It will be centrifuged 10 min at 4000 rpm, the plasma will be used as sample for calculation of total antioxidant capacity (TAC). Plasma can be stored in a freeze at -80°C in the laboratory (stable for 1 month) for future analysis if

required. After preparing the reagent provided from the test kit (DTAC-100), patient plasma will be mixed with the diluted reagent in a plate which will be read at optical density of 570nm. The result obtained will be plotted against the standard concentration and a standard curve is obtained. By using the graph plotted and a formula provided in the test kit, the serum TAC will be calculated. Serum total antioxidant capacity will be calculated at baseline and monthly for 3 months post recruitment.

4.15.6 Data Entry and Statistical Analysis

The data entry and analysis will be performed by using Statistical Package for the Social Sciences (SPSS) version 22 licensed to USM.

4.16 Plans for Minimizing Study Errors

These steps will be taken to minimize the errors while conducting the study:

The same instruments and equipment will be used for repeated measurement in this study. The measurement of IOP will be done at the same time (8am-10am) in a day for all patients (to prevent diurnal variation). OCT measurements will be performed by qualified and trained personnel only. Proper storage of serum sample and TAC test kit will be ensured.

5.0 ETHICAL CONSIDERATION

This study will be submitted to the Universiti Sains Malaysia Ethical Committee (JPeM) and Medical Research Ethics Committee (MREC), Ministry of Health of Malaysia and will be conducted in accordance to World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects. The information form will be given

to all participants and an informed and written consent will be taken prior to data collection.

5.1 Handling Privacy and Data Confidentiality

5.1.1 Confidentiality

All the information obtained in this study will be kept and handled in a confidential manner, in accordance with applicable laws and/or regulations. The confidentiality of the data will be strictly maintained, whereby only the author, supervisor and co-supervisor could access the data. Personal information and data will not be disclosed. The data collection sheets will have serial numbers instead of the names of the subjects to prevent recognition. Subjects are not given access to the personal information and study data.

5.1.2 Data Storage

Research records will be stored securely in a locked cabinet and study data will be stored in a password-protected thumb drive. Medical information will be held and processed on a computer and will be entered using unique numbers into Microsoft Excel 2016 before analysis process using SPSS. Duration of storage and archival of medical records and study data will take about 3 years after completion of study. All digital data will be deleted permanently and all data collection sheets which contain the data will be disposed after the period of storage. The participants' name will not appear on the materials published and the medical information of each subject will kept confidential unless disclosure is required by law. Participants will be offered to read the manuscripts and to see all publish materials in which they are included.

5.2 Potential Risk to Subjects

Minimal risk to participants in this study. Eye drop medications to dilate the pupil is not dangerous to user. However, the participants will be monitored by the researcher until confirmed not to have any side effect of that medications. If any side effects medical treatment will be given as necessary. However, if there are any important new information found during this study that may affect the decision in being part of the study, participants will be told about it right away.

5.3 Direct and Indirect Benefits to Subjects

Study drug and study procedures will be provided at no cost to all participants. Participants may receive information about their health from any physical examination and investigation tests to be done in this study. This intervention would increase the participant's knowledge regarding dietary antioxidant and may help towards successful POAG treatment outcome.

5.4 Incentive, Compensation and/or Reimbursement

There is no any incentive, compensation and/or reimbursement given to all participants.

