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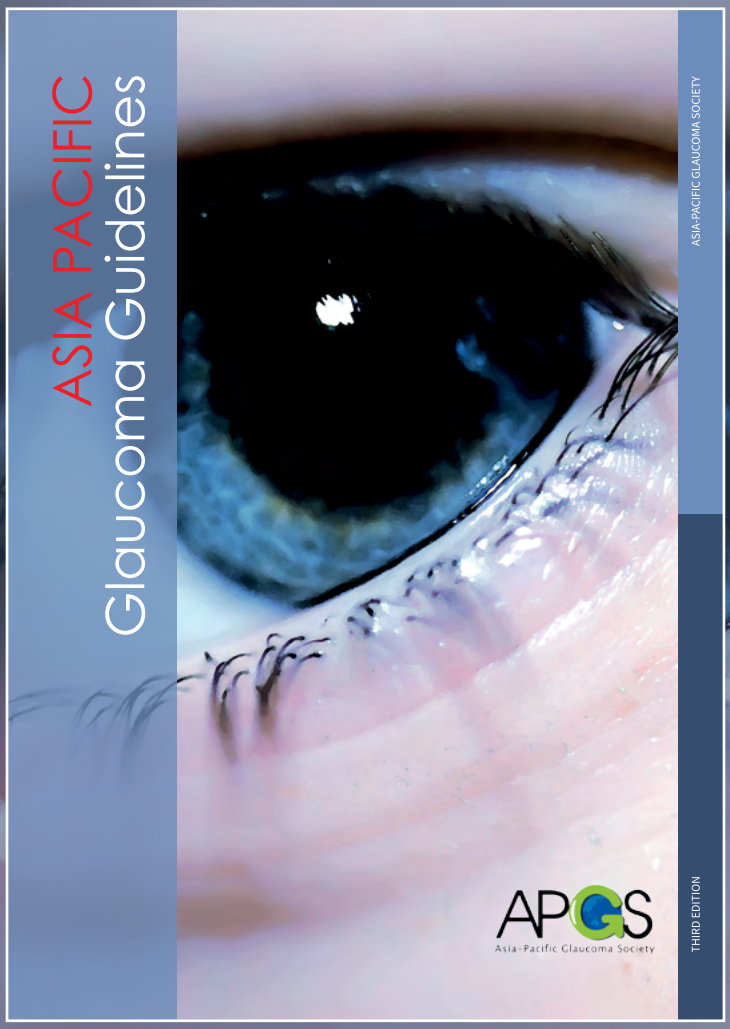

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Ocular sarcoidosis

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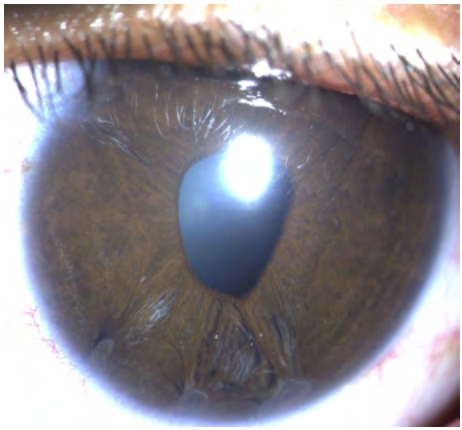

Fig. 1. Tent-shaped peripheral anterior synechiae seen in ocular sarcoidosis.

A woman in her mid-thirties presented with a history of bilaterally decreased vision since the past one year. On examination, she was found to have tent-shaped attachments of the iris to the back of the cornea, which may be seen in ocular sarcoidosis[1] (Fig. 1). They represent scarred down granulomas inferiorly that result in anterior synechiae. Other ocular findings included anterior uveitis, posterior subcapsular cataract, pars planitis, cystoid macular edema as well as raised intraocular pressures. She had a negative Mantoux test and computed tomography of the chest was suggestive of sarcoidosis. Sarcoidosis is a multisystem granulomatous disease. The frequency of ocular involvement ranges from 26% to 50%. Some patients with newly-diagnosed ocular sarcoidosis may have known sarcoidosis due to other organ involvement such as hilar lymphadenopathy. However, other patients may present with *de novo* ocular findings suggestive of sarcoidosis but without obvious extraocular disease.[2,3] Because ocular disease may be the first manifestation of sarcoidosis, physicians should be adept in looking for signs like tent-shaped peripheral anterior synechiae which, although not specific, may be particularly associated with sarcoid uveitis.

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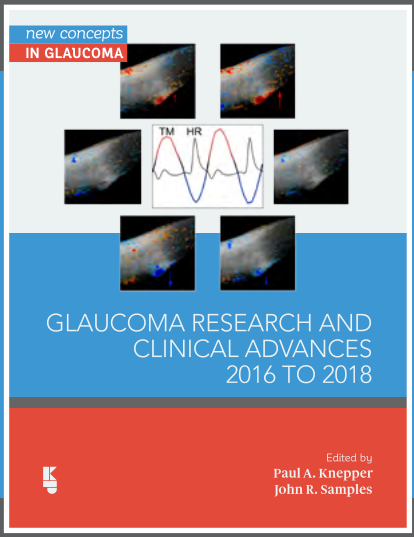

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Association of *PDGFRA* gene polymorphisms and early-onset myopia in South Sumatera, Indonesia

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Abstract

Purpose: *Myopia is a refraction error that may be caused by corneal curvature (CC) anomaly. The platelet-derived growth factor receptor alpha (PDGFRA) gene was determined to have an effect on the CC. The purpose of this study was to find a correlation between single nucleotide polymorphisms (SNP) in the PDGFRA gene and early-onset myopia in people of South-Sumatera origin, a part of an Indonesian ethnic group.*

Design*: Using a random sampling method, this population-based, case-control study included 100 subjects aged 18-40 years from Palembang, South Sumatera, Indonesia.*

Methods: *Visual acuity was measured by Snellen chart and the CC was measured by manual keratometer. DNA sample from buccal swab was investigated with Amplification Refractory Mutation System (ARMS) polymerase chain reaction (PCR) and visualized in agarose gel.*

Results: *Median of CC for the right eye was 7.73 (7.07-8.63) mm and the left eye was 7.73 (7.04-8.69) mm. There was no difference between CC in myopic and normal subjects. Distribution of mutant allele in rs17084051, rs7677751, rs7682912, and rs2114039 were higher in myopic subject compare to those of normal control. Significant association between PDGFRA gene polymorphism and early-onset myopia was found only in rs17084051 ($p = 0.009$) and rs7677751 ($p = 0.001$).*

Conclusions: *Mutant type allele A of rs17084051 and mutant type allele T of rs7677751 of PDGFRA gene polymorphism are associated with early-onset myopia in South-Sumatera tribes in this study.*

Keywords: *corneal curvature, early-onset myopia, Indonesia, myopia, polymorphism, PDGFRA, SNP*

Introduction

The eye is the most important human sensory organ that plays a critical role in human-environmental interaction. In 1996 and 1997, the WHO Program and the Task Force to the Partnership Committee of collaborating Non-Governmental Organizations launched The Global Initiative for the Elimination of Avoidable Blindness. The mission of this program is to have eliminated the main cause of all preventable and

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treatable blindness by the year 2020. Refractive error is the most common disease-causing visual impairment, especially myopia (or nearsightedness).[1]

The highest rate of myopia occurs in Bali, among the age group 11-20 years old (25.1%).[2] Some factors may play a role in myopia, such as corneal curvature (CC), lens thickness, and axial length of the eye.[3-5] The cornea in the myopic eye tends to be steeper compared to the normal eye.[6] The CC tends to be flatter in infancy and then stabilize from one year old until 18 years old.[7]

Myopia is a complex disease that may be caused by a genetic factor or environmental factors.[3,4,8] If a genetic factor was involved, myopia can be also inherited through Mendelian trait, *i.e.*, autosomal dominant, autosomal recessive, and X-linked.[3,4] Based on the onset, myopia can be divided into three groups: early-onset (< 20 years old), early-adult onset (20-40 years old), and late-adult onset (> 40 years old). So, early-onset myopia is nearsightedness that happens before the age of 20.[3,5]

There are some studies dedicated to find the genetic role in myopia. *PAX6* were determined to play a role in high myopia in Han Chinese[9] and Japanese populations.[10] *P4HA2* was associated with non-syndromic high myopia.[11] High myopia was found associated with homozygous frameshift mutation in *LRPAP1* gene.[12] The *GJD2*, *RASGRF1*, *BICC1*, *KCNQ5*, *CD55*, *CYP26A1*, *LRRC4C*, and *B4GALNT2* were associated with myopia in the Japanese population.[13] A study by Han *et al.* found polymorphisms in *FRAP1* and *PDGFRA* gene which were associated with CC in three ethnic groups in Singapore (Chinese, Malay, and India).[14] Mishra *et al.* and Guggenheim *et al.* replicated the study with different populations and found a correlation between *PDGFRA* gene and CC.[7,15]

PDGFRA gene located at chromosome 4q12 encoded a protein called platelet-derived growth factor receptor alpha. This receptor has an intracellular tyrosine kinase activity. When activated, the receptor will start a signaling process through MAP kinase, PI3 kinase, and C-gamma protein kinase. Epithelial and corneal stromal tissue are sensitive to the growth mediator which is activated by MAP and PI3 kinase.[16] PDGFRA were expressed in corneal tissue, especially in epithelial cells, stromal fibroblast, and endothelial cells.[17] Guggenheim *et al.* performed antibody labeling in cornea models and found that the most PDGFRA protein expressions were in cornea epithelial and stromal tissue.[7]

The purpose of this study was to find a correlation between SNPs in the *PDGFRA* gene and early-onset myopia in people of South-Sumatera origin.

Methods

Using a random sampling method, this population-based, cross-sectional study included 100 subjects aged 18-40 years from Palembang, South Sumatera, Indonesia. Myopic subjects were selected from the academic community of Faculty of Medicine Muhammadiyah University Palembang, South Sumatera, Indonesia. The selection was based on a visual acuity test in both eyes, using a Snellen chart. Inclusion criteria for the case group were male or female of South-Sumatera origin with a visual acuity of < 6/6 (in meters) in both eyes, a positive response with

spherical concave lens, age at examination 18-40 years, and literacy.

Because people of South-Sumatera origin with normal eyes were limited in the academic community of the Faculty of Medicine Muhammadiyah University of Palembang, the control subjects were selected from a community outside the case population. Inclusion criteria for the control group were male or female of South-Sumatera origin with visual acuity 6/6 (in meters), negative response with spherical concave and convex lens in both eyes, age at examination 18-40 years, and literacy. Exclusion criteria for both groups were subject with early-adult and late-adult onset of myopia, hyperopia, myopia only in one eye, a history of ophthalmic surgery, and ophthalmic disease during examination.

The history of the selected subjects was taken and keratometry with manual keratometer (Takagi, Japan) was performed. This study followed the tenets of the Declaration of Helsinki. Informed consent was obtained from each subject after the explanation of the nature and possible consequences of the study. This study was approved by the Committee of Bioethics, Humanities, and Islamic Medicine of Faculty of Medicine Muhammadiyah University Palembang.

DNA was extracted from a buccal swab using a Genenaid Presto™ Buccal Swab DNA extraction kit following the manual instructions. DNA quantification was performed using Nanovue Plus. This study investigated seven SNPs: rs7676985, rs17084051, rs7677751, rs2307049, rs7682912, rs7660560, and rs2114039 in the *PDGFRA* gene. Molecular investigation was done using Amplification Refractory Mutation System PCR (ARMS PCR). Primers for ARMS PCR were designed by http://www.primer1.soton.ac.uk/primer1.html.

The PCR reagents mixture consisted of 5 µl KAPA SYBR Fast Universal qPCR kit, 1 µl for each 5 pmol/µl primer, 1 µl DNA sample 10 µg/ µl, and 1 µl H2O PCR grade. PCR reaction for rs17084051 was hold 1 95°C 3′, 40 times (95°C 10″, 60°C 30″, 72°C 30″), and hold 2 72°C 2′. PCR reaction for rs7677751 was hold 1 95°C 3′, 40 times (95°C 10″, 55°C 30″, 72°C 30″), and hold 2 72°C 2′.

Results

This study recruited 100 South-Sumatera people, 50 people were myopic and 50 people were control; 67 (67%) were female and 33 (33%) were male (Table 1).

Table 1. Distribution of sex, visual acuity, and corneal curvature.

Classification	Case Group	Control Group			
	Frequency	Percentage (%)	Frequency	Percentage (%)	
Sex					
Male	41 persons	82	26 persons	52	
Female	9 persons	18	24 persons	48	
Total	50 persons	100	50 persons	100	
Visual acuity					
< 6/6	100 eyes	100	0 eyes	0	
6/6	0 eyes	0	100 eyes	100	
Total	100 eyes	100	100 eyes	100	
Myopia					
< 3 Dioptri	81 eyes	81			
3-6 Dioptri	13 eyes	13			
> 6 Dioptri	6 eyes	6			
Total	100 eyes	100			
Corneal curvature					
Right eye	≤ 7.8 mm	35 eyes	35	29 eyes	29
	> 7.8 mm	15 eyes	15	21 eyes	21
Left Eye	≤ 7.8 mm	34 eyes	34	30 eyes	30
	> 7.8 mm	16 eyes	16	20 eyes	20
Total	100 eyes	100	100 eyes	100	

Median of the CC (interquartile range) for the right eye was 7.72 (7.60-7.93) mm and for the left eye 7.73 (7.59-7.91) mm. The mean value of the CC radius in this study for the right eye was 7.75 ± 0.24 mm and for the left eye 7.76 ± 0.25 mm.

We distinguished different genotypes of subjects using ARMS PCR. The accuracy of this method was confirmed by sequencing of positive control samples (Figs. 1 and 2).

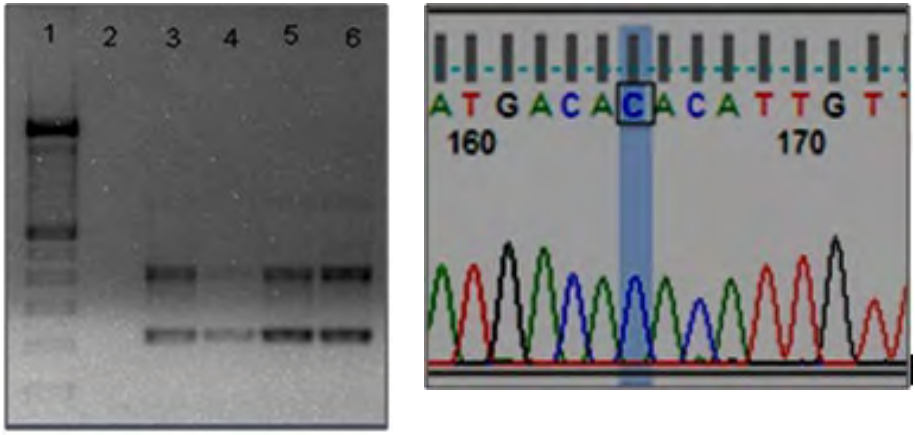

Fig. 1. Electrophoresis result of rs7677751 ARMS PCR for wild type allele. Left: Wild type allele C shown as control band at 409 bp and inner band at 212 bp. (Lane 1: Marker ladder 100bp, 2: Blank, 3: Positive control, 4-6: Wild type allele positive). Right: Positive control showed allele C in the sequencing result.

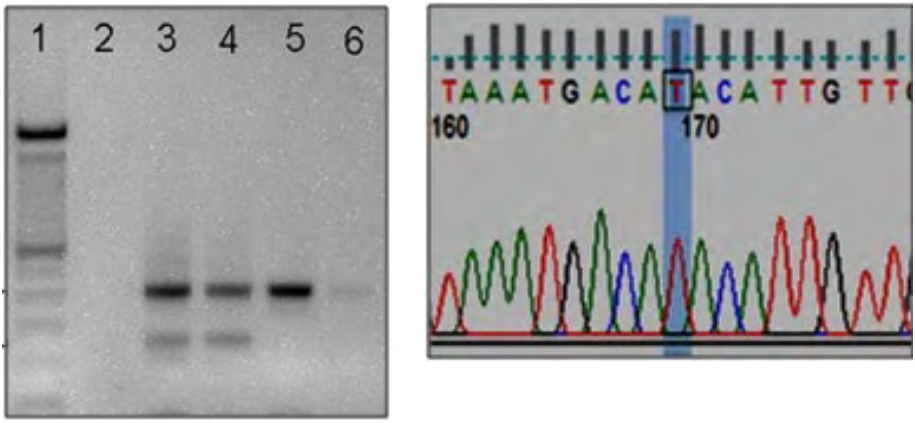

Fig. 2. Electrophoresis result of rs7677751 ARMS PCR for mutant type allele. Left: Mutant allele T shown as control band at 409 bp and inner band at 250 bp. (Lane 1: Marker ladder 100bp, 2: Blank, 3: Positive Control, 4: Mutant allele positive, 5-6: Mutant allele negative). Right: Positive control showed allele T in the sequencing result.

A significant *p* value of < 0.05 was found in two SNPs: rs17084051 and rs7677751. Those two SNPs had a positive association with early-onset myopia. There was no correlation between the seven SNPs with CC in the people of South-Sumatera origin (p > 0.05 for both eyes).

Table 2. Association between mutant allele of SNPs and early-onset myopia.

