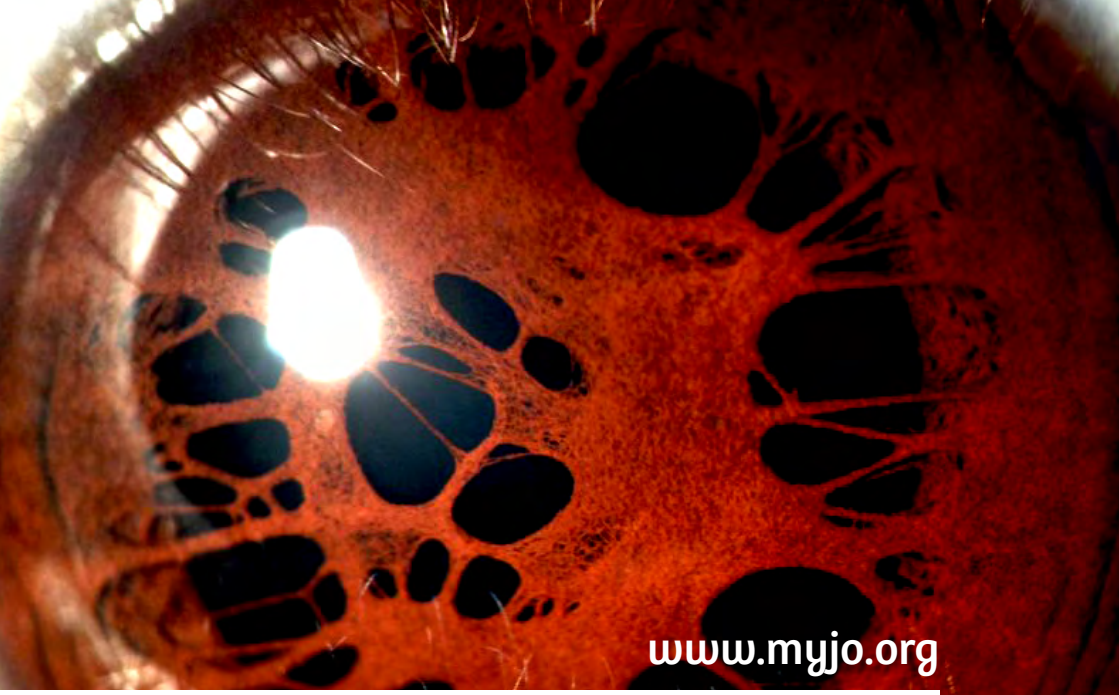
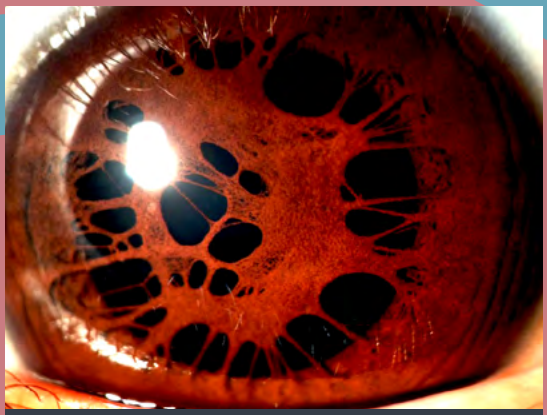


www.myjo.org

Malaysian Journal of Ophthalmology

Volume 1 • Issue 2 • 2019

KUGLER PUBLICATIONS
www.kuglerpublications.com



ABOUT THE COVER IMAGE
Blooming web by Dr. Chan Jan Bond, ophthalmologist from the International Specialists Eye Centre, Kuala Lumpur, Malaysia.

Malaysian Journal of Ophthalmology

The Malaysian Journal of Ophthalmology (MyJO) is the official journal for the Malaysian Society of Ophthalmology (MSO), College of Ophthalmologists Malaysia, and Malaysian Universities Conjoint Committee in Ophthalmology (MUCCO). MUCCO is the national board responsible for training ophthalmologists in Malaysia, comprising the Universiti Kebangsaan Malaysia, Universiti Malaya, and Universiti Sains Malaysia, as well as the Ministry of Health.

MyJO aims to provide a platform for ophthalmologists, clinicians, researchers, trainees, students, optometrists, and eye care providers to publish their work and to promote knowledge enhancement among ophthalmologists and eye care providers in Malaysia.