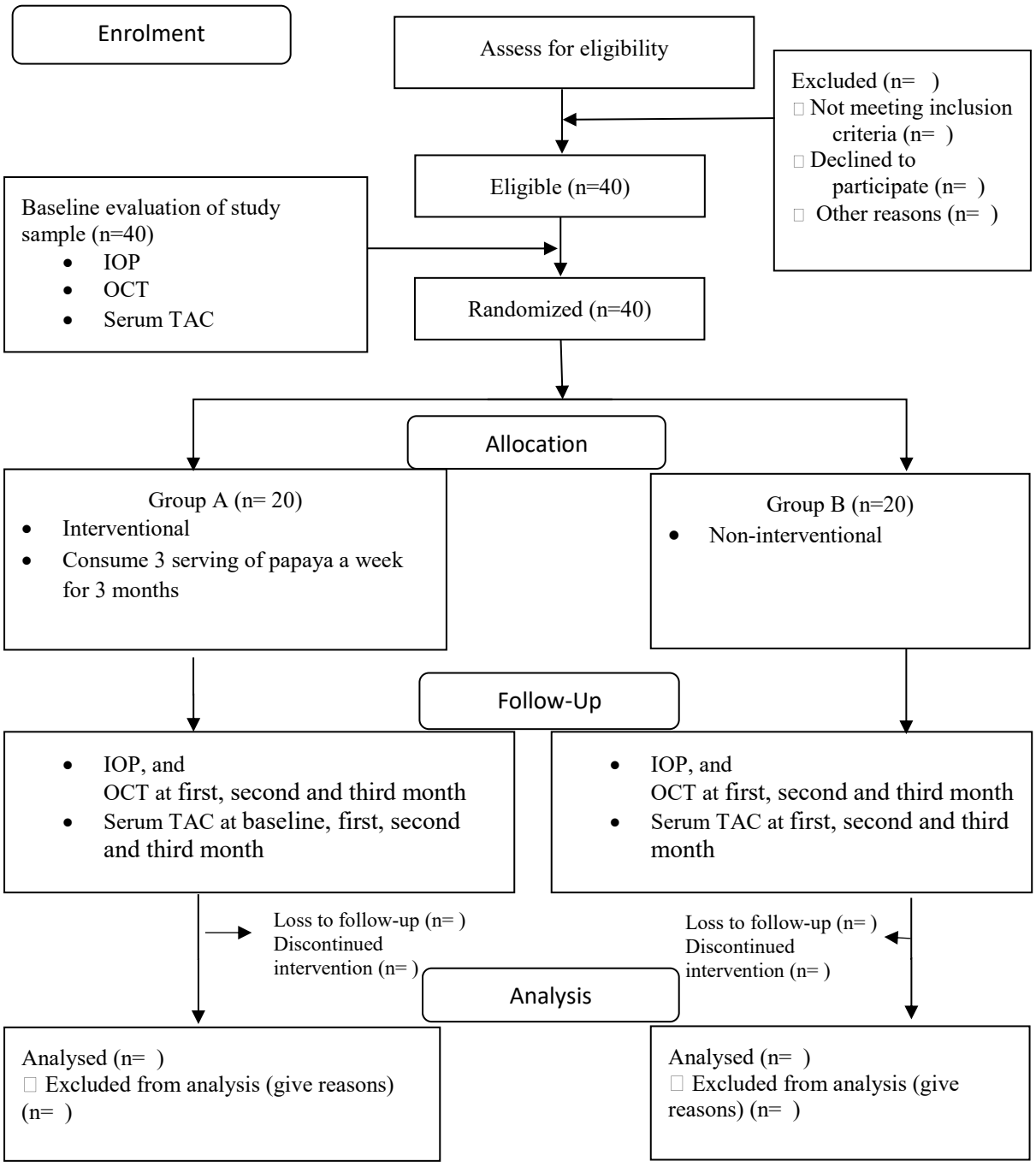
5.5 Declaration of Conflict of Interest

No conflict of interest is present.

5.6 Collaborative Study Term of Reference

There is no collaboration between researchers and other institute or ministry in this study since it is only will be done at Hospital USM.

6.0 FLOW CHART OF STUDY



7.0 EXPECTED RESULT

Table : Mean IOP and serum TAC between Group A and B

	Time (months)	Group A	Group B	p value
IOP (mean, SD)	Baseline			
	1			
	2			
	3			
Serum TAC (mean, SD)	Baseline			
	1			
	2			
	3			

Statistical analyses were performed using the Paired t-test

$p<0.05$ is significant

Table : Monthly comparison of mean IOP difference for Group A and B

	Group A				Group B			
Time Difference *	Mean IOP difference (SD) (mmHg)	95% CI of the difference		p-value	Mean IOP difference (SD) (mmHg)	95% CI of the difference		p-value
		Lower Bound	Upper Bound			Lower Bound	Upper Bound	
Vo-V1								
Vo-V2								
Vo-V3								
V1-V2								
V1-V3								
V2-V3								

*Vo- Baseline visit, V1-month 1 visit, V2-month 2 visit, V3-month 3 visit

A one-way repeated measures analysis of variance (RM ANOVA) with Bonferroni correction

$p<0.05$ is significant

Table : Mean RNFL and ONH parameters for Group A and B

	Time (months)	**Group A**	**Group B**	**p value**
RNFL thickness (µm) (Mean, SD)	Baseline			
	1			
	2			
	3			
ONH parameters				
Cup disc ratio (Mean, SD)	Baseline			
	1			
	2			
	3			
Rim area (mm^2) (Mean, SD)	Baseline			
	1			
	2			
	3			

Statistical analyses were performed using the Paired t-test

$p<0.05$ is significant

Table : Monthly comparison of mean RNFL difference for Group A and B

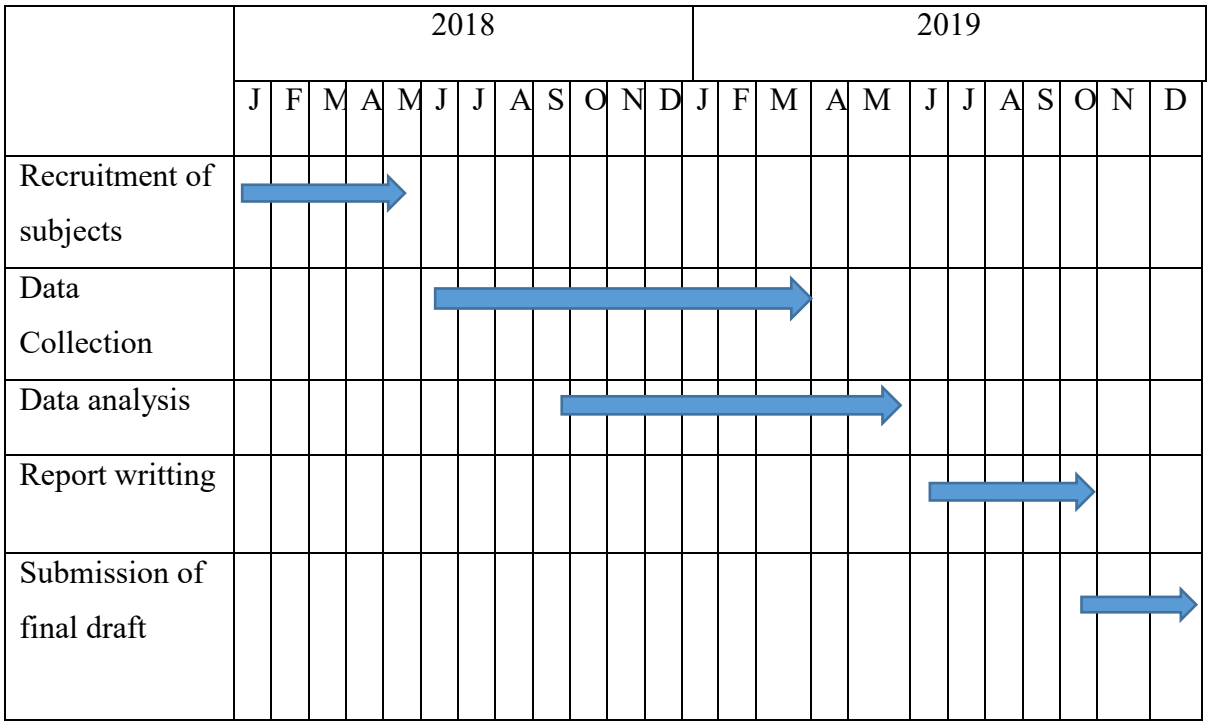
	Group A				Group B			
Time Differ-ence*	Mean RNFL difference (SD) (µm)	95% CI of the difference		p-value	Mean RNFL difference (SD) (µm)	95% CI of the difference		p-value
		Lower Bound	Upper Bound			Lower Bound	Upper Bound	
Vo-V1								
Vo-V2								
Vo-V3								
V1-V2								
V1-V3								
V2-V3								