SNP	Mutant Allele	MAF in South Sumatera population	*p* value for early-onset myopia	PR	CI 95%	*p* value for CC	
						Right eye	Left eye
rs7676985	A	0.18	0.137	0.53	0.23-1.23	0.958	0.958
rs17084051	A	0.29	0.009	2.94	1.30-6.65	0.738	0.738
rs7677751	T	0.24	0.001	4.15	1.80-9.57	0.423	0.701
rs2307049	A	0.43	0.130	0.52	0.22-1.22	0.331	0.331
rs7682912	G	0.45	0.159	1.77	0.80-3.92	0.933	0.933
rs7660560	A	0.20	0.838	1.09	0.49-2.43	0.384	0.657
rs2114039	C	0.32	0.161	1.76	0.80-3.89	0.423	0.701

MAF = Minor allele frequency; PR = prevalence risk; CI = confidence interval; CC = corneal curvature.

Minor allele frequency (MAF) of seven SNPs from all South-Sumatera subjects were resumed and compared to Malay Singaporean subjects from previous study (Table 2).[9] There were four SNPs in the South-Sumatera tribe that have high MAF compared to the Malay Singaporean population.

Discussion

The mean value of the CC radius in this study was higher than the mean value of the CC in three major ethnic groups in Singapore based on a previous study. In the Malay Singaporean population, the mean value was 7.66 mm, in the Indian Singaporean population 7.62 mm, and in the Chinese Singaporean population 7.73 mm.[14]

Some studies found an association between the CC and refractive anomaly. The myopic eye has a steeper CC than the normal eye.[6,7,18] Carney *et al.* (1997) found a difference between the mean value of the CC in emmetropic people and high myopic people.[6] Saw *et al.* (2002) in their study found the mean value of the CC in higher myopia was 7.67 mm and in lower myopia 7.71 mm. They also found a *p* value of 0.001 for the CC in higher myopia.[19] The CC in higher myopia and lower myopia was classified as steeper cornea (≤ 7.8 mm). This study found no correlation between CC and early-onset myopia (*p* value > 0.05). When we tried to classify myopia into high myopia (> 6 D) and low myopia (≤ 6 D), we found no difference between the CC in high myopia and low myopia (*p* value > 0.05). Perhaps that is why in this study we could not find a correlation between the CC and SNPs in the *PDGFRA* gene.

A study in Taiwan, Australia, and China also found that males had a higher CC radius

compared to females.[20,21,22] The CC radius of males of South Sumatera origin was higher (right eye mean 7.79 ± 0.23 mm, left eye mean 7.83 ± 0.26 mm) compared to the CC of females (both eyes mean 7.72 ± 0.23 mm). However, in Nigerians, females had a higher CC radius.[23] Although the male CC was higher than the female CC, this study found no differences in statistics between male and female CC ($p > 0.05$), just like a study in Taiwan school children that found no difference in CC radius between boys and girls.[22]

There were no SNPs of the *PDGFRA* gene that had an association with the CC in people of South Sumatera origin in this study. However, this study found a positive association between mutant allele in rs17084051 and mutant allele in rs7677751 with early-onset myopia. People with positive mutant allele in rs17084051 and rs7677751 was having 2.94 and 4.15 greater risk, respectively, for having early-onset myopia. Because those both SNPs were not associated with CC, perhaps they were affecting another path of myopia pathophysiology, such as axial length or lens thickness. Based on the Ocular Tissue Database, the lens had the highest expression of PDGFRA protein with 745.489 PLIER while the cornea only had 88.85 PLIER.[24] PDGFRA protein also expressed in lens epithelium and conducted of hyper-proliferation and ectopic differentiation into lens fiber cells.[25] rs7677751 was found to be associated with corneal astigmatism in the Singapore population,[26] but not in the Australian population.[27]

SNP rs17084051, rs2307049, rs7682912, and rs2114039 were having MAF 0.29, 0.43, 0.45, and 0.32 respectively. These MAFs were higher than MAF in the Malay Singaporean population. This condition described that the mutant allele A in these SNPs was more existing in the South Sumatera population, Indonesia. The difference in MAF in those SNPs between these two populations may be caused by different ethnicity.

As a multifactorial disease, myopia could be caused by a genetic factor and/or an environmental factor. There was lack of information about genetic involvement in myopia development in Indonesian population. The limitations of this study were the small sample size and the fact that this study only focused on the genetic factor, comparing the genetic susceptibility between people of South Sumatera origin and other ethnicities. For further studies, the environmental factor can be investigated together with the genetic factor so we can resumed which factor that play the biggest role in early onset myopia among South Sumatera tribe population in a bigger sample size.

Conclusion

Mutant type allele A of rs17084051 and mutant type allele T of rs7677751 *PDGFRA* gene polymorphism are associated with early-onset myopia in people of South Sumatera origin, Indonesia.

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Adult orbital tumors: a Southeast-Asian experience

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Abstract

Aim: *A study of the prevalence, demographics, clinical and histopathological features of orbital tumors biopsied in Serdang Hospital, a national Oculoplastic referral center in Malaysia.*

Methods: *This was a retrospective, observational study on all the orbital biopsies performed in Serdang Hospital from January 2008 to December 2014. Outcome measures included demographic data and histological diagnosis of the biopsied orbital lesions.*

Results: *Among the 136 cases, there is slight male preponderance (59.6%). Malays were the commonest racial group (58.8%) followed by Chinese (32.4%). Lymphoproliferative lesion was the most common category (34.6%), followed by inflammatory (10.3%) and lacrimal lesion (9.6%). Benign lymphoid hyperplasia (16.9%) and non-Hodgkin lymphoma (NHL) (16.2%) were the most common histology diagnoses. Benign lymphoid hyperplasia was seen among younger patients (mean 56 years) compared to NHL cases (60.6 years old). Benign lymphoid hyperplasia occurred with the same frequency in the orbit and lacrimal gland, while NHL was mostly located within the orbit. Lymphoproliferative disorders were more common among Chinese males. Cavernous haemangioma, pleomorphic adenoma and solitary fibrous tumor were seen at an earlier age than their Western counterparts. Benign orbital lesions were most common, but may be associated with significant visual and orbital comorbidities.*

Conclusions: *Lymphoproliferative lesions occur at a higher incidence among Southeast-Asian patients, especially among Chinese and male. Our patients also develop cavernous haemangioma, pleomorphic adenoma and solitary fibrous tumor at an earlier age compared to their Western counterparts. There was an ethnic difference among different orbital pathologies. Delayed presentation of benign orbital lesions lead to significant morbidity and even exenteration. Financial restraints may hinder precise histological diagnosis in developing countries.*

Introduction

Orbital tumors encompass a broad spectrum of benign and malignant lesions. It can be intrinsic to the orbit, like cavernous haemangioma, Schwannomas, glioma and inflammation, or it can also arise from adjacent periocular structures such as skin, nose, sinuses and cranial bones with secondary orbital invasion. Metastasis from breast, lung, prostate and skin are also reported. Lymphoproliferative lesions are common.[1]

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Orbital tumors can cause a variety of symptoms. Their space-occupying nature can lead to proptosis, eye displacement, swelling, diplopia, conjunctival congestion, chemosis and exposure keratopathy with associated pain and discomfort. The impingement on adjacent structure can lead to visual loss, dilated pupil and diplopia. The involvement to bone or nerve can also lead to pain. The malignant nature of some disease may even lead to mortality.

Histological examination of orbital biopsies are required to achieve a diagnosis. Orbital biopsy can be incisional or excisional depending on potential diagnosis, size of the lesions and level of infiltration towards normal structures. The surgical incision approach towards orbital biopsy usually depends on the location and size of the lesions. The recent advances in immunohistochemical staining and gene arrangement studies have helped to further characterize lesions and allow more targeted treatment. The classic examples are CD34 used to characterize haemangioma, haemangiopericytoma and solitary fibrous tumor, S100 protein for Schwannoma, neurofibroma, desmin for smooth and skeletal muscle[2] and the recently much discussed Ig G4 staining of IgG4 related disease in idiopathic sclerosing orbital inflammation.[3] Demonstration of light chain restriction using *in- situ* hybridization together with Immunoglobulin heavy chain gene monoclonal rearrangement via southern blot hybridization analysis allows B cell lymphoma to be identified from benign lymphoid hyperplasia.[2]

The types and frequencies of orbital tumors cited in studies vary according to sources, geographical location and age of the study cohort. While there are several publications addressing the incidence of space occupying lesions of the orbit in developed countries[1,4-7] and other parts in the world,[8-10] there is no such data describing incidences of space occupying tumor in South East Asia regions. Malaysia is a country consisting of three main different ethnic groups: Malay (67.4%), Chinese (24.6%), and Indian (7.3%)[11] with a relatively even distribution which provides a meaningful comparison between ethnicities.

The main objective of this retrospective study is to identify the incidence, clinical and histopathological features of orbital tumor among adult patients who had orbital biopsies in Serdang Hospital, a national Oculoplastic referral center in Malaysia. The secondary objective is to identify demographics data associated with various orbital pathologies in Malaysia and to compare the prevalence of orbital tumor with various published data in the world.

Subjects and methods

This is a retrospective, observational study on all the consecutive orbital lesions biopsies performed from January 2008 to December 2014. The informed consent was obtained orally and the subjects do not receive any stipend. This research adhered to the tenets of the Declaration of Helsinki as amended in 2008. The data is obtained via computer data surgical record. The setting is Serdang Hospital, a tertiary referral center for the orbital cases.

Serdang Hospital has been the national oculoplastic referral center for Malaysia since 2006. It receives referrals from all over Malaysia in relation with suspicious

orbital tumors. Therefore data from Serdang Hospital will serve as a useful reference point on the demographics of orbital tumor in Malaysia.

Data collected included patients' age at the time of orbital diagnosis, gender, race, ophthalmic symptoms, laterality, best corrected Snellen visual acuity, location of involved orbital contents (extraocular muscle, lacrimal gland, conjunctiva, eyelid), general histopathological category diagnosis, treatment and follow-up period. The statistical comparison with various countries was based on reports of case studies in those countries.

Inclusion criteria were age above 18, patients who had orbital biopsy and confirmed histology findings. Those who were under 18 years old and declined orbital biopsy were excluded.

Results

In total, 136 patients had orbital tumors biopsied during this period with slight male preponderance (59.6%) as compared to female 40.4%. Sixty-eight patients (50%) had lesions on the right compared to 66 patients (48.5%) patients had lesions in the left, two patients (1.5%) had bilateral lesions. Eighty patients were Malay (58.8%), 44 were Chinese (32.4%), nine were Indians (6.7%), two were Indonesians (1.5%) and one was from Myanmar. The mean follow up period was 7.67 months (median: two months, ranges: one day to 76 months). The presenting symptoms are proptosis 96 (70.6%), reduced vision 80 (59%), eyelid swelling 11 (8%) and ophthalmoplegia 38 (28%).

All patients had CT and MRI scans prior to biopsies. 27.2% of the lesions were intraconal compared to 61.8% extraconal and 11% of the lesions located both intraconally and extraconally. CT/MRI can usually diagnose lymphoproliferative disease well. However, it does not differentiate lymphoma from benign lymphoproliferative lesion. The other lesions that correlate well with CT/MRI are cavernous haemangioma and adenoid cystic carcinoma. Otherwise, CT/MRI more serves to outline the location of lesions rather than informing us about the nature of the lesion.

Seventy (51.5%) patients had incisional biopsies and 55 patients (40.4%) underwent excisional biopsies, whilst 11 patients (8.1%) had exenteration. All patients who had exenteration had malignant lesions except for one patient who had an arteriovenous malformation leading to orbital apex syndrome with no perception of light.

The demographic details of different categories are in Table 1.

Table 1. Classification of 136 consecutive patients with orbital lesions.

Category	No. of patients (%)	Mean age (years; median, range)	Race
Lymphoproliferative	47/136 (34.6%)	57.5 (57; 27-87)	M:25 C: 20 I: 1. Indo 1
Inflammatory	14/136 (10.3%)	44.7 (44; 24-69)	M:11 Chinese 2 Myanmar 1
Lacrimal gland lesion	13/136 (9.6%)	42.8 (36; 21-65)	M: 7 C:2 I: 3, Indo 1
Secondary orbital tumor	10/136 (7.4%)	58.77 (56; 41-86)	M: 10
Vasculogenic lesion	9/136 (6.6%)	42.9 (41: 26-60)	M: 7 C: 2
Cystic tumor	6/136 (4.4%)	35.5 (38; 24-44)	M: 2 C: 1 I: 1
Optic nerve sheath/ meningeal lesion	6/136 (4.4%)	46.8 (45; 29-50)	M:2 C: 2 I: 1
Fibrocystic lesion	4/136 (2.9%)	31.2 (32.5; 25-33)	M: 2 C: 2
Peripheral nerve lesion	4/136 (2.9%)	32 (33.5; 23-38)	M: 2 C: 2
Lipocytic/myxoid lesion	3/136 (2.2%)	48.7 (52; 27-62)	M: 2 C:1
Fibro-osseous lesion	1/136 (0.7%)	23	M: 1
Myogenic lesion	1/136 (0.7%)	32	M: 1
Melanocytic lesion	1/136 (0.7%)	66	M: 1
Metastatic lesion	1/136 (0.7%)	67	C: 1
Histiocytic lesion	1/136 (0.7%)	40	I: 1
Miscellaneous	14/136 (10.3%)	47.3 ($2; 37-72)	M: 3 C: 9 I: 2

M: Malay; C: Chinese; I: Indian.

As for the specific histological diagnosis, lymphoid hyperplasia and NHL constitute 16.9% and 16.2% respectively. Median age of patients who develop benign lymphoid hyperplasia (57 years old) was younger than that of NHL (60 years old). This is followed by chronic inflammation (10.3%), cavernous haemangioma (5.1 %), and pleomorphic adenoma (3.7%). Dermoid cyst, Schwannoma extra pleural solitary fibrous tumor and pleomorphic adenoma shared the same occurrence (2.9%) (Table 2).

Stratification of orbital lymphoproliferative lesions was performed to further characterize the lesions (Table 3). Benign lymphoid hyperplasia usually affected the

orbit (7.4%) and lacrimal gland (6.6%) equally. Patients with lacrimal gland involvement were slightly older (63.4 years old) compared with orbital involvement (58.6 years old). In comparison, NHL usually affect orbit only (14% of total orbital lesions) with a mean age of 59.7 years.

Table 2. Frequency of patients with orbital lesions according to histology diagnosis (commonest nine only).

	Diagnosis	No. (%)	Mean age (yrs, median, range)	Sex	Race
1	Benign lymphoid hyperplasian (BLH)	23 (16.9%)	56 (57; 27-79)	M: 18 F: 5	M: 13 C: 9
2	Non-Hodgkin lymphoma (NHL)	22 (16.2%)	60.6 (60; 42-87)	M: 13 F: 9	M: 11 C: 9 I: 1
3	Chronic inflammation	14 (10.3%)	45.8 (46; 24-71)	M: 7 F: 6	M: 11 C: 2 Myn: 1
4	Cavernous haemangioma	7 (5.1%)	42.9 (41; 33-64)	M: 1 F: 6	M: 5 C: 2
5	Pleomorphic adenoma	5 (3.7%)	38.2 (34; 26-65)	M: 5	M: 3 I: 1 Indo: 1
6	Dermoid cyst	4 (2.9%)	35.5 (38; 24-44)	M: 3 F: 1	M: 2 C: 1 I: 1
7	Schwannoma	4 (2.9%)	52.2 (51.5; 29-77)	M: 4	M: 2 C: 2
8	Extrapleural solitary fibrous tumor	4 (2.9%)	31.2 (32.5; 25-35)	M: 2 F: 2	M: 2 C: 2
9	Ca ex pleomorphic adenoma	4 (2.9%)	53 (50; 33-74)	M: 3 F: 1	M: 2 C:2

Table 3. Stratification of 47 patients with lymphoproliferative lesions.

Category	No. (% of patients)	Mean age (median, range)	Race
Benign lymphoid hyperplasia			
Orbital	10 (7.4%)	58.6 (60.5; 27-78)	M: 6, C: 4
Orbital, lacrimal gland	4(2.9%)	46 (49.5; 27-58)	M: 3 C: 1
Lacrimal gland	9 (6.6%)	63.4 (39-79)	M: 5 C: 4
Non-Hodgkin lymphoma			
Orbital	19 (14%)	59.7 (57; 42-87)	M: 9 C: 9
Orbital/lacrimal gland	1 (0.7%)	61	M: 1
Lacrimal gland	1(0.7%)	75	C: 1

For those who had NHL, the majority (18/22 or 81.8%) had low grade lesions (namely extranodal marginal zone B cell lymphoma, small lymphocytic B cell lymphoma and nodular low grade B cell lymphoma). All those with aggressive lymphoma had diffuse large B cell lymphoma. Only two patients had pre-existing orbital lymphoma and none had systemic NHL prior to diagnosis. As for pre-existing disease prior to diagnosis, three patients had diabetes mellitus and one patient had a history of intravenous drug use but a negative test for HIV. All patient had localized orbital disease except for one with diffuse large B cell lymphoma with lung nodules. All were referred to an oncologist for further management.

Chronic inflammation presented as a heterogeneous group involving various structures in the orbit. All of our patients had either proptosis or dysmotility. Pain was not a predominant feature. Four lesions (30.7%) involved the lacrimal gland with others affecting the intraconal area, extraocular muscle such as inferior rectus and also extraconal lesions. As for the histological appearance, majority of the lesions (8/14 or 61.5%) were of lymphocytic lineage. Intense fibrosis was found to be present in 5/14 (38.5%) of the sample. One patient was pANCA positive and subsequently developed lupus nephritis.