I: https://myjo.org
E: hello@myjo.org

Copyright

ISSN
Online: 2665-9565
Print: 2665-9557

Malaysia
Online: 2716-5329
Print: 2716-5248

Publisher
Malaysian Society of Ophthalmology
Unit #UG33, PJ Midtown, Jalan Kemajuan, Seksyen 13, 46200 Petaling Jaya, Selangor
admin@mso.org.my

Kugler Publications
P.O. Box 20538
1001 NM Amsterdam
The Netherlands
info@kuglerpublications.com
www.kuglerpublications.com

Manuscript submissions
Author guidelines and templates are available via the website, through which all manuscripts should be submitted. For inquiries please contact us via e-mail.

Publication frequency
MyJO is published four issues per year (quarterly) electronically.

Advertising inquiries
MyJO offers online and in print sponsorship and advertising opportunities. Please contact Kugler Publications for inquiries.

Open access policy

MyJO provides immediate open access to its content after (free) registration, on the principle that making research freely available to the public supports a greater global exchange of knowledge. There are no fees required to publish in the journal.

Article referencing

MyJO is primarily an electronic journal that will occasionally publish print issues. Articles may be referenced by appending the article ID included in the first page header of each article (*i.e.*, © Malaysian Journal of Ophthalmology 2019; #articleID) using the following url: https://www.MyJO.com/index.php/myjo/article/view/#articleID.

Disclaimers

Sponsors

MALAYSIAN SOCIETY OF OPHTHALMOLOGY

COLLEGE of OPHTHALMOLOGISTS
ACADEMY OF MEDICINE, MALAYSIA

UNIVERSITI KEBANGSAAN MALAYSIA
National University of Malaysia

UNIVERSITY OF MALAYA
The Leader in Research & Innovation

UNIVERSITI SAINS MALAYSIA

APEX™

Kami Sedia Membantu
KEMENTERIAN KESIHATAN MALAYSIA

Table of contents

Case reports

Malaysian Journal of Ophthalmology

Editorial

Role of HLA-DRB1*04 in Malay patients with Vogt-Koyanagi-Harada syndrome

Victor Menezo[1], Simon RJ Taylor[2]

[1]Institut Catala di Retina, Barcelona, Spain; [2]University of Surrey, Guildford, Surrey, UK.

In this issue of the Malaysian Journal of Ophthalmology, Alvernia *et al.* report the results of their study into the association between HLA-DRB1*04 and Vogt-Koyanagi-Harada (VKH) syndrome in patients of Malay descent. VKH is a useful condition on which to focus this type of study as, unlike many types of uveitis, it has validated international diagnostic criteria.[1,2] VKH is also among the most common causes of uveitis in Asia, where it accounts for almost one-third of all causes of panuveitis.

The combination of a genetic predisposition, including HLA haplotype as well as other genetic polymorphisms, and environmental factors is generally held responsible for the breakdown of tolerance and the development of autoimmunity in many disease entities. However, with the exception of acute anterior uveitis and HLA-B27, there is a disappointing lack of correlation between clinical phenotype, disease outcome, and HLA haplotype in uveitis. This lack of clarity also applies to VKH: there are distinct HLA associations across different ethnic populations, as has been shown in Hispanic and Japanese VKH patients with their HLA-DRB1*01 and *0405 associations; similarly, whilst VKH shares almost identical phenotypical and histopathological findings with sympathetic ophthalmia, and both share an association with HLA-DR1*0405 subtypes, they have completely different precipitating disease triggers.

Unsurprisingly, the exact aetiology of VKH remains unknown. Several immunological and histopathological studies suggest a T-cell mediated process directed against different antigens associated with melanocytes, including tyrosine or tyrosine-related proteins, in the presence of the DRB1*0405 peptide-binding motif.3 A systematic review and meta-analysis by Shi *et al.* showed an association with VKH disease and various HLA-DRB1*04 subtypes among different races, but other HLA associations, such as -DR53 or -DQ, have also been described, making it difficult to establish risk correlations. This suggests that specific HLA associations are not sufficient to explain the complex pathogenesis of VKH and that other genetic variations may also play a role. In support of this, upregulated Th17 responses and increased IL-17 production from T-cells have been associated with VKH disease in the Chine Han population, particularly in patients homozygous for the IL-17F-

rs763780 allele T.[4] Similarly, two separate studies have suggested the involvement of killer cell immunoglobulin-like receptors cluster (KIR), with an increased frequency of activating receptor KIR2DS3 in Saudi Arabian patients and the KIR gene cluster 3DS1-2DL5-2DS1-2DS5 in Japanese VKH patients. (Interestingly, there was a predominance of KIR2DL/2DL3+HLA-C1 in the Saudi Arabian control group of this study, suggesting a possible preventive role for this variant.)[5,6]