*Vo- Baseline visit, V1-month 1 visit, V2-month 2 visit, V3-month3 visit

A one-way repeated measures analysis of variance (RM ANOVA) with Bonferroni correction

$p<0.05$ is significant

8.0 GANNT CHART



9.0 KEY MILESTONES

Expected Achievement	Date
Recruitment of subjects	May 2018
Data collection	March 2019
Data analysis	May 2019
Report writing and correction	October 2019
Submit final draft	December 2020

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Chapter 5

Appendices

5.1 Data Collection Form

Index No. : _____

Title : Effect of Papaya Intake on Intraocular Pressure, Retinal Nerve Fibre Layer Thickness, and Serum Total Antioxidant Capacity in Primary Open Angle Glaucoma

A) Demographic Data

Name :_____

Date of examination :_____

Registration No. : _____

Age :_____

Gender :_____

Contact No. : _____

B) Comorbid

DM	
HPT	
HPL	
IHD	
CRF	

Others :_____

C) Examination

		BASELINE	1st MONTH	2nd MONTH	3rd MONTH
VA	RE				
	LE				
IOP	RE				
	LE				

D) Investigations

	BASELINE	1st MONTH	2nd MONTH	3rd MONTH
1) ONH CDR/rim area RE				
LE				
RNFL RE				
LE				
2) SERUM TAC				

E) Treatment

	RE	LE
Timolol		
Xalatan		
Travatan		
Trusopt		
Alphagan		
Lumigan		

F) Ocular characteristic

Lens status_____

Diagnosis_____

Duration of diagnosis_____

5.2 Dietary Diary

Diari Amalan Pemakanan
Jika anda mengambil betik 3 kali seminggu, dan setaip kali makan, anda mengambil sebanyak sekeping. Tuliskan '3' pada kolum **seminggu** dan tuliskan '1' pada kolum **Jumlah sajian**.

Bil.	Buah-buahan	Berapa kali kekerapan pengambilan dalam **seminggu**	Rujukan saiz hidangan	**Jumlah sajian** (setiap kali makan)
1	Betik		Potong	
2	Mangga		Potong	
3	Nenas		Potong	
4	Tembikai		Potong	
5	Buah naga		Potong	
6	Tembikai susu		Potong	
7	Rock Melon		keping	
8	Jambu batu		Keping	
9	Jambu air		Biji	
10	Limau		Biji	
11	Pisang(pisang segar, pisang goring, pisang salai dan sebagainya)		Biji	
12	Belimbing		Biji	
13	Epal		Biji	
14	Oren		Biji	
15	Pir		Biji	
16	Anggur		Biji	
17	Rambutan		Biji	
18	Mata kucing segar		Biji	
19	Laici segar		Biji	
20	Manggis		Biji	
21	Durian		Ulas	
22	Nangka/Cempedak		Ulas	
23	Buahan dalam tin(laici,longan dan sebagainya)		Sudu makan	
24	Buahan kering (kurma,prun,kismis dan sebagainya)		Sudu makan	
25	Buahan jeruk/acar		Sudu makan	
26	Kelapa muda		Sudu makan	

5.3 Research Information

5.3.1 English Version

RESEARCH INFORMATION

Research Title : Effect of Papaya Intake on Intraocular Pressure, Retinal Nerve Fiber Layer, Optic Nerve Head Parameters and serum Total Antioxidant Capacity in Primary Open Angle Glaucoma Patients

Researcher's Name : Dr. Chan Hui Tze ( ))

Supervisor's Name : Prof. Dr. Liza Sharmini Ahmad Tajudin

Assoc. Prof. Dr. Hamid Jan bin Jan Mohamed

INTRODUCTION

You are invited to take part voluntarily in a research study on Effect of Papaya Intake to Total Antioxidant Capacity, Intraocular Pressure, and Retinal Nerve Fiber Layer in Primary Open Angle Glaucoma Patients. Before agreeing to participate in this research study, it is important that you read and understand the information written on this form. It describes the purpose, procedure, and benefit about the study. If you agree to participate in this study, you will receive one copy of this form to keep for your records.

PURPOSE OF THE STUDY

The purpose of this study is to examine the effect of papaya intake to total antioxidant capacity, intraocular pressure and retina nerve fiber layer. It is possible that information collected during this study will be analysed by researcher in the future to help in treatment of primary open angle glaucoma patients.

QUALIFICATION TO PARTICIPATE

The doctor in charge of this study or a member of the study staff has discussed with you the requirements for participation in this study. It is important that you are completely truthful with

the doctor and staff about your health history. You should not participate in this study if you do not meet all qualifications.

The requirements to be in this study are:

4.9.1.6 Confirmed cases of POAG

4.9.1.7 Age between 40 and 70 years old

4.9.1.8 Patients on medical treatment and achieved target IOP

4.9.1.9 Good compliance to pressure lowering agents

4.9.1.10 Patients who are willing to self-sustain intake of papaya

The exclusion criteria for this study:

1. Active smoker
2. History of previous intraocular surgery other than uncomplicated cataract within past 6 months for example trabeculectomy, glaucoma drainage device implantation and etc.
3. Patients with media opacities that affect the reliability of Optical Coherence Tomography (OCT) measurements
4. History of allergic to papain
5. Poorly controlled diabetes mellitus with HbA1c of>9.0%
6. History of taking antioxidant supplement 3 months prior to Recruitment, and not taking antioxidant supplement during the study period.
7. Known case of irritable bowel disease
8. Patient in Group B who has been taking papaya ≥ 3 servings/week or 12 servings/month during study period.

STUDY PROCEDURES

If you agree to participate in this study, you will be asked to provide information about your medical history. At your first visit, you will undergo full eye examination and blood taking at Hospital Universiti Sains Malaysia (USM). Study procedures are as follows:

1. Visual acuity assessment

This is done by using Snellen's chart that needing you to recognize alphabet, number and E

chart form 6 meters distance. Each eye is examined in turn. This examination takes for about 1 to 2 minutes.

2. Eye examination and intraocular pressure measurement

Anterior and posterior segments of eye examination is done using slit lamp biomicroscope in sitting position. Each eye takes for about 5 to 20 minutes.