With regard to the prevalence of benign lesion compared with malignant lesions, 86/136 (63.2%) of the patients were benign compared with 50/136 (36.8%) being malignant. Morbidities among benign lesions were proptosis 61/86 (70.9%), eyelid swelling 27/86 (31.4%), ophthalmoplegia 22/86 (25.6%) and reduced vision 26/86 (30.2%). Most of our patients with lymphoproliferative lesions present with proptosis in the absence of any inflammatory symptoms. Some of them were not reversible even after surgical removal. The authors had attempted to analyze the correlation of different ocular symptoms (proptosis, reduced vision and diplopia) and systemic symptoms (*e.g.*, loss of appetite/weight), but were unable to find any correlations between them. We believe the different symptoms are more related

with the location of lesions rather than the nature of the lesions.

Various techniques have been adopted to remove orbital masses. The choice of procedure, whether through subbrow, lateral orbitotomy or subciliary incision depends on the location of lesion. Eleven patients with malignant lesions had exenteration.

Discussion

This is the first data for adult orbital tumor in South East Asia region. There was a wide variety of tumors encountered. The commoner frequency of benign lesion (61.8%) among our patients was comparable to Bonavoluta *et al.* data (68%),[4] but was slightly different from Shinder's data[7] (37%) (Table 4).

Table 4. Comparison incidence with other published data.

Name of data	Country	Benign *vs* malignant	Type of tumor	Commonest tumor
Shinder *et al.*[7]	Texas, USA	37% benign; 63% malignant	64% primary orbital; 26% secondary orbital; 10% metastasis	1) Secondary orbital tumor 26%; 2) Lyphoproliferative 25%; 3) Metastasis 10%; 4) Epithelial lacrimal gland tumor 10%; 5) Inflammation 8%
Shields *et al*[1] (1264)	Wills Eye Hospital, USA	52.5% benign; 47.5 % malignant		1) Lymphoid tumor 11%; 2) Idiopathic orbital inflammation 11%; 3) Cavernous haemangioma 6%; 4) Lymphangioma 4%; 5) Meningioma 4%
Johansen[5] 1974-1997	Denmark	55% benign; 45% malignant	43% primary orbital; 48% secondary orbital; 9% metastasis	
Bonavolunta[4] (2480) (1976-2011)	Italy	68% benign; 32% malignant		1) Dermoid cyst 14%; 2) NHL 12%; 3) Cavernous haemangioma 9%

Name of data	Country	Benign *vs* malignant	Type of tumor	Commonest tumor
Shikishima[8]	Japan	65% benign; 35% malignant	47% primary; 30% secondary; 22% inflammation	1) Inflammatory pseudo-tumor 18%; 2) Malignant lymphoma 12%; 3) Pleomorphic adenoma 7%
Ni[9] 1953-1992	China			1) Cavernous haemangioma 36%; 2)Malignant lacrimal gland tumor 32%; 3) Malignant lacrimal sac tumor 26%
Our study	**Malaysia**	**63.2% Benign; 36.8% malignant**	**92.6% primary; 7.4% secondary**	**1) BLH 16.9%; 2) NHL 16.2%; 3) inflammation 10.3%**

As for the category of lesion, lymphoproliferative lesions were the most common pathology, just like our western counterparts.[1,7] However, our prevalence of 34.6% was much higher compared to Western country counterparts, 10% in Shields' data[1] and 25 % in Shinder's.[7] Both benign lymphoid hyperplasia and NHL were the most common lesions found. As benign lymphoid hyperplasia and NHL falls within the same spectrum of the disease,[12-14] it is understandable that benign lymphoid hyperplasia occurs at slightly a younger age (57 years old) compared to NHL (60 years old). One patient with benign lymphoid hyperplasia progressed to extranodal marginal zone lymphoma within the period of two years. Hence if there is any suspicion on progression of lesion, further biopsy is required. The majority (81.8%) of our patients had low-grade lymphoma which was very similar to the published literature.[15,16] However, systemic work-up for staging is important, especially those with high grade lymphoma, as the patient was found to have a concurrent lung disease.

The high prevalence of chronic inflammation also warranted attention especially as the majority of the inflammation is of lymphocytic lineage and pain is not a predominant complaint among those patients. Of our patients, 38.5% had sclerosing orbital inflammation, which may represent a different subtype and separate entity, such as IgG4-related disease.[17] Unfortunately, we are yet to have the financial resources for the IgG4 staining in the public hospital in Malaysia. The recent case report of IgG4-related dacryoadenitis that regressed without steroid but with anti-allergic administration may provide a useful alternative treatment for our patients who are not able to tolerate steroids.[18] Recent evidence has suggested that chronic inflammation may lead to or mimic lymphoma, three patients who had

the histological appearance of IgG4 disease also showed immunoglobulin heavy-chain rearrangement and light chain restriction.[19,20] Regular vigilant follow-up is required to monitor the progress of the patients and re-biopsy may be required if the disease progresses.

Another important observation is that there were four cases of orbital extrapleural solitary fibrous tumors (SFT), which is being increasingly recognized. It is a rare spindle-cell benign lesion with only 80 cases encountered in the orbit so far.[21] It is commonly described as a slow-growing painless orbital mass and/or facial deformity. On imaging, it presents as a well-defined soft tissue mass with strong enhancement on CT and MRI imaging.[22] If extensive bone remodeling is present or a long-standing lesion is noted, the possibility of malignant transformation should be suspected, particularly if a partial excision had been attempted.[22] Histologically, it displays haphazardly arranged fibroblast like cells (spindle) indistinct nucleoli, variable stromal collagen, and prominent vasculature with perivascular fibrosis which shares many similarities with other pathologies.[21] However, it has a characteristic immunohistochemical staining pattern which is strong positivity towards CD34 (90-100%) and CD 99 (70%) with about one third positive for Bcl-2. It is not immunoreactive for S100 protein like Schwannoma.[21] It is a benign lesion if completely excised. Unfortunately, in three of our four patients, they were recurrent lesions previously operated elsewhere and adhered to periosteum and extraocular muscle making complete excision impossible. Lifelong follow up is required as there is risk of malignant transformation.[21]

Age distribution for various types of lesions was also analyzed and compared with the western counterparts in the published literature. Pleomorphic adenoma occurs at a younger age (mean age 38 years old) compared to Western counterparts (48 years old[1]). A similar trend was noted with cavernous haemangioma (42.9 years old vs 48 years old[1]) and extrapleural solitary fibrous tumor (31.2 years old vs 43 years old[21]).

As for racial distribution, it is found that lymphoproliferative disorder seems to have equal distribution between Malay and Chinese. However, if one takes into account the constitution of races in Malaysia in which Malay constitutes 60.3% and Chinese only constitutes 22.9% of population,[11] it is clear that there is a preponderance of such condition among Chinese. Our results share similarity with Shikishima's data from Japan where no obvious reasons can be found.[8] Hence there should be a low threshold to perform orbital biopsy for some patients with these demographics who present with symptoms of orbital tumor. Malays had a higher incidence of orbital chronic inflammation and secondary orbital tumors at 78.6% and 100% respectively in our study which is yet to be reported.

In the west, adult patients were more likely to have a higher incidence of orbital metastases and lymphoma.[6] However, in our series, patients were more likely to have lymphoproliferative disorders and inflammation.

Orbital tumors including those of a benign nature can create a significant amount of morbidity among patients. Whilst they may not be related with reduced lifespan, morbidities such as proptosis, ophthalmoplegia and reduced vision may not be

reversible despite surgical intervention.

The advent in immunophenotyping of orbital diseases have allowed rare lesions such as solitary fibrous tumor to be recognized. It also aided in differentiation of benign lymphoid hyperplasia from lymphoma. The recent recognition of IgG4 as a subgroup of orbital inflammation[3] will provide more insight onto orbital inflammation although we are yet to have the relevant immune-essays and immunostaining to analyze this disease due to financial constraints. Our series highlighted the common problems faced by clinicians in developing countries in which financial constraints in obtaining tissue staining are one of the huddles of getting a precise diagnoses of orbital lesions.

We admit the limitation of the retrospective nature of our study and we are in the process of establishing national orbital biopsy data with prospective collection of data including those from the private sector. We also admit that we may miss out on a group of patients who may decline orbital biopsy or has been treated empirically with steroid by medical practitioners elsewhere. It is hoped that the publication of this data will educate the local clinicians to refer patients with orbital tumors to qualified oculoplastic surgeons for further investigative management. We will also seek to collaborate with hematologist to identify the long-term survival outcome of those with lymphoproliferative orbital lesions.

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Conflict of interest: Ho SF, None; Radzlian Othman, None.
Dr Ho SF conceived the ideas of this research, collected the data, wrote and edited the manuscript. Dr Radzlian Othman edited the manuscript. Dr Ho SF and Dr Radzlian Othman had full access to the data.

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Comparison of efficacy and safety of Difluprednate 0.05% and Nepafenac 0.1% in reducing macular thickness and volume after cataract surgery

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Abstract

Aim or Purpose: *To evaluate and compare efficacy and safety of topical Difluprednate ophthalmic emulsion 0.05% with Nepafenac ophthalmic suspension 0.1% in patients of uneventful cataract surgery with respect to postoperative macular thickness and volume.*

Design: *A prospective, single centric, tertiary care center-based, comparative, interventional study from August 2013 to July 2014.*

Subjects: *Total 206 (Group N = 106, Group D = 100) patients were followed-up, who completed their 12 weeks follow-up.*

Methods: *Surgery was performed by phacoemulsification technique by clear corneal incision with foldable PCIOL implantation by a single surgeon having ten years of surgical experience. Postoperative patients were divided into two groups. Group N were given topical treatment with Nepafenac ophthalmic suspension 0.1% TID starting 24 hours before surgery and continued postop four weeks. Group D were given Difluprednate ophthalmic emulsion 0.05% QID post-surgery for two weeks followed by BID for two weeks.*

Main outcome measures: *Postoperative assessment of patients were done on first day and on first, eighth and 12th weeks after the surgery for best corrected visual acuity (BCVA) by logMAR, intraocular pressure by applanation tonometry and macular thickness and volume by SD-OCT.*

Statistical test used was sample unpaired and paired 't' test and statistical analysis was done with SPSS 20.0 (IBM, USA).

Results: *There was increase in the measured mean central subfield thickness (CST) at eight and 12 weeks as compared to one week, in both study groups ($P < 0.05$). On comparing the volume (in mm^3) and average thickness (in µm) at one week, it was observed that the thickness of group N (266.82 ± 25.06 µm) was statistically higher than that of group D (253.14 ± 22.21 µm) ($P = 0.03$). The comparison of best corrected visual acuity (LogMAR) and the intraocular pressure recordings showed no difference between the patients of two studied groups recorded at one, eight and 12 weeks.*

Conclusion: *Both Nepafenac ophthalmic suspension 0.1% and Difluprednate ophthalmic emulsion 0.05% are equally effective in controlling macular thickness change after uneventful cataract surgery.*

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Introduction

Incidence rate of increase in macular thickness post cataract surgery ranges from 1-6% in uncomplicated cataract surgery to 5-10% after posterior capsule rupture.[1,2] Therapeutic interventions are based on the proposed pathogenesis of edema, mainly inflammation and vitreous traction.[3] Inflammation after cataract surgery is generally managed by topical anti-inflammatory drugs such as corticosteroids or NSAIDS.[4] The majority of physicians employ a prophylactic regimen of anti-inflammatory medications in the pre-operative and post-operative period.

Currently, no standardized protocol exists for the prophylaxis and management of increased macular thickness because of a lack of prospective randomized clinical trials. The purpose of this study was to evaluate and compare efficacy and safety of topical Difluprednate ophthalmic emulsion 0.05% with Nepafenac ophthalmic suspension 0.1% in patients of uneventful cataract surgery with respect to postoperative macular thickness and volume.

Material and methods

The study was done as per tenets of Helsinki. It was a prospective, single centric, tertiary care center-based, comparative, interventional study from August 2013 to July 2014**.** The sample size was calculated on the basis of the assumption of a difference of 10 nm of macular thickness between two study groups and a SD within 10% difference and 95% confidence limit. Calculated sample size for each group was 81 patients. Cases were randomized into two groups by using computer generated random numbers: Group N [n = 81] and Group D [n = 81]. All patients above 40 years of age with senile cataract grading NS I-III, undergoing cataract surgery for visually significant cataract (phacoemulsification with PCIOL implantation) in the department of ophthalmology were included in the study. Patients receiving treatment for any other co-existing ocular pathology, having a history of any recent intraocular surgery, systemic illness which may increase macular thickness, hypersensitivity to the drugs used in the study were excluded. Patients having pre-existing macular disease or taking systemic medications which may affect macular thickness were also excluded. Patients with macular disease as seen on clinical evaluation at one week were excluded from the study (especially patients with dense cataract in whom preoperative fundus evaluation was not possible).

Written informed consent was taken for inclusion from all patients for participation in the study. Assessment of patients was done on the preoperative day by detailed history and clinical examination. Surgery was performed by phacoemulsification technique by clear corneal incision with foldable PCIOL implantation by a single surgeon having ten years of surgical experience. Postoperatively, patients were assessed for iris trauma, posterior capsular rupture and/or vitreous loss. Moxifloxacin hydrochloride ophthalmic suspension 0.5% QID for two weeks starting 24 hours prior to surgery and Cyclopentolate hydrochloride ophthalmic suspension 1.0% HS for one week were given to both groups. Group N were given topical treatment with Nepafenac ophthalmic suspension 0.1% TID starting 24

hours before surgery and continued postoperatively for four weeks. Group D were given Difluprednate ophthalmic emulsion 0.05% QID post-surgery for two weeks followed by BID for two weeks. Postoperative assessment of patients were done on the first day and on one, eight and 12 weeks after the surgery for best corrected visual acuity (BCVA) by logMAR, intraocular pressure by Applanation tonometry and macular thickness and volume by SD-OCT.

OCT protocol

OCT 512x128 scans were done with CIRRUS SD-OCT (Zeiss, USA) for macular thickness assessment.[5] Macular thickness was reported in a modified Early Treatment of Diabetic Retinopathy Study macular map with the central subfield one mm in diameter and the inner and outer subfields having diameters of three mm and six mm, respectively [Figs.1a, 1b, 1c]. The retinal thickness in the inner and outer subfields, the central foveal thickness (CFT), the center point thickness (CPT), and the macular volume were calculated. CPT was defined as average of six radial scans centered at the foveola, whereas the CFT was defined as the average of all points within the central one mm diameter circle surrounding fixation.[6]

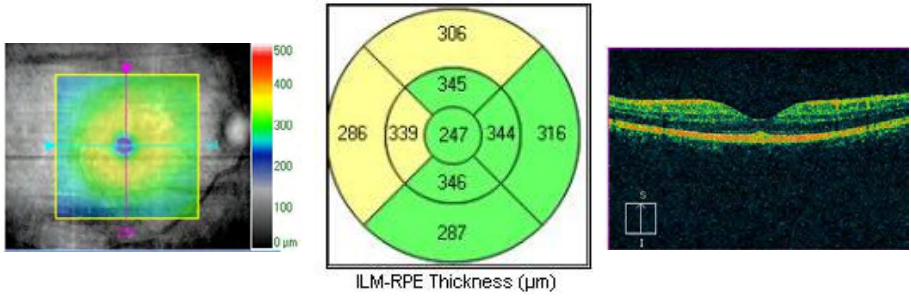

Fig. 1. Macular thickness map using ETDRS circles of one mm, three mm, and six mm showing the mean thickness in each of the nine subfields in a participant.

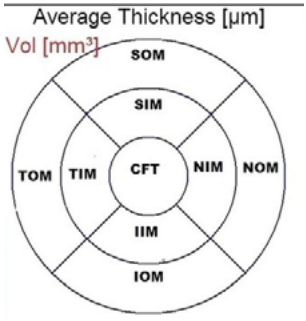

Fig. 2. The standard ETDRS subfields dividing the macula into central fovea, inner macula, and outer macula. CFT: Central foveal thickness; SIM: Superior inner macula; NIM: Nasal inner macula; IIM: Inferior inner macula; TIM: Temporal inner macula; SOM: Superior outer macula; NOM: Nasal outer macula; IOM: Inferior outer macula; TOM: Temporal outer macula.

Statistical analysis

Average macular thickness and volume by OCT were recorded on each visit for both groups and compared by statistical test by two sample unpaired and paired 't' tests. BCVA and IOP were recorded on each visit and compared between two groups by unpaired and paired 't' test. $P < 0.05$ was considered for level of significance during statistical analysis. Statistical analysis was done with SPSS 20.0 (IBM, USA).

Results

A total of 300 patients were screened. Seventy-two patients were excluded (senile cataract of grade NS-IV - 42, and diabetes mellitus and/or hypertension 30), so 228 patients were enrolled in the study: 118 in group N and 110 patients in group D. Four patients were discontinued from study after recruitment (perioperative complications - one, retinal pathology postoperatively - one, and other complications revealed on the first postoperative day - two). Two hundred twenty-four patients were included in the study for further follow-up (114 patients in group N and 110 patients in group D). Eight patients from group N were lost to follow-up before completion of 12 weeks and excluded from the study. Ten patients from group D were lost to follow-up before completion of 12 weeks and excluded from the study. A total of 206 (N = 106, D = 100) patients were followed-up who completed their 12 weeks follow-up after which the data was compiled and statistical analysis was done (Fig. 1).

There was no statistically significant difference in baseline (first day) measurements of central subfield thickness (CST), macular volume, average thickness and intra ocular pressure between group D and group N (Tables 2, 4, 6, 9). The two study groups were comparable in terms of age and the gender ratio of the study patients as shown in Table 1. No statistical difference was observed in the mean CST recorded at one, eight and 12 weeks in both the study groups ($P > 0.05$) (Table 2). There was increase in the measured mean CST at eight and 12 weeks as compared to one week, in both the study groups ($P < 0.05$). But there was no difference from one to eight and 12 weeks among the two study groups (Table 3).