The importance of defining these associations between HLA and clinical entities lies in the potential to provide new insights into the pathogenesis of the disease, and to be able to identify those populations at risk of developing the disease or of having worse clinical outcomes. Identification of alternate targets, such as KIR genes that encode inhibitory and activating receptors on natural killer cells, and therefore may affect susceptibility or even influence disease severity, thus provides an exciting avenue for research. It is in this context that the current study provides useful and interesting information that should assist in the search for the pathogenesis of this and similar autoimmune uveitides.

References

1. Read RW, Holland GN, Rao NA, et al. Revised diagnostic criteria for Vogt-Koyanagi-Harada disease: report of an international committee on nomenclature. Am J Ophthalmol. 2001;131(5):647-52.
2. Yang P, Zhong Y, Du L, et al. Development and evaluation of diagnostic criteria for Vogt-Koyanagi-Harada disease. JAMA Ophthalmol. 2018;136(9):1025-31.
3. Kobayashi H, Kokubo T, Takahashi M, et al. Tyrosinase epitope recognized by an HLA-DR-restricted T-cell line from a Vogt-Koyanagi-Harada disease patient. Immunogenetics. 1998;47(5):398-403.
4. Shu Q, Yang P, Hou S, et al. Interleukin-17 gene polymorphism is associated with Vogt-Koyanagi-Harada syndrome but not with Behcet's disease in a Chinese Han population. Hum Immunol. 2010;71(10):988-91.
5. Levinson RD, Okada AA, Ashouri E, Keino H, Rajalingam R. Killer cell immunoglobulin-like receptor gene-cluster 3DS1-2DL5-2DS1-2DS5 predisposes susceptibility to Vogt-Koyanagi-Harada syndrome in Japanese individuals. Hum Immunol. 2010;71(2):192-4.
6. Sheereen A, Gaafar A, Iqneibi A, et al. A study of KIR genes and HLA-C in Vogt-Koyanagi-Harada disease in Saudi Arabia. Mol Vis. 2011;17:3523-8.

Malaysian Journal of Ophthalmology

© Malaysian journal of Ophthalmology 2019; 2:84-97
Original article

Association between *HLA-DRB1*04* and Malay patients with Vogt-Koyanagi-Harada syndrome in Malaysia: A case-control study

Alvernia M Samy[1,2], Safinaz Mohd Khialdin[2], Nazila Ahmad Azli[1], Noor Hamidah Hussin[3], Azrena Anee[3], Nor Fariza Ngah[1,4]

[1]Department of Ophthalmology, Hospital Selayang, Selangor, Malaysia; [2]Department of Ophthalmology, Faculty of Medicine, The National University of Malaysia Medical Centre, Kuala Lumpur, Malaysia; [3]Department of Diagnostic Laboratory Services, Hematology Unit, The National University of Malaysia Medical Centre, Kuala Lumpur, Malaysia; [4]Department of Ophthalmology, Hospital Shah Alam, Selangor, Malaysia

Abstract

Introduction: Vogt-Koyanagi-Harada (VKH) is an autoimmune disorder affecting melanocyte-containing tissues. Major histocompatibility complex, class II, DR beta 1 *(HLA-DRB1)*04* and its suballele *HLA-DRB1*0405* were found to be associated with VKH in many studies.
Purpose: To determine the association of *HLA-DRB1*04* and its suballele *HLA-DRB1*0405* with VKH patients of Malay descent.
Materials and methods: A case control study was conducted among VKH patients of Malay ethnicity attending Ophthalmology Clinic, Hospital Selayang, Malaysia from December 2016 to December 2017. *HLA-DRB1*04* allele-specific typing was performed on 14 Malay patients with VKH and 14 healthy controls using the polymerase chain reaction sequence-specific primer method. The data was then analysed using Fisher's Exact test.
Results: The frequency of *HLA-DRB1*04* was noted to be higher in patients (42.9%) compared to controls (14.3%), but was not statistically significant (p = 0.209). The

Correspondence: 1. Safinaz Mohd Khialdin, The National University of Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Kuala Lumpur, Malaysia.
E-mail: drsafinaz_1978@yahoo.com.my;
2. Nor Fariza Ngah, Hospital Shah Alam, Persiaran Kayangan, Seksyen 7, 40000 Shah Alam, Selangor, Malaysia.
E-mail: drfarizangah@gmail.com

frequency of suballele *HLA-DRB1*0405* was also increased in patients (42.9%) vs controls (7.1%); however, the results were not significant (p = 0.077).
Conclusion: In conclusion, although the findings were not statistically significant, the increased frequency of both HLA-DRB1*04 and its suballele *HLA-DRB1*0405* may suggest a possible cause for the development of VKH among Malay patients.