3. Retinal Nerve Fiber Layer

This assessment is done using Optical Coherence Tomography (OCT) machine.

4. Total Antioxidant Capacity

Three (3) mls of blood sample will be taken in EDTA tube and sent to the lab for analysis by using QuantiChrom^{TM} Antioxidant Assay Kit (DTAC-100), the total antioxidant capacity in blood will be determined. Specimen will be discarded at the end of the study.

5. Papaya Intake

Three (3) portions of papaya will be supplied to subject weekly, and patient will be taking it once a day, 3 days in a week. Supply of papaya will be provided throughout study period.

6. Reassesment of Intraocular pressure, Retinal Nerve Fiber Layer and Total Antioxidant Capacity during subsequent follow up at first, second and third month.

These reassessement will be using the methods as previously. It takes for about 20 to 30 minutes.

All study findings and measurement will be recorded to the specific document for every patient.

All procedures will be done by trained staff, optometrist and doctor after consented by patient and the patient is fit to perform the above mentioned procedures.

RISKS

Visual acuity examination using Snellen's chart is very easy and safe to be done. This examination is very important to assess someone's visual acuity capacity.

Eye examination including intraocular pressure measurement using slit lamp biomicroscope will give a clear image and bigger image of eye structure. This machine is not dangerous, comfort and safe to be used. There is no direct contact to the eyeball. This is a routine eye examination that is being practised by ophthalmologist all over Malaysia and the world.

Examination of retinal nerve fiber layer using OCT machine is safe. This examination is frequently used in eye clinic that has this facility.

Intake of papaya is safe as it is within the range of recommendation in Malaysia Dietary Guideline version 2013.

However, if there are any important new information found during this study that may affect you decision in being part of the study, you will be told about it right away.

ALTERNATIVE TREATMENT

This study is not a type of treatment for your disease or condition. There are other treatment and therapy for your disease, including the one you are undergoing currenly. The doctor involved in this study may discuss your treatment and therapy with you.

REPORTING HEALTH EXPERIENCES

If you have any injury, bad effects, or any other unusual health experience during this study, make sure that you immediately contact the following person:

Dr. Chan Hui Tze
Department of Ophthalmology
School of Medical Sciences
UniversitiSains Malaysia,Health Campus.

PARTICIPATION IN THE STUDY

Your taking part in this study is entirely voluntary. You may refuse to take part in the study or you may stop participation in the study at any time, without a penalty or loss of benefits to which you are otherwise entitled. Your participation also may be stopped by the study doctor or sponsor without your consent. Expected duration of participation is 4 months.

POSSIBLE BENEFITS [Benefit to Individual, Community, University]

Study procedures will be provided at no cost to you. You may receive information about your health from any physical examination and investigation tests to be done in this study.

The information achieve from this research will beneficial to provide treatment to future patients.

QUESTIONS

If you have any question about this study or your rights, please contact:

Dr. Chan Hui Tze (
Department of Ophthalmology
School of Medical Sciences
Universiti Sains Malaysia,Health Campus.

If you have any questions regarding the Ethical Approval, please contact:

Mr. Mohd Bazlan Hafidz Mukrim
Secretary of Human Research Ethics Committee USM
Centre for Research Initiatives, Clinical & Health Sciences
USM Health Campus

CONFIDENTIALITY

Your medical information will be kept confidential by the study doctor and staff and will not be made publicly available unless disclosure is required by law.

Data obtained from this study that does not identify you individually will be published for knowledge purposes.

Your original medical records may be reviewed by the researcher, the Ethical Review Board for this study, and regulatory authorities for the purpose of verifying clinical trial procedures and/or data. Your medical information may be held and processed on a computer.

By signing this consent form, you authorize the record review, information storage and data transfer described above.

SIGNATURES

To be entered into the study, you or a legal representative must sign and data the signature page (ATTACHMENT S and ATTACHMENT P).

5.3.2 Malay Version

MAKLUMAT KAJIAN

Tajuk Kajian **:** Kesan Pengambilan Betik Kepada Jumlah Kapasiti Antioksidan, Tekanan Intraokular, dan Lapisan Urat Retina di Pesakit Glaukoma Sudut Terbuka.

Penyelidik Utama : Dr. Chan Hui Tze -

Penyelidik Bersama: Prof. Dr. Liza Sharmini Ahmad Tajudin
Assoc. Prof. Dr. Hamid Jan bin Jan Mohamed

Pengenalan

Anda dipelawa untuk menyertai satu kajian penyelidikan secara sukarela. Kajian ini dijalankan untuk meneliti kajian: Kesan pengambilan betik kepada jumlah kapasiti antioksidan, tekanan intraokular, dan lapisan urat retina di pesakit glaukoma sudut terbuka. Sebelum anda bersetuju untuk menyertai kajian penyelidikan ini, adalah penting anda membaca dan memahami borang ini. Ia menghuraikan tujuan, prosedur, manfaat, risiko, ketidakselesaan dan langkah berjaga-jaga kajian ini. Ia juga turut menerangkan prosedur alternatif yang terdapat untuk anda dan hak anda untuk menarik diri dari kajian ini pada bila-bila masa. Sekiranya anda menyertai kajian ini, anda akan menerima satu salinan borang ini untuk disimpan sebagai rekod anda.

Tujuan Kajian

Kajian ini bertujuan untuk mengkaji kesan pengambilan betik selama 4 bulan kepada pesakit glaucoma dari segi jumlah kapasiti antioksidan, tekanan inraokular, dan lapisan urat retina. Terdapat kemungkinan maklumat yang dikumpulkan dalam kajian ini akan dianalisa oleh pihak penyelidik pada masa depan dan dapat menghasilkan rawatan baru kepada pesakit glaukoma

Apakah Kesan-kesan Sampingan?

Berdasarkan kepada maklumat yang diperolehi, tiada kesan sampingan yang akan timbul daripada ujian-ujian yang akan dilakukan.

Siapa yang tidak layak menyertai kajian ini?