On comparing the volume (in mm^3) and average thickness (in µm) at one week, it was observed that the thickness of group N (266.82 ± 25.06 µm) was statistically higher than that of group D (253.14 ± 22.21µm) ($P = 0.03$). Otherwise there was no statistical difference in the volume (in mm^3) and average thickness (in µm) of patients in two study groups at one week, eight weeks and 12 weeks ($P > 0.05$) (Tables 4 and 6). In either of the studied groups no statistical change in the volume (in mm^3) and average thickness (in µm) of patients was observed between one week and eight weeks and between one week and 12 weeks ($P > 0.05$) as shown in Table 5 and Table 7, respectively. The comparison of best corrected visual acuity (LogMAR) and the intraocular pressure recordings showed no difference between the patients of two studied groups recorded at one week, eight weeks and 12 weeks as shown in Table 8 and Table 9, respectively.

Discussion

Ocular inflammation after cataract surgery is generally managed by topical anti-inflammatory drugs such as corticosteroids and NSAIDS. Pre and postoperative treatment with anti-inflammatory drops is now standard in many centers to reduce surgically induced inflammation. Control of postoperative inflammation is important in ensuring a successful outcome after cataract surgery. Deciding which anti-inflammatory agent is to be used as standard in patients undergoing cataract surgery is important to ensure a favorable outcome. Current guidelines do not provide specific recommendations concerning the postoperative management of inflammation.[7]

There are multiple reports comparing role of various steroid (Betamethasone, Dexamethasone, Fluorometholone, Rimexolone) with different types of NSAIDs (Diclofenac, Ketorolac, Bromfenac, Indomethacin and Flurbiprofen) in controlling post cataract surgery inflammation.[8-13] To the best of our knowledge, there is only one report in which Nepafenac has been compared with a steroid (Fluorometholone).[14] Corticosteroids are typically the cornerstone of these treatment regimens because of their broad anti-inflammatory activity.[15]

Difluprednate 0.05% ophthalmic emulsion is a potent new topical synthetic difluorinated prednisolone derivative steroid that exhibits enhanced penetration, better bioavailability, rapid local metabolism, and strong efficacy with low incidence of adverse effects. It has been have incorporated into their standard anti-inflammatory treatment regimen for postoperative inflammation.[7] Difluprednate, being a steroid derivative, can also be associated with elevated IOP. Thus, standard care of practice must be employed, with frequent measurement of eye pressure for anyone using this medication.

Nepafenac ophthalmic suspension 0.1%, a topical prodrug, is the first prodrug ophthalmic NSAID formulation approved for use in the US for the treatment of postoperative pain and inflammation after cataract surgery. The theoretical advantage offered by Nepafenac over other existing NSAIDs is in corneal penetration, providing a better bio-availability. Prophylactic use of Nepafenac prior to cataract surgery may in fact lessen postoperative inflammation avoiding intraocular pressure-related complications incurred with frequent administration of high dose corticosteroids postoperatively.

This is the first study planned to evaluate and compare efficacy and safety of topical Difluprednate ophthalmic emulsion 0.05% with Nepafenac ophthalmic suspension 0.1% in patients of uneventful cataract surgery with respect to postoperative macular thickness and volume. In the present study we observed that central subfield thickness recorded in patients were reported to be the maximum at eight weeks after which there was a fall in central subfield thickness values in Nepafenac 0.1% group (group N) and unchanged in Difluprednate group 0.05% (group D) (Tables 2 and 3). Previous studies have also reported that macular thickness, as assessed by OCT in patients without pseudophakic cystoid macular edema, peaks at approximately four to six weeks postoperatively.[16-18] Our finding is supported by earlier fluorophotometric findings, that an earlier

re-establishment of the blood-aqueous barrier occurs in NSAID-treated patients compared with steroid-treated patients.[19] We failed to observe any difference in the visual acuity, macular volume and average macular thickness, between two study groups. Despite an initial fall in the central subfield thickness by Nepafenac group compared to Difluprednate ophthalmic emulsion 0.05%, the final average macular thickness, macular volume and visual acuity showed no difference in both the groups. Thus, both Nepafenac ophthalmic suspension 0.1% and Difluprednate ophthalmic emulsion 0.05% are equally effective in controlling macular thickness change after uneventful cataract surgery.

The risk of rise of IOP after steroid use has tempted many surgeons to turned to NSAIDs to control inflammation after cataract surgery.[20] But we did not observe any statistically significant difference between IOP recorded in both the groups during the 12-weeks follow-up. Also there was no evidence of increased risk of adverse events with the use of NSAID, although previous reports have indicated that prolonged use of topical NSAIDs may be associated with a risk of corneal melts and impaired corneal wound healing.[21,22]

Conclusion

Nepafenac ophthalmic suspension 0.1% offers no advantage over Difluprednate ophthalmic emulsion 0.05% in reducing macular thickness and volume post uneventful cataract surgery. Both Difluprednate ophthalmic emulsion 0.05% and Nepafenac ophthalmic suspension 0.1% are comparable in terms of elevating IOP. Thus, both have equal efficacy and safety in reducing postoperative macular thickness and volume in patients of uneventful cataract surgery.

Fig. 1. Flowchart for patient recruitment.

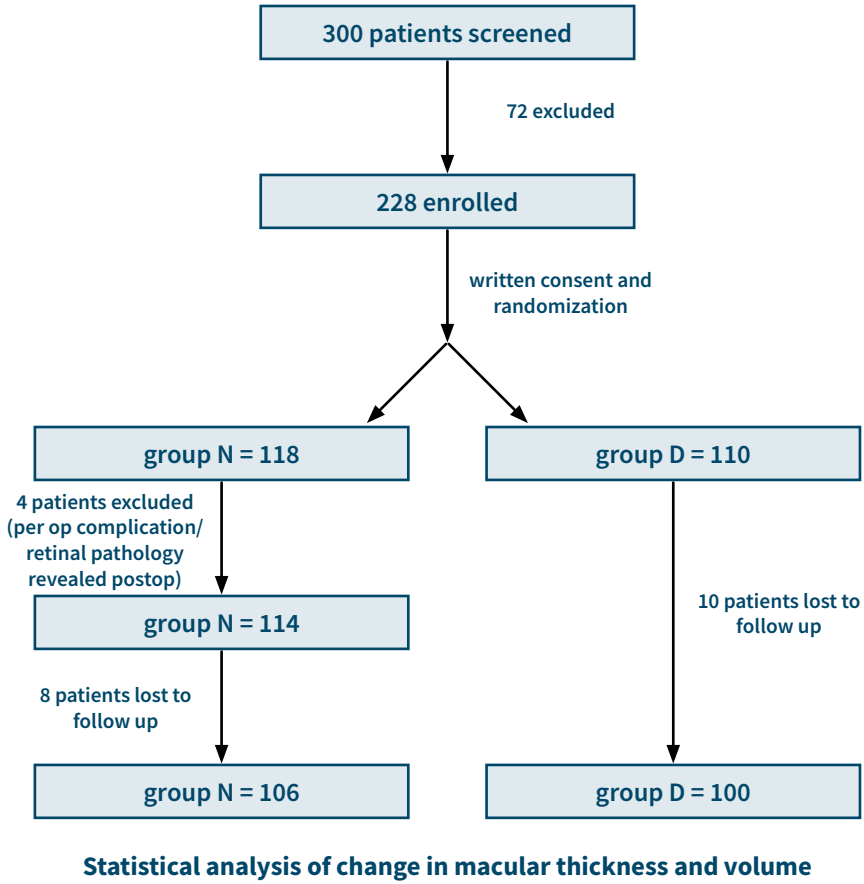

Table 1. Comparison of age distribution of the patients in two groups.

	Group N (n = 56)	Group D (n = 50)	p-value
Age in years	56.43 ± 9.11	59.00 ± 10.38	0.32[1]
Gender ratio (M/F)	20/36	16/34	M: 0.56[1] F: 0.58[1]

Group N: Nepafenac 0.1%; Group D: Difluprednate 0.05%; [1]Unpaired t-test; [2]Chi-square test.

Table 2. Comparison of central subfield thickness (in µm) between the groups across the time interval.

	Group N (n = 106)	Group D (n = 100)	p-value
Baseline (1st day) 1 week	240.36 ± 24.23 238.36 ± 25.23	241.12 ± 23.65 239.07 ± 26.74	0.86 0.91
8 weeks	248.21 ± 24.74	245.57 ± 22.24	0.67
12 weeks	244.93 ± 19.97	245.61 ± 18.95	0.89

Group N: Nepafenac 0.1%; Group D: Difluprednate 0.05%; [1]Unpaired t-test.

Table 3. Average percent change in central subfield thickness (in µm) from 1 week to 8 and 12 weeks.

	Group N (n = 106)	Group D (n = 100)	p-value[1]
1 week	-	-	
8 weeks	3.8 ± 7.1	2.7 ± 3.6	0.48
12 weeks	2.6 ± 7.4	2.7 ± 5.7	0.92
p-value[2]			
1 week to 8 weeks	0.009*	0.001*	
1 week to 12 weeks	0.05	0.02*	

Group N: Nepafenac 0.1%; Group D: Difluprednate 0.05%; [1]Unpaired t-test; [2]Paired t-test.

Table 4. Comparison of volume (in mm^3) between the groups across the time interval.

	Group N (n = 106)	Group D (n = 100)	p-value
Baseline (1st day) 1 week	9.68 ± 0.80 9.62 ± 0.80	9.42 ± 0.78 9.32 ± 0.66	0.87 0.91
8 weeks	9.46 ± 0.73	9.23 ± 0.80	0.67
12 weeks	9.51 ± 0.53	9.16 ± 0.72	0.89

Group N: Nepafenac 0.1%; Group D: Difluprednate 0.05%; [1]Unpaired t-test.

Table 5. Average percent change in volume (in mm^3) from 1 week to 8 and 12 weeks.

	Group N (n = 106)	Group D (n = 100)	p-value[1]
1 week	-	-	
8 weeks	*1.8 ± 7.3*	*0.4 ± 9.3*	*0.53*
12 weeks	*1.2 ± 8.6*	*1.4 ± 12.1*	*0.94*
p-value[2]			
1 week to 8 weeks	*0.22*	*0.98*	
1 week to 12 weeks	*0.48*	*0.73*	

Group N: Nepafenac 0.1%; Group D: Difluprednate 0.05%; [1]Unpaired t-test; [2]Paired t-test.

Table 6. Comparison of average thickness (in µm) between the groups across time interval.

Baseline (1st day)	Group N (n = 106) 264.80 ± 23.06	Group D (n = 100) 264.50 ± 22.99	p-value 0.90
1 week	266.82 ± 25.06	253.14 ± 22.21	0.03*
8 weeks	260.29 ± 24.11	257.32 ± 23.17	0.64
12 weeks	266.39 ± 19.56	256.21 ± 21.71	0.07

Group N: Nepafenac 0.1%; Group D: Difluprednate 0.05%; [1]Unpaired t-test.

Table 7. Average percent change in average thickness (in µm) from 1 week to 8 and 12 weeks.

	Group N (n = 106)	Group D (n = 100)	p-value[1]
1 week	-	-	
8 weeks	2.8 ± 9.1	1.1 ± 9.7	0.11
12 weeks	0.57 ± 11.0	0.42 ± 12.9	0.75
p-value[2]			
1 week to 8 weeks	0.13	0.35	
1 week to 12 weeks	0.93	0.60	

Group N: Nepafenac 0.1%; Group D: Difluprednate 0.05%; [1]Unpaired t-test; [2]Paired t-test.

Table 8. Comparison of best corrected visual acuity (LogMAR) between the groups across the time interval.

	Group N (n = 106)	Group D (n = 100)	p-value
1 week	0.57 ± 0.07	0.60 ± 0.17	0.07
8 weeks	0.50 ± 0.23	0.45 ± 0.25	0.44
12 weeks	0.37 ± 0.15	0.27 ± 0.24	0.06

Group N: Nepafenac 0.1%; Group D: Difluprednate 0.05%; [1]Unpaired t-test.

Table 9. Comparison of IOP (mmHg) between the groups across the time intervals.

	Group N (n = 106)	Group D (n = 100)	p-value
Baseline (1st day) 1 week	13.14 ± 2.80 13.07 ± 2.24	13.50 ± 3.00 12.42 ± 2.57	0.50 0.32
8 weeks	13.07 ± 2.26	13.09 ± 3.24	0.37
12 weeks	14.14 ± 0.91	14.18 ± 3.14	0.39

Group N: Nepafenac 0.1%; Group D: Difluprednate 0.05%; [1]Unpaired t-test.

Disclaimer- The authors have no financial interest and no competing of interest

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The use of bone marrow derived mesenchymal stem cell for cornea regeneration in rabbit model

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Abstract

Aim or purpose: *To evaluate the use of autologous bone marrow derived mesenchymal stem cells (BM-MSCs) to treat cornea stromal defect in a rabbit model.*

Methods: *A non-randomized interventional controlled animal study involving twenty-one adult New Zealand white rabbits. Corneal deep lamellar dissections were created in three groups of rabbits and treated accordingly. Group I: Autologous bone marrow derived MSCs with autologous fibrin and human amniotic membrane. Group II: Autologous fibrin with human amniotic membrane without MSCs. Group III: No treatment. Clinical outcome was evaluated by corneal re-epithelization, corneal opacity, corneal thickness and histology.*

Results: *BM-MSCs were successfully isolated from bone marrow of seven rabbits based on the adherence property of the cells to the plastic of the cell culture plate. At day 60 corneal thickness was significantly thicker in Group I. The localization of PKH26-labeled BM-MSCs showed an increase in cell density at the transplanted site, proving its role in cornea stromal regeneration. Although the cornea clarity was not achieved in this study, we believe that cornea stromal remodeling requires many months to years to regain its original optical quality.*

Conclusion: *Locally transplanted BM-MSCs may be a useful source for cornea stromal regeneration. The use of autologous BM-MSCs offers a promising option for treating corneal disorder without the risk of immune-rejection and calcification.*

Keywords*: BMSCs, corneal stroma, rabbit, tissue engineering, transplantation*

Introduction

The cornea is damaged in various diseases, such as trauma and injuries. Corneal melt is a debilitating disease resulting in severe loss of vision. Such cases usually result in scarred and vascularized corneas or evisceration. At present, the only treatments available are inorganic tissue glue or corneal transplantation. However, the main risk of corneal transplantation in an inflamed eye is tissue rejection. Furthermore, in many countries access to corneal tissue is severely hampered by the lack of organ donors. The use of inorganic tissue glue is also hampered by the fact that

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they are very toxic to the underlying cell. This may sometimes result in a larger corneal hole than before. Using a person's own cells in corneal regeneration may address such problems.

Autologous corneal tissue engineering is a promising therapeutic approach to overcome the problems of primary immune rejection and the limitation of corneal donors. Norzana *et al.*[9] reported that corneal epithelium can be serially expanded in serum-free and feeder layer-free culture system, and be used for corneal tissue engineering. In clinical cases, the epithelial cells can be obtained from donors or from the healthy eye of the same patient. However, the former may cause tissue rejection, whilst the latter may not be suitable in cases where both eyes are damaged.

A recent report suggested that transplantation of human mesenchymal stem cells could reconstruct the damaged cornea.[8,12,14] Ye *et al.*[14] showed that systemically transplanted MSCs can engraft to an injured cornea and promote wound healing by differentiation, proliferation and synergizing with hematopoietic stem cells. The therapeutic effect of the transplantation may be associated with the inhibition of inflammation and angiogenesis. However, these studies are mainly looking at cornea epithelial regeneration as opposed to stromal regeneration. The latest study by Gu *et al.*[5] showed MSCs could differentiate into corneal epithelial like cells *in vivo* and *ex vivo*. However, most corneal diseases involve the corneal stroma, which accounts for 90% of corneal thickness. Diseases that can cause cornea melt include autoimmune keratitis, infection and ectatic diseases. Arnalich-Montiel *et al.*[1] did a study to look at stroma regeneration. Their results showed that adipose-derived stem cells could be a source for cellular therapy for the corneal stroma.

Barbosa and co-workers[2] suggested that corneal myofibroblasts, which make up the corneal stromal layers, can be derived from MSCs in a chimeric mice study. MSCs also have the capacity to differentiate to corneal stromal cells when the cells were intravenously introduced, as reported by Harada.[6] A recent study by Park *et al.*[11] reported that MSCs are able to be differentiated to keratocyte-like cells *in vitro* by using keratocyte conditioned-medium (KCM).The MSCs differentiated keratocyte-like cells expressed both keratocan and ALDH1A1 and consistently upregulated lumican. This study showed that conditioned medium promotes differentiation of human MSCs to corneal keratocyte-like cells *in vitro*.

The ability to bioengineer a person's own cornea using autologous MSCs in improving visual outcome will represent a significant improvement in corneal tissue engineering. This may delay or reduce the need for corneal transplantation and improves the patient's quality of life. It may also prevent the risk of corneal rejection, which is the main cause of graft failure. It also avoids the controversy surrounding the use of embryonic stem cell. The primary aim of a bioengineered cornea is to overcome the undesirable outcome of corneal melt following insult and the secondary aim is to restore the corneal thickness and clarity.

The objective of this study was to evaluate the use of autologous bone marrow derived mesenchymal stem cells (BM-MSCs) to treat cornea stromal defect in a rabbit model.

Materials and methods

Animals experiment approval

This study involved 21 adult male New Zealand white rabbits weighing 1.8-2.3 kg, obtained from Medical Faculty UKM Animal Unit after the approval of Universiti Kebangsaan Malaysia Animal Ethics Committee. They were treated according to the *Guide for The Care and Use of Laboratory Animals* (National Academy of Science 1996). The animals were randomly divided into three groups of seven rabbits each. Group I: Autologous BM-MSCs with autologous fibrin glue and human amniotic membrane (HAM); Group II: Autologous fibrin glue with human amniotic membrane without BM-MSCs; Group III: No treatment. Bone marrow was harvested from the left iliac crest bone in all the rabbits in group I. Four ml of blood were collected to produce autologous fibrin from all the rabbits in group I and II.

Isolation, expansion and labeling of BM-MSCS

Autologous BM-MSCs aspiration from the Group I was harvested and expanded in the laboratory using widely accepted protocol and commercially available tissue culture media.[13] The animals were being anaesthetized by intramuscular injection of a mixture of zoletil 50 mg/kg (Virbac), ketamine hydrochloride 50 mg/kg (Bioketan) and 10 mg/kg of xylazine (Troy Lab) (0.2 ml/kg body weight). Once they were sedated, the animals were then placed in a wooden box restrainer. Intravenous animal anesthetic drugs regime (0.1 ml/kg body weight of the mixture) was given through the lateral marginal ear vein to ensure the animals were deeply sedated. Left iliac crest region of the rabbit was shaved, painted and draped. One centimeter skin incision was made over the iliac crest. Four ml of rabbit bone marrow was harvested using a percutaneous 18-G needle aspiration from the iliac crest. The bone marrow was kept in vacutainers containing 0.1 ml of heparin (3000 U/ml-B/Braun) at room temperature and transferred to the cell culture facility.

Isolation and expansion of BM-MSCs were performed as previously described.[13] They were isolated solely based on their plastic adherent property. All cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Fresh medium was added on the third day. Medium was changed upon substantial cell attachment and later, twice a week in a 75 cm^2 flask for the second passage and in a 175 cm^2 flask for the third passage. Haematopoietic cells and non-adherent cells were removed along with the media. Upon 80%-90% cell confluency, cells were detached by the addition of 0.05% trypsin-EDTA (Gibco USA) and viable cells counted using trypan blue dye-exclusion-method. Cells were subsequently sub-cultured at a standard density of 10,000 cells/cm^2. Adherent cells after the third passage referred to as third-generation MSCs, were used for this experiment.

Preparation of autologous fibrin

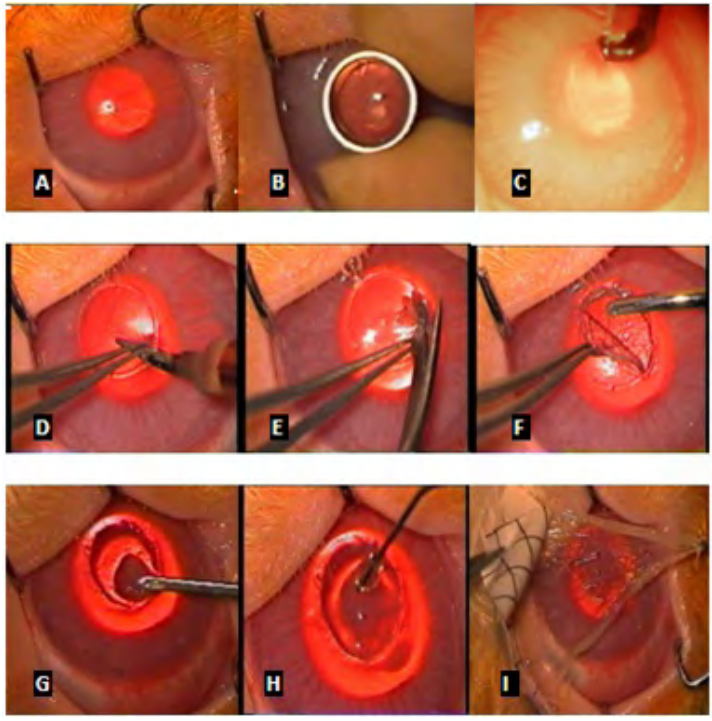
An amount of four ml of fresh rabbit blood was collected through the lateral marginal artery from the rabbit's ear. Blood was collected in sodium citrate tubes followed by rapid inversion of the tube to deter blood from clotting and kept at room temperature. It was then centrifuged at 3000 rpm for five minutes. The plasma

layer (top yellowish layer) was gently transferred to a new tube with a pipette without disturbing the bottom layer (red layer). This process was repeated three times to ensure complete removal of residual cellular components. The plasma was sterile-filtered using a 0.2 µ syringe filter (Sartorius, USA) to remove the cell debris that may cause spontaneous clotting of the plasma. Plasma was stored at -20 ˚C until the day of transplantation.

Animal model

The rabbits were anesthetized and the quality of ocular surface was evaluated by the degree of smoothness using slit lamp microscopy (SL-DC 1, Topcon, Japan) with a Topcon anterior segment camera. The cornea thickness was measured by anterior segment optical coherence tomography (OCT-Visante Carl Zeiss). The left eye was cleaned and draped and was kept open by a speculum. Left cornea defect was created by deep lamellar dissection (Fig. 1A-F). A five-mm trephine was used to standardize the size of the defect. A guarded diamond knife was calibrated to a desired corneal thickness. An incision of 80% to 90% thickness of the rabbit's cornea was created. The cornea was then dissected using Melles' corneal lamellar dissector and the edge was cut by corneal scissors. The thickness of lamellar dissection was then evaluated by anterior segment OCT and slit lamp to assure the desired corneal thickness was removed.

Fig. 1.



In-vivo transplantation

Regarding the rabbits in Group I, BM-MSCs were transplanted with an autologous fibrin. On the day of transplantation, monolayer cells were trypsinized and centrifuged at 3000 rpm for five minutes to obtain a cell pellet of approximately ten million cells. Autologous fibrin derived from plasma was used as BM-MSC cell carrier. Stored plasma was thawed on the day of surgery. Calcium chloride (0.5 mmol/l) was added to one ml of plasma to initiate the polymerization process. Cell fibrin mixtures were then filled into the cornea defect. A fresh frozen human amniotic membrane (HAM) was sutured over the site using 10/0 nylon sutures to be held in place for seven days (Fig. 1G-I).

In Group II, corneal defects were treated with the autologous fibrin without cells. As in group I, the treated eyes were covered with HAM. The HAM was removed on day seven post transplantation. Group III consisted of cornea defects without treatment and were left to heal on their own. All rabbits were returned to their cages after the procedure and were allowed to move freely. Topical dexamethasone 0.1% (Alcon®) and moxifloxacin 0.5% (Alcon®) were used four times daily for two weeks. Only dexamethasone was continued twice daily for another month to all corneas.

Follow-up and clinical evaluation

Each eye of the cornea defect underwent slit lamp examination, fluorescein staining and viewing under blue filter, and anterior segment OCT at postoperative day 0, 7, 14, 30, and 60. The corneal epithelial integrity was quantified by the ratio of the epithelial defect area to the total cornea using simple Imaging Measurement Software (Topcon, US). The corneal opacity was assessed using published corneal opacity grading technique by visualizing the ocular tissue beneath the cornea.[4] The corneal clarity was graded as: grade 0, totally clear with no opacity seen by any method of slit lamp microscopic examination; grade 1, haze of minimal density seen with difficulty with direct and diffuse illumination; grade 2, mild haze easily visible with direct focal slit illumination; grade 3, moderately dense opacity that partially obscured the iris details; grade 4, severely dense opacity that completely obscured the details of intraocular structures. Corneal thickness was measured with anterior segment OCT at preoperatively and post operatively day 30 and 60. Six readings were taken of the center corneal thickness and mean thickness was used for the data.

Statistical analysis

Epithelial defect size and cornea thickness were compared with repeated measure ANOVA. To compare statistical association of cornea opacity among group, we used the Fisher exact test.

Histology examination and localization of MSC cells in the cornea

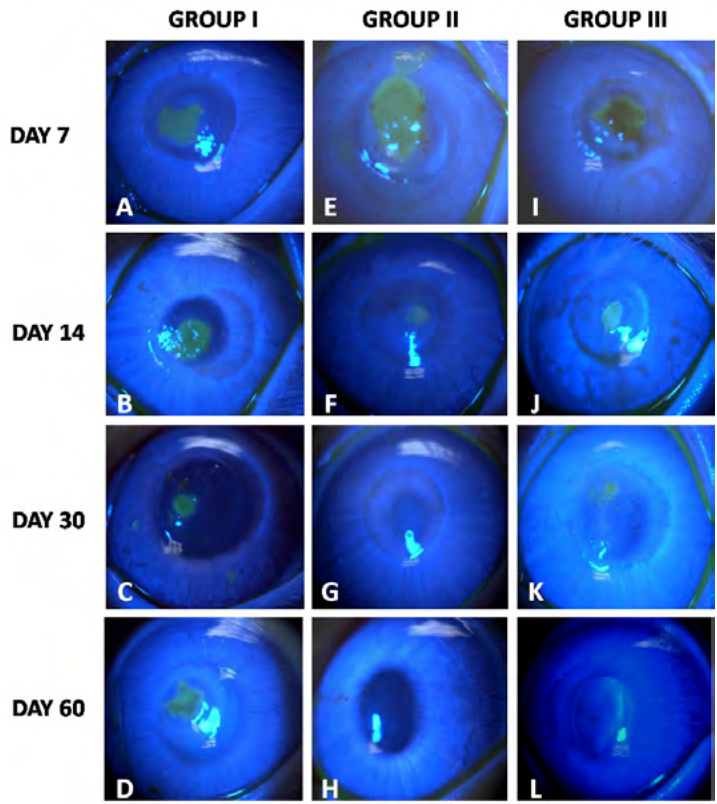
For localization of the BM-MSC cell experiment, one rabbit in group I was euthanized after two weeks and one after four weeks and the remaining were euthanized 60 days after cell transplantation. Several sections of each cornea were stained with hematoxylin and eosin and von Kossa staining for light microscopy examination

(Olympus BX51; Olympus Co. Ltd., Tokyo, Japan). DAPI stain was used to label all cells nuclei in the corneas in the biosafety experiment. Localization of PKH26 labeled BM-MSC cells using Red Fluorescent Cell Linker Kit (Sigma-Aldrich, Inc. Saint Louis, Missouri, USA) was achieved using confocal microscope (LSM 510 Meta, Zeiss). This technique was performed according to the manufacturer's instruction. Cell tracking was observed at week 2 and 4, following transplantation.

Result

The treatment groups were successfully performed, *i.e.*, Group 1: autologous BM-MSCs with autologous fibrin glue and human amniotic membrane (HAM); Group II: autologous fibrin glue with human amniotic membrane without BM-MSCs; Group III: no treatment. The mean area of baseline epithelial defect ranged from 19.04 mm^2 to 19.51 mm^2. The statistical analysis revealed there was no significant difference in baseline epithelial defect area among the three groups. This was not surprising as all the defects are created in a well-defined area using a trephine (Fig. 2). All the rabbits showed significant improvement in corneal re-epithelization by day 60 among their group.

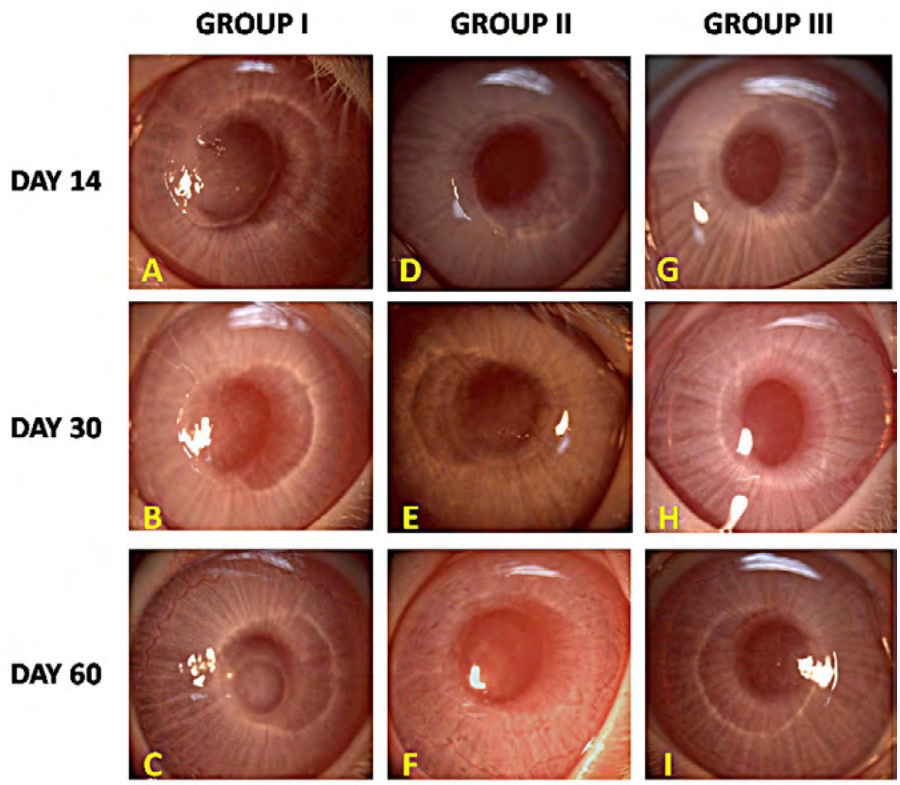
Fig. 2.



However, overall cornea re-epithelization in the three groups did not show any statistical significance (Table 1). The results showed that BM-MSCs transplantation did not improve the rate of epithelial healing.

Corneal opacity was observed in all three groups during follow-up. Fig. 3 shows a photography of one of the rabbits in each group on day 14, day 30 and day 60 of the follow-up. In group III, the corneas were much clearer and only one rabbit developed moderately dense opacity at day 30. In addition, the opacities observed in group I tended to be denser at the edge of the dissected cornea, forming a ring appearance. However, at day 30 the corneal opacity in the three groups did not show any statistical significance (Table 2).

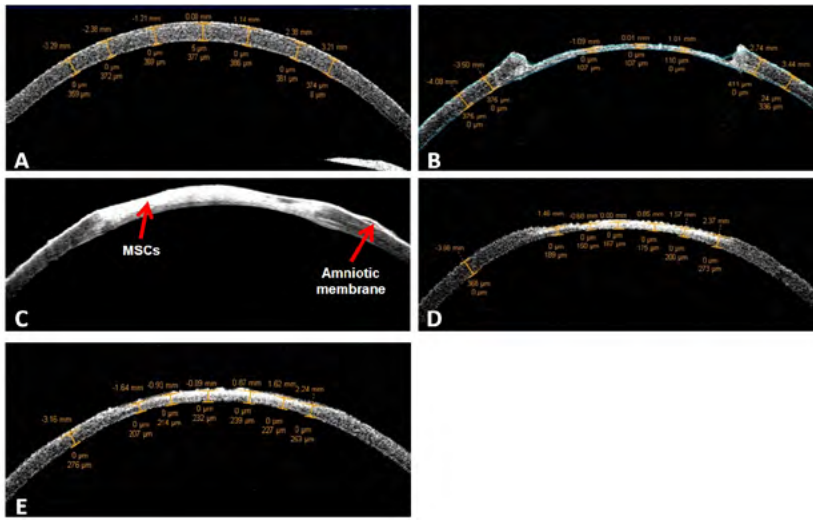
Fig. 3.



The normal corneal thickness in all rabbits ranges from 405 µm to 342 µm with mean thickness of 369 µm. The mean corneal thickness preoperatively in group I: 351 µm; group II: 372 µm and group III: 374 µm. We removed almost 80% of corneal thickness during deep lamellar dissection. There appeared to be a significant increase in the corneal thickness in all three groups at day 30 and day 60. At day 60, the final corneal thickness was also significantly higher in group I (258.40 µm)

compared to group II (215.33 µm) and III (225.60 µm) (Table 3). With the advantage of high resolution anterior segment OCT, we managed to clearly visualize the depth of the corneal defect and the transplanted BM-MSCs covered with HAM (Fig. 4).

Fig. 4.



At day 30, a confocal microscope examination of the transplanted cornea which received a PKH26 labelled MSCs showed localization of PKH26 MSCs at the area of defect and edge of the wound (Fig. 5A). Histology staining with Hematoxylin and Eosin in all three groups (Fig. 5E-G) showed that the rabbit's epithelium had completely covered the damage cornea surface with variable epithelium layer. The epithelium in the peripheral corneal areas was normal in appearance with a total thickness of five to seven layers. It was noted that there was an increase in epithelial thickness in group II and III. Stromal regeneration was observed in the group I at the edge of the wound with increase in cellular density compared to group II and III which was lesser toward the centre.

Fig. 5.

Discussion

Our study was based on clinical observation as an objective measurement. The size of epithelial defect, corneal opacity and corneal thickness were used as parameters for stroma regeneration. In our study, corneal neovascularization was not observed in all three groups. It seemed that neither the MSCs nor fibrin scaffold are capable of angiogenesis.

Epithelial healing rates were the same in all three groups. It seemed that transplantation of BM-MSCs or fibrin alone did not affect the healing rate on epithelium.

This result differs from previous study in other groups.[10,14] A study by Ma *et al.*[8] also showed significant improvement in re-epithelization in MSCs-treated group. The re-epithelization was quantified by less than one fourth of cornea fluorescein staining. It differs from our study method in that we use autologous fibrin as a scaffold and then mixed with autologous MSCs pellets before transplantation. Their method uses amniotic membrane as a cultivating sheet for the MSCs before being transplanted. This may be due to the preservation of stromal layer in their cornea rabbit model whereas in our study, we removed almost 80% of the stroma layer. We postulated that cellular migration in our study might be inhibited by the corneal construct. Instead of enabling the epithelial cells to grow over the stroma as in group III, the cells in group I and II have to grow over the cell-fibrin and fibrin matrix, respectively.