Kriteria Kelayakan

I. Pesakit yang berumur 40 tahun dan keatas
II. Pesakit yang disahkan dengan diagnosis POAG
III. Pesakit yang mematuhi rejim ubat anti-glaukoma
IV. Pesakit yang mengambil ubat dan sudah mencapai IOP sasaran
V. Pesakit rela untuk mengambil papaya diri sendiri lepas 2 bulan

Kriteria Ketidaklayakkan

I. Perokok aktif atau pasif
II. Menghidap glaukoma mutlak
III. Pesakit yang mempunyai sejarah pembedahan mata selain daripada pembedahan katarak.
IV. Pesakit yang mempunyai masalah media mata yang menyebabkan pengukuran Optical Coherence Tomography (OCT) tidak tepat
V. Alahan kepada papain
VI. Kencing manis yang tidak terkawal, HBA1c > 9.0 %
VII. Pernah ambil ubat supplement ketika kajian atau 3 bulan sebelum tarikh kajian.
VIII. Pesakit group B yang ambil betik lebih daripada 3 kali seminggu atau lebih daripada 12 kali sebulan
VIII. Masalah dengan usus

Prosedur Kajian

Sekiranya subjek bersetuju dan memenuhi syarat kelayakan, subjek akan ditemuduga dan memberikan maklumat tentang sejarah perubatan yang lengkap. Subjek akan diminta datang ke Klinik Mata Hospital Universiti Sains Malaysia, dan pemeriksaan akan dijalankan seperti berikut :

1. Pemeriksaan keupayaan penglihatan

Subjek akan diminta untuk membaca huruf atau simbol pada Carta Snellen pada jarak 6 meter bagi setiap mata kanan dan kiri secara berasingan. Pemeriksaan ini akan mengambil masa 1

hingga 2 minit.

2. Pemeriksaan struktur bola mata dan tekanan mata

Pemeriksaan struktur bola mata bahagian hadapan dan bahagian belakang akan dilakukan dengan menggunakan mesin yang dikenali sebagai *'slit lamp biomicroscope'*. Pemeriksaan ini akan dilakukan dalam keadaan pesakit sedang duduk. Bacaan tekanan mata dan sebarang struktur yang abnormal akan direkodkan. Pemeriksaan ini akan mengambil masa lebih kurang 5 hingga 20 minit.

3. Pemeriksaan lapisan urat retina

Seterusnya pesakit akan menjalani pemeriksaan lapisan urat retina dengan menggunakan mesin OCT (Optical Coherence Tomography).

4. Pemeriksaan jumlah antioksidant

Sampel darah sebanyak 3mls akan diambil dan disampan dalam tabung EDTA. Sampel darah akan disimpan di makmal dan dikaji dengan menggunakan QuantiChrom^{TM} Antioxidant Assay Kit (DTAC-100), jumlah antioksidant dalam darah akan didapatkan. Specimen akan dibuang apabila kajian ini berakhir.

5. Penghantaran betik untuk subjek

Subjek akan dibekalkan 3 sajian betik setiap minggu untuk 3 bulan yang pertama. Subjek perlu mengambil satu sajian betik setiap hari untuk 3 hari seminggu.

6. Pemeriksaan jumlah antioksidant, tekanan bola mata dan lapisan urat mata pada bulan

pertama, kedua dan ketiga.

Pemeriksaan ini akan dilakukan di HUSM pada bulan ke-dua dan ke-empat. Pemeriksaan ini hanya mengambil masa selama lebih kurang 20-30 minit sahaja

Semua hasil kajian dan bacaan akan direkodkan dalam dokumen khas untuk setiap pesakit. Setelah kajian, doktor atau staf mungkin akan menghubungi anda untuk mendapatkan maklumat tentang pengalaman anda semasa kajian ini atau keadaan semasa anda.

Risiko

Pemeriksaan tahap penglihatan mata dengan menggunkan carta Snellen adalah amat mudah dan selamat dijalankan. Pemeriksaan ini penting untuk menentukan tahap keupayaan penglihatan pada setiap individu.

Pemeriksaan struktur bola mata dan tekanan mata menggunakan mesin *'slit lamp biomicroscope*' akan memberikan imej struktur mata yang lebih jelas dan besar. Alat ini tidak berbahaya, selesa dan selamat untuk digunakan. Ia tidak akan menyentuh bola mata secara langsung. Kedua-dua pemeriksaan ini adalah pemeriksaan rutin yang dilakukan oleh pakar-pakar mata di seluruh Malaysia dan dunia.

Pemeriksaan mata menggunakan OCT adalah selamat. Pemeriksaan ini dapat membantu memberikan maklumat dalam diagnosa, penilaian dan pemantauan mata pesakit. Pemeriksaan ini kerap dijalankan di klinik-klinik mata yang mempunyai kemudahan ini.

Pengambilan darah untuk jumlah antioksidant di darah akan dilaksanakan oleh doktor yang berpengalaman, tiada risiko dalam pengambilan sampel darah.

Jumlah betik yang dibekalkan di kajian ini adalah dalam lingkungan cadangan Garis Panduan Pemakanan Malaysia versi 2013, jadi adalah selamat untuk makan 3 kali betik yang dibekalkan

dalam seminggu.

Sekiranya penemuan sebarang maklumat penting semasa kajian ini dijalankan yang mungkin mengubah persetujuan anda untuk terus menyertai kajian ini, anda akan diberitahu dengan secepat mungkin.

Melapurkan Pengalaman Kesihatan

Jika anda mengalami gejala yang luarbiasa dan tidak diingini semasa dalam kajian ini, maka anda hendaklah melaporkan kepada penyelidik kajian secepat mungkin.

Nombor telefon penyelidik adalah seperti yang tertera di bawah:

Dr. Chan Hui Tze
Jabatan Oftalmologi
Pusat Pengajian Sains Perubatan
Universiti Sains Malaysia, Kampus Kesihatan.

Anda boleh menghubungi penyelidik pada bila-bila masa tidak kira malam atau siang untuk melaporkan gejala tersebut.

Rawatan Lain

Kajian ini bukan merupakan satu rawatan bagi penyakit atau keadaan anda. Terdapat rawatan dan terapi lain untuk penyakit anda, termasuk rawatan yang sedang anda jalani. Doktor kajian boleh membincangkan rawatan dan terapi ini dengan anda.

Penyertaan Dalam Kajian

Penyertaan anda dalam kajian ini adalah secara sukarela. Anda boleh menolak penyertaan dalam kajian ini atau anda boleh menamatkan penyertaan anda dalam kajian ini pada bila-bila masa, tanpa sebarang hukuman atau kehilangan sebarang manfaat yang sepatutnya diperolehi oleh anda.

Jika anda berhenti menyertai kajian ini, doktor kajian atau salah seorang kakitangan akan berbincang dengan anda mengenai semua isu perubatan berkenaan dengan pemberhentian penyertaan anda.

Manfaat Kajian

Prosedur kajian akan diberikan kepada anda tanpa kos. Anda mungkin menerima maklumat tentang kesihatan mata anda dari hasil pemeriksaan kajian ini.

Maklumat yang didapati dari kajian ini akan memanfaatkan penaja kajian ini dan mungkin memanfaatkan pesakit lain pada masa depan.

Persoalan

Sekiranya anda mempunyai sebarang soalan mengenai prosedur kajian ini atau hak-hak anda, sila hubungi:

Dr. Chan Hui Tze

Jabatan Oftalmologi
Pusat Pengajian Sains Perubatan
Universiti Sains Malaysia, Kampus Kesihatan.

Sekiranya anda mempunyai sebarang soalan berkaitan kelulusan Etika kajian ini, sila hubungi:

En. Mohd Bazlan Hafidz Mukrim

Setiausaha Jawatankuasa Etika Penyelidikan (Manusia) USM
Pusat Inisiatif Penyelidikan -Sains Klinikal & Kesihatan
USM Kampus Kesihatan.

Kerahsiaan

Maklumat perubatan anda akan dirahsiakan oleh doktor dan kakitangan kajian dan tidak akan didedahkan secara umum melainkan jika ia dikehendaki oleh undang-undang.
Rekod perubatan anda yang asal mungkin akan dilihat oleh Lembaga Etika Kajian ini dan pihak berkuasa regulatori untuk tujuan mengesahkan prosedur dan/atau data kajian. Maklumat perubatan anda mungkin akan disimpan dalam komputer dan diproses dengannya.

Dengan menandatangani borang persetujuan ini, anda membenarkan penelitian rekod, penyimpanan maklumat dan pemindahan data seperti yang dihuraikan di atas.

Tandatangan

Untuk dimasukkan ke dalam kajian ini, anda atau wakil sah anda mesti menandatangani serta menarikhkan halaman tandatangan. (lihat Borang Keizinan Pesakit di LAMPIRAN S atau LAMPIRAN P).

5.4 Consent Form
5.4.1 English Version

ATTACHMENT S

Patient/ Subject Information and Consent Form
(Signature Page)

Research Title : Effect of Papaya Intake on Intraocular Pressure, Retinal Nerve Fiber Layer, Optic Nerve Head Parameters and serum Total Antioxidant Capacity in Primary Open Angle Glaucoma Patients

Researcher's Name : Dr. Chan Hui Tze

Supervisor's Name : Prof. Dr. Liza Sharmini Ahmad Tajudin

Assoc. Prof. Dr. Hamid Jan bin Jan Mohamed

To become a part this study, you or your legal representative must sign this page. By signing this page, I am confirming the following:

- I have read all of the information in this Patient Information and Consent Form including any information regarding the risk in this study and I have had time to think about it.
- All of my questions have been answered to my satisfaction.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the doctor, nurses, or other staff members, as requested.
- I may freely choose to stop being a part of this study at anytime.
- I have received a copy of this Patient Information and Consent Form to keep for myself.

_____ _____
Patient Name (Print or type) Patient Initials and Number

_____ _____
Patient I.C No. (New) Patient I.C No. (Old)

_____ _____
Signature of patient or Legal Representative Date (DD/MM/YY)
(Add time if applicable)

_____
Name of Individual
conducting Consent Discussion (Print or Type)

_____ _____
Signature of Individual Date (DD/MM/YY)
Conducting Consent Discussion

_____ _____
Name & Signature of Witness Date (DD/MM/YY)

Note: **i) All subject/patients who are involved in this study will not be covered by insurance.**
ii)Excess samples from this research will not be used for other reasons and will be destroyed with the consent from the Research Ethics Committee (Human), USM.

ATTACHMENT P

Patient's Material Publication Consent Form
Signature Page

Research Title : Effect of Papaya Intake on Intraocular Pressure, Retinal Nerve Fiber Layer, Optic Nerve Head Parameters and serum Total Antioxidant Capacity in Primary Open Angle Glaucoma Patients

Researcher's Name : Dr. Chan Hui Tze

Supervisor's Name : Prof. Dr. Liza Sharmini Ahmad Tajudin
Assoc. Prof. Dr. Hamid Jan bin Jan Mohamed

To become a part this study, you or your legal representative must sign this page.

By signing this page, I am confirming the following:

- I understood that my name will not appear on the materials published and there have been efforts to make sure that the privacy of my name is kept confidential although the confidentiality is not completely guaranteed due to unexpected circumstances.
- I have read the materials or general description of what the material contains and reviewed all photographs and figures in which I am included that could be published
- I have been offered the opportunity to read the manuscript and to see all the materials in which I am included, but have waived my right to do so.
- All the published materials will be shared among the medical practitioners, scientists and journalist worldwide.
- The materials will also be used in local publications, book publications and accessed by many local and international doctors worldwide.
- I hereby agree and allow the materials to be used in other publications required by other publishers with these conditions:
- The materials will not be used as advertisement purposes nor as packaging materials.
- The materials will not be used out of contex – i.e.: Sample pictures will not be used in an article which is unrelated subject to the picture.