Stroma remodeling is a major factor in contributing to the transparency of the cornea. Based on our result, we observed that the MSCs group developed more corneal opacity. This is consistent with other study by Gu *et al.*[5] in that they also showed that groups treated with MSCs developed cornea opacity. In contrast, another study by Ye *et al.*[14] showed significantly clear corneal in the treated group with MSCs compared to those without MSCs. These differences may be due to different methods of transplantation.

In the present study, we observed that MSCs tend to distribute more at the edge of the defect. The cellular distribution was not even due to the curvature of the stromal defect created. It also explained why the scarring tends to be annular in shape in the treated group. It developed opacity, but from the increase in corneal thickness, a new stromal matrix must have been produced.

The optical coherence tomography (OCT) can visualize the transplanted MSCs within the lamellar dissected cornea and amniotic membrane overlying it. We found that the normal corneal thickness in experimental rabbits range from 408 µm to 342 µm, which is similar to the findings in other studies.[3,7]

We found that the corneal thickness had significantly increased from day 30 to day 60 and at day 60 in group I, the newly regenerated cornea is marked by the presence of a thick stroma layer with an increase in cellular density in the region close to the corneal defect that was created. It showed that MSCs-treated group has participated in stroma regeneration. The difference of corneal thickness in BM-MSCs treated group had significantly increased at day 60 compared to day 30, indicating that stromal regeneration continues throughout the time frame of this experiment. In groups II and III, corneal thickness also increased at day 30 but had not increased significantly at day 60. Further, epithelial hyperplasia noted histologically in group II and III. This study also found that stroma took a long time to regenerate as the thickness from postoperatively to day 30 was not significant between all groups.

The present study had the following limitations: transplantation method of MSCs results in excessive accumulation of cells at the edge of the wound and less remaining in the central region. We did not investigate whether the transplanted MSCs differentiated into functional keratocytes or if they exert a paracrine effect in promoting wound healing. Despite the limitations, we showed that transplanted

BM-MSCs are capable of regenerating corneal stroma. However, this beneficial effect comes at the expense of increased corneal haze.

Conflict of interest

The authors have no conflict of interest to declare in the conduct of the study.

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Visual outcome and ocular survival in pediatric ocular trauma

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Abstract

Purpose: *Ocular injuries in children are a major under-recognized cause of ocular morbidity and can be prevented by identifying the etiology and implementing measures to prevent these injuries. This study intends to assess the visual outcome and ocular survival in pediatric ocular trauma.*

Design: *A descriptive study set in a tertiary care teaching hospital in Southern India.*

Methods: *A total of 56 children less than 18 years of age with ocular trauma during the period August 2010 to August 2012 were studied. Injuries were classified as per modified Ocular Trauma Classification System (OTCS). Final visual outcome was assessed at the end of one, three and six months.*

Results: *Of the 56 children assessed, 87.5% were boys. About 12% were under-fives and almost 66% were in the age range of 5-14 years. Majority (47.5 %) had sustained trauma in the streets. Closed globe injury predominated (69.5%). At presentation, grade I injury was noted in 28 eyes (47.5%), grade II in one (1.7%), grade III in three (5.1%), grade IV in eight (13.5%) and PL negative in three (5.1%) eyes. Sixteen children were not cooperative for assessment of visual acuity (27.1%).*

At six months, good vision was noted in 44 (74.6%), moderate in five (8.5%), poor vision in one (1.7 %), PL negative in four (6.8%) eyes and four children were un-cooperative (6.8%). Four eyes had phthisis (6.8%) and one eye (1.7%) was eviscerated.

Conclusions: *Ocular trauma in childhood was more common in the male child, mostly due to road traffic accidents. Visual acuity at presentation is one of the major criteria for the final visual outcome.*

Key words: *causes, ocular trauma, paediatric age group, preventive measures*

Introduction

Eye injuries are a major under-recognized cause of disabling ocular morbidity affecting the young and are a leading cause of acquired unilateral blindness in childhood.[1] However, nearly 90% of eye injuries can be prevented by relatively simple measures, although these may not always be possible.[2] By identifying the factors in the etiology of these injuries, it may be possible to determine the most effective methods of reducing the incidence of visually damaging trauma. This study was carried out in order to assess the causes, demographic profile, visual outcome and complications of mechanical pediatric ocular trauma presenting to a tertiary care hospital.

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Methods

The study was carried out at the eye department of a tertiary care hospital, from August 2010 to August 2012. The study protocol was approved by the Ethics Committee of the hospital. A total of 56 children aged one to 17 years, treated for eye injury, formed the subjects of the study. At the time of presentation, name, age, gender, hospital number of the patients , mode of injury, time of injury, previous ocular complaints, place of injury, previous treatment if any were recorded. Cases of chemical injuries and trauma in previously diseased or injured eyes were excluded. Patients were divided into three age groups: pre-schoolers (one to four years), schoolchildren (five to 14 years) and adolescent (15 to 17 years).

Complete ophthalmic evaluation done at presentation included the evaluation of initial visual acuity, bony orbit, lids and adnexa, visual axis, ocular movements and anterior - posterior segment examination. Various methods of evaluating the vision included Snellen chart, random E s, Allen pictures, finger counting and assessment of light perception and projection of rays. For children too young to understand and cooperate with the above, vision was assessed by their ability to fix and follow light. Anterior segment evaluation included-location (zone) of the wound, presence of contusion, superficial foreign body, lamellar laceration, hyphema, relative afferent pupillary defect, presence or absence of cataract and subluxation or dislocation of lens . Posterior segment evaluation included – examination of the fundus for the detection of vitreous hemorrhage, retinal detachment, intraocular foreign body, signs of endophthalmitis and any other pathology. X ray and CT scan of the orbit were done where necessary, as in cases of orbital fracture or suspected intraocular foreign body. The eyes were categorized according to 'The Ocular Trauma Classification system' with a modification to include two more categories—eyelids and orbital injuries.[3]

All cases of open globe injury underwent primary corneal/corneoscleral tear repair, under general anaesthesia on an emergency basis. Patients were followed-up over a period of one month, three months and six months depending upon the severity of injury. For minor injuries, one month follow-up was adequate. Final best corrected visual acuity and complications like phthisis, evisceration etcetera, if any, were noted at the end of the study period. The final visual acuity was graded according to the categories of visual impairment by the World Health Organization. In this study, vision greater than or equal to 6/18 was taken as good visual outcome and vision less than 3/60 was considered as poor visual outcome. Vision in between these two categories was considered as moderate visual outcome. Ocular survival (*i.e.*, preservation of the globe) was studied.

Results

Of the 56 children recruited into the study, 49 (87.5%) were boys and seven (12.5%) were girls; the total eyes evaluated were 59 since three children sustained injury to both eyes. Twenty-six children (44.1%) encountered trauma to the right eye, 30 (50.8%) to the left eye and three of them (5.1%) to both eyes.

The details of injuries are as follows:

The majority of injuries (66%) occurred in the age group of 5-14 years and most of these occurred on the street (43.2%). Almost all the injuries (85.7%) among the pre-schoolers except one occurred at home. Details of these findings are depicted in Table 1.

Table 1. Age and place of injury of the children recruited into the study (N = 56.)

PLACE OF INJURY	0- 4 YEARS N (%)	5-14 YEARS N (%)	15-17 YEARS N (%)
HOME	06 (85.71)	13 (33.33)	01 (08.33)
SCHOOL	00	02 (05.40)	00
PLAYGROUND	00	07 (18.91)	02 (16.66)
STREET	01 (14.29)	17 (43.58)	10 (76.92)

The time delay between sustaining the trauma and reporting to the hospital was as follows:

Twenty-five children (44.6%) presented within six hours of injury to the hospital while another 14 (25%) presented within 24 hours. However, it is of concern that about 30% of the children presented not earlier than after two days of the injury.

With respect to the type of injury, the observations were:

The most common kind of injury was blunt trauma (74.6%) followed by penetrating trauma (18.6%) and the remaining (5.1%) were due to animal bites. One child with penetrating trauma had retained intraocular foreign body (IOFB) and the cause of injury was not known in one child who presented to us with a repaired corneal tear.

The most common cause of injury was found to be road traffic accidents (42.9%) where children in the age group of 15 to 17 years were riding a motor bike/bicycle or were pillion riders. There were no reported gunshot injuries or those due to glass shards. Details of the injuries and the causes are illustrated in Table 2.

Table 2. Nature and cause of injury among the study recruits (N = 56.)

	NATURE OF INJURY		
CAUSE OF INJURY	**BLUNT n (%)**	**PENETRATING n (%)**	**IOFB n (%)**
Road traffic accident (RTA)	26 (100)	00	00
Sticks & pencils	1 (25.0)	2 (50.0)	1(25.0)
Toys (bow & arrow)	00	1 (100)	00
Cans / stones	08 (100)	00	00
Fireworks	01 (33.3)	02 (66.7)	00
Sharp tools	00	03 (100)	00
Falls	08 (88.9)	01 (11.1)	00
Animal & bird attacks	00	01 (25)	00
Unknown	00	01 (100)	00

The injuries were typed applying the Modified Ocular Trauma Classification System and the majority (69.5%) were observed to have closed-globe injuries, while another 20% were open-globe injuries. Four cases were restricted to trauma to the eyelids and another two had orbital injuries.

Visual acuity assessment at presentation revealed the grades of injury as follows:
Of the 59 eyes, 28 eyes (47.5%) (24 of closed globe, two of lid and orbit, respectively) had good vision (grade I), one moderate (grade II), 11 poor (grade III and IV) and three were blind (Grade V injury). Vision could not be assessed in sixteen children as they were uncooperative.

On correlating the grade of injury to the type of injury, it was found that all the patients with open-globe injury had a vision ranging from 6/30 to no light perception, while 85% of those with closed-globe injury had good vision (≥ 6/12) (Table 3).

Table 3. Ocular features associated with blunt and penetrating trauma.

	Visual acuity at presentation	Closed globe (no of eyes)	Open globe (no of eyes)	Lid injury	Orbital injury
Grade of injury	> 6/12 (20/40) grade I	24	0	2	2
	6/15-6/30 (20/50 to 20/100) grade II	1	0	0	0
	< 6/30 to 1.5/60 (19/100-5/200) grade III	1	2	0	0
	1.2/60 to light perception (LP) Grade IV	3	5	0	0
	No LP, Grade V	1	2	0	0
	No response (not cooperative)	11	3	2	0
Zone	I	31 (75.6%)	8 (66.7%)	-	-
	II	3 (7.3%)	3 (25%)		
	III	7 (17.1%)	1 (8. 3%)		
Pupil	RAPD +	5	2	0	0
Cataract		0	3		
Retinal detachment		0	03	0	0
Endophthalmitis		0	4	0	0
Hyphema		3	4	0	0

NB 16 patients were uncooperative for vision assessment.

Patients with relative afferent pupillary defect (RAPD) were classified as pupil positive. Two children among the open-globe injury and five among closed-globe injury had RAPD.

Assessment of the zones of injury showed that among the open-globe type, 66.7% were in zone I (isolated to cornea) and 25% in zone II (corneo scleral limbus to a point five mm posterior into the sclera) and 8.3% in zone III (posterior to anterior five mm of the sclera). Among the closed-globe type 75.6% were in zone I (external), 7.31% in zone II (anterior segment) and 17.1% in zone III (posterior segment).

The ocular features associated with trauma at presentation were as follows (Table 3):
RAPD was noted in seven children, cataract in three, retinal detachment in three, endophthalmitis in four, IOFB in one and hyphema in seven. The three cases in which cataract was noted had penetrating injuries with respectively knife, bow-and-arrow and in one child, the cause was unknown. The three cases of retinal detachment were caused by blunt trauma secondary to a tennis ball injury, a cricket ball injury and a stone injury. All these three patients underwent retinal detachment surgery.

All four cases of endophthalmitis received intravitreal antibiotics (0.1 ml of 1.0 mg vancomycin and 0.1 ml of 2.25 mg ceftazidime) and two of them were culture-positive for Klebsiella and Microsporidia.

The underlying cause of endophthalmitis in these four cases was as follows:
In case one, the child had sustained trauma with a broom stick and presented with grade-IV injury with visual acuity of perception of light (PL) positive and projection of rays (PR) being inaccurate. This child presented to us 43 hours after the trauma and received intravitreal antibiotics on the day of admission. Here, the culture was positive for Klebsiella. However, after two days it progressed to panophthalmitis and was eviscerated immediately.

In case two, the trauma was with broom stick and presented with grade-IV injury with vision of PL-positive and PR-accurate. The child presented seven hours after the trauma. Two doses of intravitreal antibiotics were given 72 hours apart. However, on day seven the eye became PL-negative and on review at one month, the eye was phthisical.

In case three, the trauma was due to bow-and-arrow with grade-IV injury and presenting visual acuity of counting fingers at one meter. The child had intravitreal antibiotics and was discharged on day six with a visual acuity of hand movements. However, the child was PL-negative after six months and phthisical at eight months.

In case four, the child presented to us one week following trauma with a repaired corneal tear which was done outside. The visual acuity at presentation to us was hand movements positive. Child underwent pars plana vitrectomy with silicone oil injection and the visual acuity improved to 6/24 at six month follow-up. The vitreous culture was positive for microsporidia.

Of the four cases, one underwent evisceration while the rest were subjected to vitrectomy; eventually however, two of the three eyes resulted in phthisis bulbi.

Of the seven cases that presented with hyphema, four had open-globe injury and three closed-globe injury. One patient had wooden IOFB, following a broom stick injury. The foreign body was removed at the time of primary repair but after one month the eye resulted in phthisis bulbi.

Follow-up was scheduled at the end of one, three and six months, depending on the severity of injury. Fifty-five children came for the first follow-up (post one month), 46 cases for the second (post three months) and another 46 cases came for the third follow-up, which was after six months. Visual acuity was assessed at each of these visits and the results are shown in Table 4. Forty-four cases complied with all three follow-ups, while one patient visited only once after six months. Eighteen

patients with minor ocular injuries were advised only one follow-up visit at the end of one month. There appeared to be a significant improvement in the vision during the follow-up phase with 50% eyes demonstrating good vision at the end of six months (Table 4).

Table 4. Visual acuity at follow-up.

VISUAL ACUITY	BASELINE n (%)	1 MONTH n (%)	3 MONTHS n (%)	6 MONTHS n (%)
Good	28 (47.5)	36 (62.1)	30 (65.2)	31 (67.4)
Moderate	01 (1.7)	06 (10.3)	04 (8.7)	04 (8.7)
Poor	11 (18.6)	02 (1.7)	03 (4.3)	03 (4.3)
Blind	03 (5.1)	05 (8.6)	03 (4.3)	04 (8.7)
Uncooperative	16 (27.1)	09 (15.5)	06 (13.1)	04 (8.7)
TOTAL	**59**	**58**	**46**	**46**

The numbers of eyes with poor vision decreased over a period of time, from almost 19% at baseline to 7% at six months follow-up. However, the improvement appeared to plateau at the three months to six months follow-up period with no further improvement in the outcome.

Seven patients had poor vision at six months follow-up, four of whom had phthisis bulbi, one patient had undergone evisceration, one was post retinal detachment surgery with aphakia with best corrected vision of 3/60 and one more had significant irregular astigmatism due to corneal scarring (this child's parents refused further management).

There were four children with moderate vision during the final follow-up and they did not undergo amblyopia therapy. The details are as follows: one had injury due to fireworks and had multiple intra-corneal deposits and was advised penetrating keratoplasty. Two children with moderate visual outcome had sustained missile injury and had macular grade corneal opacity involving the pupillary area. The fourth child was injured by a stone and had central leucomatous grade corneal opacity which accounted for the moderate visual outcome.

Discussion

Eye injury in children is a menace and a common problem worldwide. In the present study, the maximum number of injuries was in the school-going age group of 5-14 years and the minimum in children under four years of age. This is similar to other studies done previously.[4,5,9] Children under four years had lower incidence due to more protected and cocooned environment at home with their parents.

In this study, it was found that the male-to-female ratio was around 7:1. Many studies have shown that boys tend to be affected more commonly than girls, with male:female ratio varying from 2:1 to 4:1.[6-8] This can be attributed to the greater

physical activity and a more adventurous or aggressive nature of young boys during playing and interacting with their peer group.

The present study noted that the most common place of injury was in streets due to road traffic accidents (34%). This is quite contrary to the results of other studies like those by MacEwen *et al.* and by Kaur and Agrawal, where home was the most common place of injury (45.6% and 51%, respectively).[4,9]

In the above-mentioned studies, the most common place of injury was home and the injury at home was attributed to trauma with toys, sticks, stone, glass pieces and falls. In this study, home being the second most common place also emphasizes the fact that even home is not the safest place for young, active and curious children.

The most common mechanism of injury was blunt trauma (73%). This is in accordance with previous studies by MacEwen *et al.* in Scotland (65%) and other studies, but contrary to a study done in KGMU Lucknow (16%), where penetrating trauma was more common (74%).[4,10]

In this study, the most common cause of injury was found to be road traffic accidents (43%). This can again be attributed to lenient traffic rules in this country. In other studies where penetrating injuries were more common, injury due to stick, wood etcetera, while playing were more common probably due to the rural setting of these studies.[9] In the present study, three children had trauma while playing with a knife unsupervised at home.

Other studies found high prevalence of ocular trauma in sport-related activity (MacEwen *et al.*, Grin *et al.*) like baseball, tennis and basketball.[4,11] In the present study, 13% of children had ocular injury due to cricket and tennis balls.

Uncommon causes of injury in this study were trauma with bird's beak and dog bite. Two children had injury due to fireworks and both became PL-negative. None of the children in this study sustained trauma due to gunshot.