_____	_____
Patient Name (Print or type)	Patient Initials or Number
_____ _____	_____
Patient I.C No. Patient's Signature	Date (DD/MM/YY)
_____	_____
Name and Signature of Individual Conducting Consent Discussion	Date (DD/MM/YY)

Note: **i)** **All subject/patients who are involved in this study will not be covered by insurance.**

5.4.2 Malay Version LAMPIRANS

Borang Keizinan Pesakit/ Subjek
(Halaman Tandatangan)

Tajuk Kajian : Kesan Pengambilan Petik Kepada Jumlah Kapasiti Antioksidan, Tekanan Intraokular, dan Lapisan Urat Retina di Pesakit Glaukoma Sudut Terbuka.

Penyelidik Utama **:** Dr. Chan Hui Tze -MMC 52490

Penyelidik Bersama : Prof. Dr. Liza Sharmini Ahmad Tajudin
Assoc. Prof. Dr. Hamid Jan bin Jan Mohamed

Untuk menyertai kajian ini, anda atau wakil sah anda mesti menandatangani muka surat ini. Dengan menandatangani muka surat ini, saya mengesahkan yang berikut:

- Saya telah membaca semua maklumat dalam Borang Maklumat dan Keizinan Pesakit ini termasuk apa-apa maklumat berkaitan risiko yang ada dalam kajian dan saya telah pun diberi masa yang mencukupi untuk mempertimbangkan maklumat tersebut.
- Semua soalan-soalan saya telah dijawab dengan memuaskan.
- Saya, secara sukarela, bersetuju menyertai kajian penyelidikan ini, mematuhi segala prosedur kajian dan memberi maklumat yang diperlukan kepada doktor, para jururawat dan juga kakitangan lain yang berkaitan apabila diminta.
- Saya boleh menamatkan penyertaan saya dalam kajian ini pada bila-bila masa.
- Saya telah pun menerima satu salinan Borang Maklumat dan Keizinan Pesakit untuk simpanan peribadi saya.

_____ Nama Pesakit (Dicetak atau Ditaip)	_____ Nama Singkatan &No. Pesakit
_____ No. Kad Pengenalan Pesakit (Baru)	_____ No. K/P (Lama)
_____ Tandatangan Pesakit atau Wakil Sah	_____ Tarikh (DD/MM/YY) Masa (jika perlu)
_____ Nama & Tandatangan Individu yang Mengendalikan Perbincangan Keizinan (Dicetak atau Ditaip)	_____ Tarikh (DD/MM/YY)
_____ Nama Saksi dan Tandatangan	_____ Tarikh (DD/MM/YY)

Nota: i) Semua subjek/pesakit yang mengambil bahagian dalam projek penyelidikan ini tidak dilindungi insuran

LAMPIRAN P

Borang Keizinan bagi Penerbitan Bahan yang berkaitan dengan Pesakit/ Subjek
(Halaman Tandatangan)

Tajuk Kajian : Kesan Pengambilan Petik Kepada Jumlah Kapasiti Antioksidan, Tekanan Intraokular, dan Lapisan Urat Retina di Pesakit Glaukoma Sudut Terbuka.

Penyelidik Utama : Dr. Chan Hui Tze -

Penyelidik Bersama : Prof. Dr. Liza Sharmini Ahmad Tajudin
Assoc. Prof. Dr. Hamid Jan bin Jan Mohamed

Untuk menyertai kajian ini, anda atau wakil sah anda mesti menandatangani mukasurat ini.
Dengan menandatangani mukasurat ini, saya memahami yang berikut:

- Bahan yang akan diterbitkan tanpa dilampirkan dengan nama saya dan setiap percubaan yang akan dibuat untuk memastikan ketanpanamaan saya. Saya memahami, walaubagaimanapun, ketanpanamaan yang sempurna tidak dapat dijamin. Kemungkinan sesiapa yang menjaga saya di hospital atau saudara dapat mengenali saya.
- Bahan yang akan diterbitkan dalam penerbitan mingguan/bulanan/dwibulanan/suku tahunan/dwi tahunan merupakan satu penyebaran yang luas dan tersebar ke seluruh dunia. Kebanyakan penerbitan ini akan tersebar kepada doktor-doktor dan juga bukan doktor termasuk ahli sains dan ahli jurnal.
- Bahan tersebut juga akan dilampirkan pada laman web jurnal di seluruh dunia. Sesetengah laman web ini bebas dikunjungi oleh semua orang.
- Bahan tersebut juga akan digunakan sebagai penerbitan tempatan dan disampaikan oleh ramai doktor dan ahli sains di seluruh dunia.
- Bahan tersebut juga akan digunakan sebagai penerbitan buku oleh penerbit jurnal.
- Bahan tersebut tidak akan digunakan untuk pengiklanan ataupun bahan untuk membungkus.
- Saya juga memberi keizinan bahawa bahan tersebut boleh digunakan sebagai penerbitan lain yang diminta oleh penerbit dengan kriteria berikut:
- Bahan tersebut tidak akan digunakan untuk pengiklanan atau bahan untuk membungkus.
- Bahan tersebut tidak akan digunakan di luar konteks – contohnya: Gambar tidak akan digunakan untuk menggambarkan sesuatu artikel yang tidak berkaitan dengan subjek dalam foto tersebut.

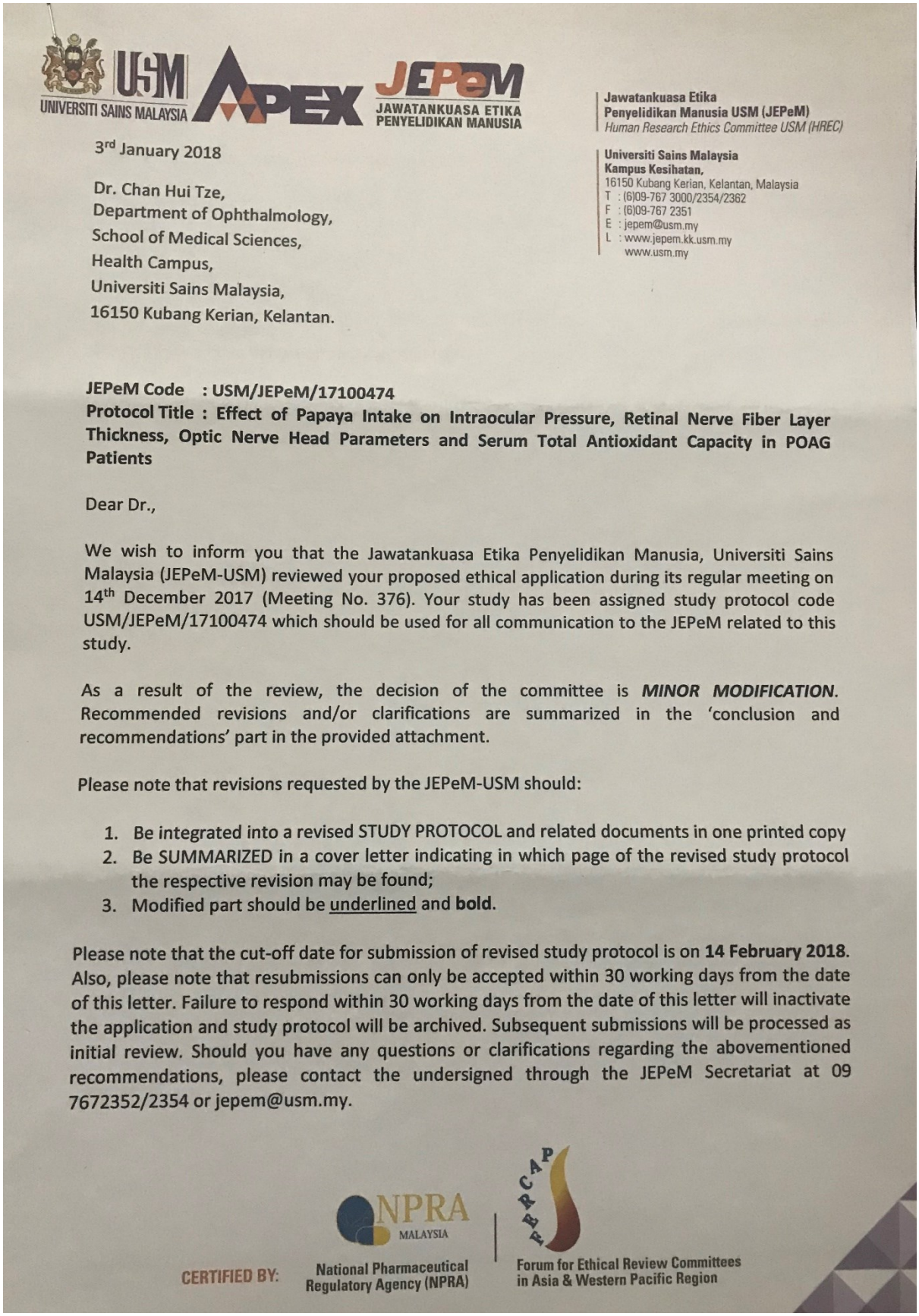
_____ _____
Nama Pesakit (Dicetak atau Ditaip) Nama Singkatan atau No. Pesakit

_____ _____ _____
No. Kad Pengenalan Pesakit T/tangan Pesakit Tarikh (DD/MM/YY)

_____ _____
Nama & Tandatangan Individuyang Mengendalikan Tarikh (DD/MM/YY)
Perbincangan Keizinan (Dicetak atau Ditaip)

Nota: i) Semua subjek/pesakit yang mengambil bahagian dalam projek penyelidikan ini tidak dilindungi insuran.

5.5 Ethical Approval Letter



UNIVERSITI SAINS MALAYSIA

JAWATANKUASA ETIKA PENYELIDIKAN MANUSIA

Jawatankuasa Etika Penyelidikan Manusia USM (JEPeM)
Human Research Ethics Committee USM (HREC)

Universiti Sains Malaysia
Kampus Kesihatan,
16150 Kubang Kerian, Kelantan, Malaysia
T : (6)09-767 3000/2354/2362
F : (6)09-767 2351
E : jepem@usm.my
L : www.jepem.kk.usm.my
www.usm.my

3rd January 2018

Dr. Chan Hui Tze,
Department of Ophthalmology,
School of Medical Sciences,
Health Campus,
Universiti Sains Malaysia,
16150 Kubang Kerian, Kelantan.

JEPeM Code : USM/JEPeM/17100474
Protocol Title : Effect of Papaya Intake on Intraocular Pressure, Retinal Nerve Fiber Layer Thickness, Optic Nerve Head Parameters and Serum Total Antioxidant Capacity in POAG Patients

Dear Dr.,

We wish to inform you that the Jawatankuasa Etika Penyelidikan Manusia, Universiti Sains Malaysia (JEPeM-USM) reviewed your proposed ethical application during its regular meeting on 14th December 2017 (Meeting No. 376). Your study has been assigned study protocol code USM/JEPeM/17100474 which should be used for all communication to the JEPeM related to this study.

As a result of the review, the decision of the committee is ***MINOR MODIFICATION***. Recommended revisions and/or clarifications are summarized in the 'conclusion and recommendations' part in the provided attachment.

Please note that revisions requested by the JEPeM-USM should:

1. Be integrated into a revised STUDY PROTOCOL and related documents in one printed copy
2. Be SUMMARIZED in a cover letter indicating in which page of the revised study protocol the respective revision may be found;
3. Modified part should be underlined and **bold**.

Please note that the cut-off date for submission of revised study protocol is on **14 February 2018**. Also, please note that resubmissions can only be accepted within 30 working days from the date of this letter. Failure to respond within 30 working days from the date of this letter will inactivate the application and study protocol will be archived. Subsequent submissions will be processed as initial review. Should you have any questions or clarifications regarding the abovementioned recommendations, please contact the undersigned through the JEPeM Secretariat at 09 7672352/2354 or jepem@usm.my.

CERTIFIED BY:

NPRA MALAYSIA
National Pharmaceutical Regulatory Agency (NPRA)

Forum for Ethical Review Committees in Asia & Western Pacific Region

The JEPeM-USM looks forward to your immediate response and action.

"ENSURING A SUSTAINABLE TOMORROW"

Very truly yours,

Mohd Bazlan Hafidz Mukrim
Secretary
On behalf of Chairperson
Jawatankuasa Etika Penyelidikan (Manusia) USM

5.6 Raw Data in SPSS