It is important that the injured follow-up at least for the first three months as this is a period of remarkable improvement in vision as depicted in the study. Visual acuity at presentation is one of the major criteria of visual outcome in cases of trauma. In open-globe injury, seven children had poor vision at presentation and six of them had poor final visual outcome. Only one child with poor vision at presentation improved to moderate visual outcome at six months follow-up. In closed globe, 24 children had grade-I injury and continued to have good vision at the final follow-up. One child with grade-V injury at presentation had no perception of light during the final follow-up. Hence the results of this study also confirm that the visual acuity at presentation is one of the major criteria predicting the final visual outcome.

Adequate steps should be taken to prevent ocular trauma in children. Prevention of injury depends, firstly, on identifying the cause and, secondly, targeting the cause by education and implementation of legislation.

Common household articles such as broom sticks, knives and wooden sticks can prove to be dangerous for the child's eye as is seen in the study. The home environment should therefore be made child-friendly by keeping sharp objects out

of reach of children and padding the corners of couches, tables and chairs. Children should be kept away from dangerous toys and handling of fireworks. Moreover, there is an urgent need to implement strict traffic rules. Wide publicity with regards to these aspects could be a way forward. The need for compliance with follow-up also needs to be stressed to the parents of injured children.

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Association between the second-to-fourth digit (2D:4D) ratio and myopia in medical students of the Medical Faculty of the Jeneral Soedirman University

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Abstract

Refractive error is one of the biggest eye problems in young people. The most prevalent refractive error, myopia, is continuously increasing. The myopia degree impact results in serious complications in the eye. Current studies report that myopia is associated with the genetic and hormonal system. The second-to-fourth digit (2D:4D) ratio on the other hand, has been reported as being related to the genetic and hormonal mechanism. This study aimed to establish an association between the 2D:4D ratio and myopia cases in medical students of the Medical Faculty of Jenderal Soedirman University. This was a quantitative observational analytic research with a cross-sectional approach. One hundred students, divided into a myopia group and an emmetropia group, were recruited for the study. Digit measurement was done using a computer-based method by two independent observers, whereas an eye refraction test was conducted by an independent refractionist. An independent t-test on the 2D:4D ratio showed no difference in values ($p > 0.05$) between the myopia group and the emmetropia group. In conclusion, the study reported that there was no significant association between the 2D:4D ratio with myopia cases in medical students of Medical Faculty of Jenderal Soedirman University.

Keywords: *Myopia, 2D:4D ratio, Jenderal Soedirman*

Introduction

Myopia makes up 54% of total eye problems and is the most frequent refractive error in the world. WHO found that myopia prevalence is 43% of the total world population.[1] Myopia prevalence is higher in the South-East Asia region.[2] The last report showed that the prevalence in Vietnam, Singapore and Indonesia was 20.4%,[3] 32.4%[4] and 3.69%,[5] respectively.

Myopia needs immediate recognition and prevention to avoid dangerous complications. High-grade myopia of more than S -6.0 diopter, especially high-grade axial

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myopia, can increase the risk for serious complications such as cataract, glaucoma, retinal ablation, chorioretinal atrophy, and lacquer cracks in the retina.[6] Elongation of the axial length can stretch the choroid and retinal epithelium, leading to complications.[7] Myopia can lead to blindness, being the number-seven cause of blindness.[8] High-grade untreated myopia also can decrease the quality of life based on eye complication and discomfort because of the use of eyeglasses.[9,10,12]

Myopia can be progressive. In China, a study found that the degree of myopia can increase up to S (-0.6)-(-0.7) diopter each year whereas in Singapore, a report said the increment was S -2.4 diopter every three years.[6,11]

Myopia is associated with genetic and hormonal factors. Prenatal androgen exposure at 13 weeks of gestation has an effect on axial length and myopia progression. The sexual steroid hormone can also alter corneal thickness and corneal curvature.[13] Genetically, MYP and Homeobox (HOX) gen can also alter scleral thickness and corneal curvature.[14] Those alterations in corneal thickness and curvature can lead to myopia.[15] SMOC1 gene can also induce bone morphogenic pathway (BMP) intrauterine to regulate eye axial length and appendicular skeleton development.[16]

Second-fourth digit ratio (2D:4D ratio) is measured by dividing the second digit length with the fourth digit ratio. Digit length is measured from the first line in basal crease line which reflects the base of metacarpophalangeal joints.[34] 2D:4D ratio is also associated with genetic and hormonal factors. Hormonally, androgen exposure in week 13 of gestation makes the fourth digit longer, so the 2D:4D ratio is lower. Androgen exposure is higher in males, so the 2D:4D ratio in males is lower than in females.[17] An adult male has 0,98 of mean 2D:4D ratio, which is lower than adult female, with one of mean 2D:4D ratio.[17] Females with congenital androgen hyperplasia have higher androgen levels and tend to have lower 2D:4D ratio and males with Kinefelter syndrome have higher 2D:4D ratio.[18,19]

Genetically, the 2D:4D ratio is associated with HOX gene and Androgen Receptor (AR) gene. HOX gene regulates anterior-posterior axis, including digit length and eye axial length, and it also regulates segmentation of body during embryonal development.[20] Androgen hormone can also induce SMOC1 gene and HOX gene, which impacts appendicular skeleton and digit development.[16] CAG sequence repeats in AR gene have minimal effect on 2D:4D ratio and is still debated. The number of CAG sequence repeat in AR gene has reduced effects on androgen level so the 2D:4D ratio is higher.[22]

This research was conducted to observe association between 2D:4D with myopia cases in medical students of the Medical Faculty of Jenderal Soedirman University. The current hypothesis about the association between 2D:4D ratio and myopia so far was mainly because of the effect of prenatal androgen steroid exposure. Prenatal androgen exposure impacts HOX gene in regulating digit length and 2D:4D ratio. It also impacts scleral tissue proliferation which alters axial length and progressing into axial myopia.[16] Recent research conducted by Krishnakumar *et al.*, (2014) shows that there was significant association between 2D:4D ratio and myopia in a female group, and the rest remains insignificant.[16]

Method

Study Design

This study was a quantitative observational analytical research with cross-sectional study design. Ethic certificate was received from ethical commission of Medical Faculty Jenderal Soedirman University.

Participants

The participants in this study were 100 students from the Medical Faculty of Jenderal Soedirman University. Complete randomized design was adopted to receive proportional to size (PPS) sampling. Inclusion criteria were: no hand anatomical congenital deformities and anomaly, no destructive hand trauma history, no joints disease history, body mass index (BMI) 18,5-23 kg/m^2, 19-21 years of age, in both the myopia and emmetropia group. Astigmatism, no responses, and no better correction (NBC) or no correction (NC) refraction status were set as exclusion criteria.

Data sources and settings

Digit length was measured by a computer-based method. Participants' right and left hand were scanned using Canon® CanoScan LiDE 120 (Vietnam). The measurement value was counted using a measurement tool on Adobe® Photoshop® CS6 in pixel(s) unit. Measurement was taken in Computer Laboratory in the Faculty of Medicine Jendral Soedirman University.

An independent refractionist conducted refraction tests using a standard trial lens, trial frame and also automatic Snellen chart projector. Myopia degree was stated in spherical negative (S-) diopters and presented in numerical value. The test was done in the Public Eye Center (Balai Kesehatan Mata Masyarakat) Purwokerto. In order to avoid measurement bias, digit length measurement was executed by two observers.

Statistical analysis

Univariate analysis consisted of mean and standard deviation of respondent age, body mass index (BMI), and myopia degree. Independent t-test used to observe 2D:4D ratio difference between myopic and emmetropic respondent. Pearson test and Spearman test were used to observe correlation between myopia degrees and 2D:4D ratio. The Spearman test was used in the total myopic and the female group, whereas Pearson test was used in the male group and used to check inter-observer correlation.

Result

Participants

The total population of students in the Medical Faculty Jenderal Soedirman University was screened using a questionnaire to get basic characteristics of respondents and temporary subjective eye refractive conditions, whether it was emmetropia, myopia, or astigmatism.

Basic respondent characteristics

Age and BMI were controlled variables, so the range is still on the controlled range, 19-21 years old and 18.5-23 kg/m^2 for BMI. In gender data, the female respondents (82 students) outnumbered the male respondents (18 students). Myopia degree for right and left eye for myopic respondents was summarized in the range of S - (2.00 ± 1.50) diopter (Table 1).

Table 1. Descriptive data of participants.

Characteristics	**Group**	
	Myopia n = 47	Emmetropia n = 53
Age	19.96 ± 0.86	19.91 ± 0.766
BMI	20.75 ± 1.36	20.73 ± 1.38
Gender		
Male	7 (38.9%)	11 (61.1%)
Female	40 (48.8%)	42 (51.2%)
Mean right eye myopia degree (S -diopter)	2.00 ± 1.55	-
Mean left eye myopia degree (S -diopter)	**1.95 ± 1.54**	**-**
Low-grade myopia respondent (S -0.25 till S -3 diopter)	**33 (66%)**	**-**
Moderate-grade myopia respondent (S -3 till S -6 diopter)	**16 (32%)**	**-**
High-grade myopia respondent (> S -6 diopter)	**1 (2%)**	**-**

Digit measurement characteristics

Calculation of 2D:4D ratio found that mean 2D:4D ratio was between 0.96-0.97. A Pearson correlation test was used to analyze inter-observer measurement correlation and the result was a significant ($p < 0.05$) and very strong ($r > 0.8$) correlation between the two observers (Table 2).

Table 2. The characteristics of digit measurement result.

Characteristics	Measurement result	Inter-observer correlation		
		R	P	
Right hand (pixel(s))	2nd digit	822.99 ± 51.12	0.984	0.000**
	4th digit	847.00 ± 55.94	0.970	0.000**
Left hand (pixel(s))	2nd digit	818.00 ± 51.39	0.985	0.000**
	4th digit	846.64 ± 55.34	0.980	0.000**
2D:4D ratio	Right hand	0.972 ± 0.028	0.914	0.000**
	Left hand	0.967 ± 0.030	0.955	0.000**
	Mean	0.969 ± 0.026	0.950	0.000**

**Significant ($p < 0.01$)

Association between 2D:4D ratio and myopia cases

Observation of association between 2D:4D ratio and myopia cases was done using t-independent test. This analysis discovered that there was no significant ($p > 0.05$) 2D:4D ratio difference between myopia and emmetropia respondents. Table 3 also shows us that in the total sample female group, the 2D:4D ratio was higher in myopic respondents than emmetropic respondents in the male group, 2D:4D ratio in myopic respondents is lower than in emmetropic respondents. We could also notice that males had lower 2D:4D ratio than females.

Table 3. The result of t-independent test to observe association between 2D:4D ratio and myopia cases.

Group	Object	Myopia (n = 47)	Emmetropia (n = 53)	*p value*
Total sample (n = 100)	Right hand	0,974 ± 0,027	0,970 ± 0,029	0,450
	Left hand	0,969 ± 0,032	0,965 ± 0,029	0,504
	Mean	0,972 ± 0,026	0,968 ± 0,027	0,436
Male (n = 18)	Right hand	0,952 ± 0,016	0,968 ± 0,034	0,249
	Left hand	0,946 ± 0,018	0,965 ± 0,031	0,176
	Mean	0,949 ± 0,014	0,967 ± 0,031	0,187
Female (n = 82)	Right hand	0,978 ± 0,026	0,971 ± 0,028	0,203
	Left hand	0,973 ± 0,032	0,965 ± 0,028	0,240
	Mean	0,978 ± 0,027	0,968 ± 0,026	0,175

Correlation between 2D:4D ratio and myopia degree

A correlation test was done using Pearson and Spearman correlation tests. The data in the male group was normally distributed so the analysis used the Pearson correlation test. The Spearman correlation test was used in the female myopia sample group because the data was not normally distributed. We found no significant (p > 0,05) correlation between 2D:4D and myopia degree. The majority of the results in Table 4 was negative correlation with the correlation coefficient mostly being very weak (r < 0,2) whereas in the male group we got weak (r : 0,2 – 0,399) and moderate correlation (r : 0,4 – 0,599) between 2D:4D ratio and myopia degree.

Table 4. Correlation test result between 2D:4D ratio and myopia degree in left and right eye.

Group	Object	Right eye	Left eye		
		r	p	r	p
Total myopia sample (n = 47)[b]	Right hand	0,029	0,0847	-0,015	0,918
	Left hand	-0,067	0,656	-0,002	0,987
	Mean	-0,031	0,836	-0,007	0,960
Male (n = 7)[a]	Right hand	-0,262	0,570	-0,535	0,216
	Left hand	0,522	0,229	0,301	0,512
	Mean	0,183	0,695	-0,108	0,818
Female (n = 40)[b]	Right hand	-0,034	0,834	-0,101	0,537
	Left hand	-0,167	0,303	-0,085	0,609
	Mean	0,135	0,465	-0,106	0,575

[a]Pearson correlation test; [b]Spearman correlation test

Discussion

BMI subjectivity arose as a problem in this study report because we got body height and weight data only from questionnaire screening. Therefore, BMI data in this research was expected not too valid because no standard measurement tool was used to count it. There is ongoing debate about the correlation between BMI and 2D:4D ratio. The first study in 2003 found that BMI had a positive correlation with 2D:4D ratio.[23] Further study a few years later found that BMI was negatively correlated with 2D:4D ratio.[24,25] Indeed, another report in 2014 showed that IMT has correlation to 2D:4D in the age of 10-18 years.[26] Many studies also reported that IMT was not a biological predictor of prenatal androgen level which has effects on 2D:4D ratio. A better predictor of prenatal androgen level was waist to hip ratio and hand grip strength.[27-29]

The female respondents inadvertently outnumbered the male respondents,

which was different from recent study (Table 1).[16] This was an ordinary result because in each cohort in 2012, 2013, and 2014, the females always outnumbered the males in a 3:1 ratio. Average myopia degree for right and left eye for myopic respondents summarized in the range of S - (2,00 ± 1,50) diopter, matching with previous studies.[16,30]

Theoretically, this research was directed to proof that 2D:4D ratio can be a good predictor for high grade myopia. Unfortunately, we only had one (2%) respondent with a high myopia grade (> S -6 diopter); low-grade myopia respondents (up to S -3 diopter) were dominating (66%). However, in the previous research conducted by Krishnakumar *et al.*,[16] there is no explanation about the number of each myopia grade as the limitation of respondents' myopia grade was only S -0,5 diopter. This condition is one of the limitations in this study.

The fourth digit length tended to be higher than the second digit length (Table 2). This tendency happened because the androgen and estrogen receptor was higher in the fourth digit.[35] As to the impact, the 2D:4D ratio was always lower than one, even though females sometimes had a 2D:4D ratio of almost one or even higher than one.[17,31] Ethnically, this research was conducted in Indonesia, which had Mongolian ethnic people so it can be compared with mean 2D:4D ratio of Mongolian. But, regrettably we could not make this comparison because no meta-analysis data exists about the 2D:4D ratio of mean Mongolian people.[32]

The result of independent t-test in Table 3 revealed that there was no significant ($p > 0.05$) association between the 2D:4D ratio and myopia cases which was different from recent similar study reports which showed significantly different results in the female group.[16] The insignificance may be because of worse measurement, ethnical difference, invalid BMI data, or menstrual cycle of women. Women dominated the number of respondents (82%), but in this research menstrual cycles were not controlled. In women, the digit length was significantly ($p < 0.05$) different up to the period of menstrual cycle.[33]

The insignificance in Table 3 was followed by analysis results in Table 4 as there was no significant correlation ($p > 0.05$) between the 2D:4D ratio and the myopia degree. Negative correlation dominated the result while in the male group there was a stronger correlation than in the other group. Negative correlation was observed because high prenatal androgen could have made higher fourth digit so 2D:4D ratio was lower whereas high prenatal androgen could also elongate the axial eye length, which could increase the degree of myopia.[16] Stronger correlation in the male group occurred because the androgen exposure was higher in males than in females.

Naturally, myopia is influenced by many factors which made it hard to correlate 2D:4D with myopia. Myopia has been well-established as a multifactorial disease with both a genetic and an environmental etiology. There are about 70 genetic loci that have been linked with myopia, such as 22q12 locus and 11p13 locus for MYP6 gene and MYP7 gene respectively, and many more. Familial aggregation studies have estimated sibling recurrence risks of common forms of refractive errors to range from 2 to 5.61 for myopia. Some systemic disorders are also associated with

myopia, such as: Marfan syndrome, Cohen syndrome, Stickler syndrome, Ehlers-Danlos syndrome, Weill-Marchesani syndrome, homocystinuria, McCune-Albright syndrome, Kniest syndrome, Down syndrome, Prader-Willi syndrome, Noonan syndrome, Rubinstein-Taybi syndrome, Cornelia de Lange syndrome and fetal alcohol syndrome.[36]

There are several environmental factors implicated in myopia, including near work, light exposure, lack of physical activity, diet, higher level education, higher socioeconomic status, greater levels of educational attainment, and visually intensive occupations. Children with myopia spent more time studying, reading, and less time playing sports than children without myopia, even though the relationship between reading, near work activity is complex and remain poorly understood. Other environmental factors, such as exercising sports and time spent outdoors, have shown protective relationships which can reduce the risk of myopia.[37] This research cannot cover the genetic and many environmental factors. Therefore, the input of genetic data and the other factors, as well as advance statistic methods such as regression models, are essential for the next study.

The research about 2D:4D and myopia has only been conducted twice so far. But, in this second research, because of the result and the limitation, we still cannot implement it as a predictor of a high myopia grade. Additional advance research still is required to confirm the results and to fix the limitation of previous research. This result also cannot be an additional proof for the 2D:4D ratio to be a potential prenatal androgen predictor.

Even while this study used computer-based measurement, which was the best measurement technique for measuring the 2D:4D ratio,[21] there were several limitations other than we have discussed above that need to be fixed in the next research. Firstly, the BMI should be objectively and precisely measured because it could be a source of confounding factors and selection bias. Secondly, as we discussed before, the theory of an association between the 2D:4D ratio and myopia actually concerns specifically the axial type of myopia. Thirdly, this research involved not enough respondents and was lacking respondents with high grade myopia (S -6 diopter or higher). So, in the next research, axial eye length should be the study target with more sophisticated ophthalmological tools to measure eye axial length, such as USG biometry. Also, we highly recommend that the next research will include a higher number of respondents and a higher number of respondents with high grade myopia.

Conclusion

There was no significant association between the second to fourth-digit ratio (2D:4D) with myopia cases in medical students of the Medical Faculty of Jenderal Soedirman University.

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Long-term survival of corneal transplants and visual outcomes among private patients of corneal surgeons in Metropolitan Manila (the Philippines)

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Abstract

Purpose: *There are currently no published studies on the outcomes of keratoplasty in the Philippines. This study aimed to report graft survival rates at years one, three and five after surgery, visual outcomes, and causes of graft failure among private patients of corneal surgeons in Metropolitan Manila receiving tissue from a single local eye bank in the period 2008-2012.*

Design: *Retrospective cohort, multi-center study.*

Methods:

Population: *Private patients of Metro Manila corneal surgeons, receiving tissue from the Santa Lucia International Eye Bank of Manila in 2008-2012. Two hundred and forty-one of 593 yielded sufficiently complete data sets.*

Procedure: *Donor and tissue characteristics, preoperative and latest follow-up characteristics/clinical findings were retrieved and processed. Data from the eye bank was merged with follow-up data from surgeons submitted March-October 2014.*

Main outcome measures: *Survival rates at one, three and five years; best-corrected visual acuity as of latest follow-up; causes of failure.*

Results: *Interval between surgery and most recent follow-up (number of patients) at less than one year = 41; at least one year but less than three years = 112; at least three years but less than five years = 61, and at least five years = 27. One-year survival: 90.4%; three-year: 71.5%; five-year: ~52.7%. Overall, 43.5% had postoperative best corrected visual acuity of 20/50 or better, and 25% counting fingers or worse, with variations across diagnoses.*

Conclusion: *Although small in sample size and response rate, survival trends parallel studies with larger populations elsewhere. Specific trends like favorable survival in keratoconus were consistent. Indications for surgery have changed little since 2005, but regraft has become the most common indication. Further data collection and completion are required for multivariate analysis on factors regarding survival. Uncontrolled intraocular pressure, Inflammation, infection, trauma, and poor adherence to medications are among the identified reasons for failure.*

Keywords: *keratoplasty, transplant, survival, Philippines*

Introduction

Corneal blindness is estimated to be the second most prevalent cause of bilateral blindness in less-developed countries. However, its true prevalence is difficult

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to assess because epidemiological data is limited and classification schemes are complicated.[1-7] In the Philippines, it is the fifth most prevalent cause of bilateral blindness, but as in the rest of the world, its actual prevalence may be much more due to shared difficulties in accurate classification.[8-10,16.]

While the profound difference between the burden of binocular and monocular blindness is obvious, vision loss in one eye still represents a significant departure from the optimal state. Factors and etiologies that bear risk for corneal blindness have yet to be eliminated in the present day and age. The importance therefore of optimizing the predominant treatment of choice, keratoplasty, remains. The urgency of this endeavor is heightened by many additional constraints yet to be resolved – the absence of universally-approved synthetic materials, scarcity of tissue worldwide, permanent graft survival not being the rule, and the lack of active and constantly updated registries to report outcomes in different populations.[10-15,17-20]

Local outcome measures have not been reported, and save for larger and more developed populations – Singapore, China, India – the literature offers little more data for Asian populations.[12] Filipino surgeons have been able to render the intervention for more than a decade now, owing to the establishment in 1995 of the Santa Lucia International Eye Bank of Manila (SLIEBM). Save for very small numbers of tissue processed by an eye bank in the city of Cebu, the SLIEBM has been the dominant source graft tissue in the Philippines ever since. It maintains operating and tissue evaluation standards acceptable to the Association of Eye Banks of Asia; each tissue released is seen and approved by both a trained technician and a cornea-external disease subspecialist.

This study, therefore, was interested in corneal transplant survival rates, postoperative visual acuity, and causes of graft failure in the capital of this developing, middle-income Asian nation. Outcomes of penetrating keratoplasty using optical corneal grafts from the single local eye bank implanted in private patients of Metropolitan Manila cornea surgeons were examined. Survival rates at one, three and five years post-surgery, and, best-corrected visual acuities achieved in the most recent follow-up were quantified. Factors associated with failure were described.

Materials and Methods

Study design:

Retrospective cohort study, multi-center. Follow-up forms were sent to all corneal surgeons (members of the local cornea and external disease subspecialty society) who per eye bank data, conducted keratoplasty using optical donor tissue in the years 2008-2012 within Metropolitan Manila. Survival status, visual acuity, and post-operative interventions and complications were inquired. Surgeries were carried out in different hospitals, hence the multi-center nature of the study.

Participants, inclusion and exclusion criteria:

Only private patients undergoing penetrating keratoplasty were included. All patients that underwent surgery with post-operative data available for all of them

at one, three and five years were included. The remaining patients with follow-up data missing were excluded. No exclusion was made based on age, race, or any demographic characteristic of either donor or recipient.

Main outcome measures:
Graft survival rates at one, three and five years, best corrected visual acuity and causes of graft failure.

Added descriptive statistics:
We also tabulated the indications for surgery, survival rates overall and per diagnosis, visual acuity postoperative, and reasons for failure.

Data and statistical analyses:
Kaplan-Meier survival analysis was used for survival-related calculations. Other measures were tabulated and summarized.

Ethics board approval:
Philippine General Hospital Expanded Hospital Research Office (ethics board) approval was granted October 10, 2013 ((OVS) 2013–295-01 Version No. 1). All conventions and regulations pertaining to donor and patient information were re-examined and applied. The principles of the Declaration of Helsinki were adhered to. All data was handled with privacy and confidentiality, with efforts taken to eliminate detail in outputs that would permit reconstruction of identities.

Results

Five hundred and ninety-three corneas for use in private cases were distributed during the period 2008 to 2012. Two hundred and fifty-two forms were returned from 25 of 50 surgeons (50%), and after exclusion for deficiencies in data, 241 were included (40.64%). The distribution of surgeries per surgeon yielded an average of 9.64 per surgeon. The surgeon with the greatest number of cases had 42, and 16 surgeons carried out less than ten surgeries. Owing to deficiencies in records, data was absent on sex, age and pre-operative best corrected visual acuities for more than two thirds of the patients.

Two hundred and twenty-five cases were full-thickness penetrating keratoplasty (PK) only, 15 cases had the added steps of lens extraction and intraocular lens implantation, and one case involved only lens extraction and anterior vitrectomy with the PK.

Interval between surgery and most recent follow-up (number of patients) at less than one year = 41, at least one year but less than three years = 112, at least three years but less than five years = 61, and at least five years = 27.

Overall one-year survival was 90.4%, three-year was 71.5%, and five-year was 52.7% (Fig. 1).

Regraft, pseudophakic bullous keratopathy (PBK), microbial keratitis, corneal scar, and Fuch's Endothelial Dystrophy were the five most common diagnoses (Table 1). These yielded survival rates at year five as low as 32% and as high as 61%.

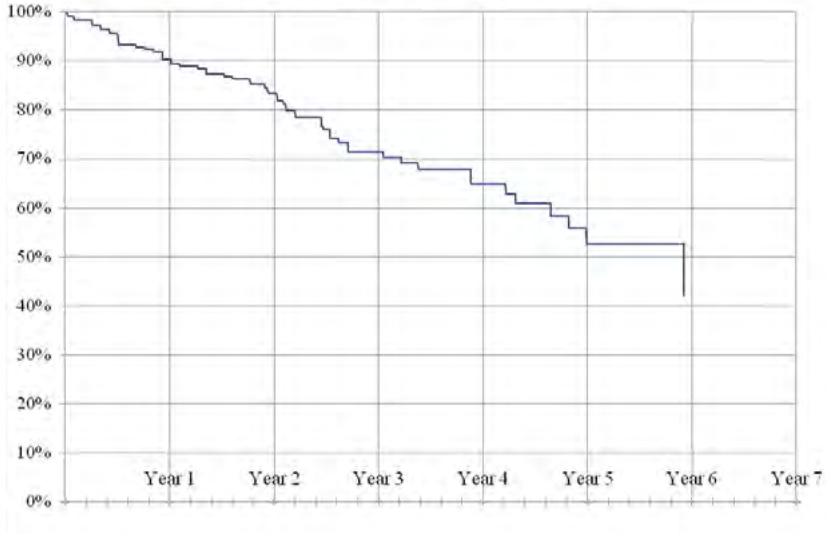

Fig. 1. Kaplan Meier Plot

Diagnosis	Year 1	Year 2	Year 3	Year 4	Year 5
1. Regraft (n=66)	88.7%	86.7%	81.1%	72.4%	52.2%
2. Pseudophakic Bullous Keratopathy (58)	94.3%	92.3%	82.3%	71.3%	61.1%
3. Corneal Scar (28)	91.5%	70.7%	43.2%	32.4%	32.4%
4. Microbial Keratitis (23)	81.7%	65.6%	52.4%	52.4%	52.4%
5. Fuch's Endothelial Dystrophy (22)	94.7%	78.3%	71.8%	71.8%	47.8%

Table 1. Survival Rates of the top 5 diagnoses at yearly intervals.

Of the patients, 43.5% had best corrected postoperative visual acuity of 20/50 (0.4) or better. However, 39.4% had postoperative vision of 20/200 or worse (0.1 or less), with 25% at counting fingers or worse (Fig. 2).

Rejection (4.9% of all grafts), uncontrolled intraocular pressure and endothelial failure were the top-listed reasons for failure observed in the postoperative period. A host of other phenomena have also been observed, with patient related factors

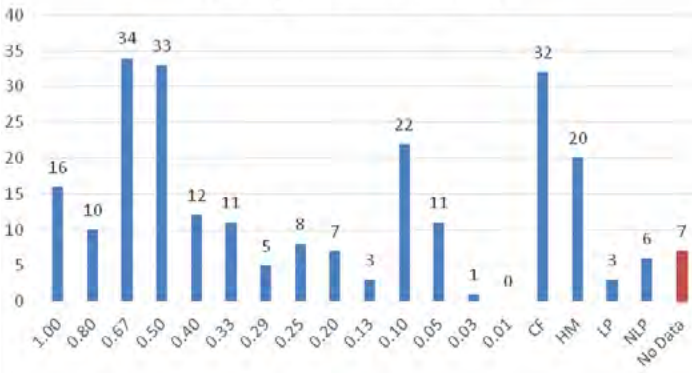

Fig. 2. Post-Operative Visual Acuity, all diagnoses.

of poor follow-up and poor compliance to medication cited seven times (Table 2).

Reason for Failure	No. Patients
Uncontrolled Intraocular Pressure	13
Rejection	12
Not Stated	12
Endothelial Failure	10
Poor Follow-Up	5
Recurrence Of Infection	3
Infection	2
Poor Compliance To Meds	2
Chronic Hypotony	2
Graft Melt	2
Ocular Surface Disease	1
Following Eye Surgery	1
Edema Following Systemic Illness	1
Silicone Oil Endotheliopathy	1
Graft Opacity	1
Trauma	1
Neurotrophic Ulcer	1
Decompensated Corneal Graft	1

Table 2. Reasons for failure post-op, as reported by surgeons.

Discussion

Results from this Filipino population of keratoplasty patients contribute to a number of study areas. While it is the first effort at reporting local outcomes, it contributes to knowledge for both the Asian population and the developing world. Uniquely, it demonstrates outcomes for private patients of a community of surgeons who have increasing expertise with keratoplasty, but perhaps relatively less experience when compared to the developed world.

The top indication for surgery in the time period was regraft, which accounts for one-fourth of the cohort. This may imply the possibility of poorer survival-to-date if the cohort were to include all past recipients, considering (1) a likely 'learning curve' period in the early years of keratoplasty in the Philippines; (2) refinements in medical technologies and techniques over those same years; and (3) the exclusion of charity/service patients. These may be the factors behind the drop to 52% survival at year five. Strength can be lent to this hypothesis by expanding the cohort's size.

This study was limited to private patients in the Metropolitan Manila area for ease of securing records. The ratio of private to service cases have varied per year; for example, the year 2013 saw 70% of cases private, and 30% service. For the year

2012, the proportion was 49% private and 51% service. The possibility that compliance and access to care of these patients were better than that of charity/service patients – and therefore would demonstrate the better local outcomes – must be considered.

With these possible limitations in mind, we note that overall, at least until year three, the population studied still yielded survival rates that are not drastically lower compared to the latest results from elsewhere (albeit gathered in the five to ten years prior). Notably, four of the top five most common indications mirrored that of our Asian comparator, Singapore.[12] Although not tested for significance, similar if not higher survival rates were observed in our sample. Perfect survival as of the five-year mark for 11 grafts done for keratoconus locally is in conformity with the global experience.[21,22] The strength of these figures however may be somewhat weakened by the lack of uniformity in terms of interval between surgery and most recent follow-up.

Tan *et al.*[12] even then did point out how direct comparisons across populations may be unwieldy if only because similarity of clinical parameters was not established. We feel, however, that the absence of gross dissimilarities at least suggests that there may be a common experience/slope of degeneration and failure, across any dissimilarities in context and environment. Certainly, there will be areas of divergence; the survival rates of 18.3% in Singapore,[12] 64% in Italy[18] and 53.5% in Australia[13] at five years for regraft compared to ours of 52.2% is one figure that seems to invite investigation. Examination of indications for the first transplant, standards for judging survival, and any difference in technique or care at all points – from harvesting, through to surgery and the postoperative period – may be in order for this.

Studies in less-developed Asian nations[9,12] – the Philippines included – frequently highlight the high occurrence of infectious keratitis. Accompanying characteristics already regarded as 'high-risk', particularly of inflammation and vascularization, are also mentioned. The varied insults reported as reasons for failure in this present study seem to agree with this perspective. The peculiarly high year-five survival rate of 74% for patients with infectious keratitis may also thus deserve further examination. Issues of classification may arise here as well, as the diagnosis of scar (which has lower survival rates) may include previous infectious or inflammatory states.

Lack of data to investigate risk/survival factors is a clear shortcoming of this present investigation; easily verified characteristics regarding the donor, tissue, and recipient would permit more extensive conclusions. Its importance is highlighted when we consider the areas of divergence already observed across populations. Fasolo *et al.* concluded from their Italian sample that indication for surgery is the primary determinant of long-term maintenance of graft clarity, with associations noted only with lens status, and previous inflammation or infection.[18] Tan *et al.*'s Singaporean study meanwhile also identified recipient age and gender, and donor endothelial cell count as factors impacting survival significantly.[12] These, along with a number of other variables – including tissue death-to-preservation time, surgeon workload, recipient comorbidities – can be secured in our sample and subsequently

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processed to permit comparison with global trends. In terms also of final visual acuity, rates of improvement are not reported as many surgeons failed to report preoperative vision. The report of postoperative VA's, therefore, serves only as a rough description of functional outcomes.

Be that as it may, this preliminary investigation for our unique sample – developing world patients but possibly 'optimized' owing to private case status, with comparatively rapid adaptation of therapeutic techniques by local surgeons – reveals that survival rates approximating the experience elsewhere are being achieved. Further contributions to describing the local/regional experience, as well as to confirm factors for survival would be made possible by mining more data and expanding the dataset. In this era when the demand for this resource-heavy intervention persists, it is clearly an effort that deserves a follow-through, and that must be sustained.

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CORNEAL BIOMECHANICS

from theory to practice

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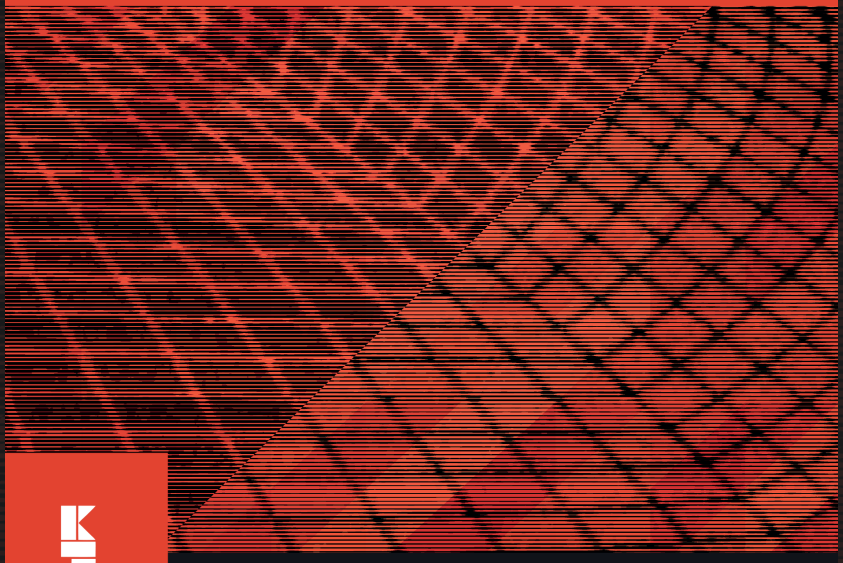
Jun Liu



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