*Keywords: HLA-DRB1*04*, *HLA-DRB1*0405*, Malay, Malaysia, Vogt-Koyanagi-Harada

Hubungkait di antara HLA-DRB1*04 dan pesakit Melayu dengan sindrom Vogt-Koyanagi-Harada di Malaysia: Kajian kawalan-kes

Abstrak

Pengenalan: Vogt-Koyanagi-Harada (VKH) adalah gangguan autoimun yang mempengaruhi tisu yang mengandungi sel melanosit. Kompleks histokompatibiliti utama, kelas II, DR beta 1 *(HLA-DRB1)*04* dan subalel *HLA-DRB1*0405* didapati dikaitkan dengan VKH dalam banyak kajian banyak kajian terdahulu.
Tujuan: Bagi menentukan menentukan hubungkait *HLA-DRB1*04* dan subalelnya *HLA-DRB1*0405* dengan pesakit VKH berketurunan Melayu.
Bahan dan kaedah: Kajian kawalan kes dijalankan di kalangan pesakit VKH etnik Melayu yang menghadiri Klinik Oftalmologi, Hospital Selayang, Malaysia dari Disember 2016 hingga Disember 2017. *HLA-DRB1*04* jenis khusus alel dilakukan pada 14 pesakit Melayu dengan VKH dan 14 subjek kawalan yang sihat menggunakan kaedah primer turutan pra-tindak balas rantai polimerase. Data tersebut kemudian dianalisis menggunakan ujian Fisher's Exact.
Keputusan: Kekerapan *HLA-DRB1*04* diperhatikan lebih tinggi pada pesakit (42.9%) berbanding dengan subjek kawalan (14.3%), tetapi tidak signifikan secara statistik (p = 0.209). Kekerapan subalel *HLA-DRB1*0405* juga meningkat pada pesakit (42.9%) vs subjek kawalan (7.1%); Walau bagaimanapun, hasilnya tidak signifikan (p = 0.077).
Kesimpulannya: Walaupun penemuan itu tidak signifikan secara statistik, peningkatan frekuensi *HLA-DRB1*04* dan subalel *HLA-DRB1*0405* yang ditunjukkan ada kemungkinan menjadi penyebab VKH di kalangan pesakit Melayu.

*Kata kunci: HLA-DRB1*04*, *HLA-DRB1*0405*, Bangsa Melayu, Malaysia, Vogt-Koyanagi-Harada

Introduction

Vogt-Koyanagi-Harada (VKH) syndrome is a multisystem, autoimmune disorder, which affects melanin-containing cells in the uveal tract, skin, meninges, and inner ear.[1,2] Racial groups that are darkly pigmented, such as Asians, Hispanics, Native Americans, and Indians, are more frequently affected. The disease is more common in women and disease onset usually occurs from 20 to 50 years old. VKH is most prevalent in Japan, where 6.8-9.2% of uveitis cases are due to VKH.[1] The prevalence of VKH in Singapore was found to be similar to the Japanese population.[3]

VKH is characteristically manifested as bilateral panuveitis.[1,2] Posterior uveitis is a major cause of blindness worldwide (responsible for 10% of all cases of blindness).[4] A census done at the Singapore National Eye Centre found VKH to be the commonest cause of panuveitis (32.8%), which accounts for 7.2% of all uveitis cases that were treated from 1997-2001.[3]

VKH is associated with multifocal serous retinal detachment, signs of meningismus, and other extraocular manifestations, such as auditory derangement and integumentary changes.[1,2] VKH syndrome has four stages or phases, which are the prodromal, uveitic, convalescent, and chronic recurrent phase.[1] Ocular complications include glaucoma, cataract, subretinal fibrosis, and choroidal neovascularisation.[1,2] Factors that contribute to poor prognosis are initial presence of ocular complications or severe presentation, multiple recurrences, inadequate treatment, and the presence of suballeles *HLA-DRB1*0405* and *0410*.[2]

The diagnosis of VKH syndrome is usually made based on clinical findings. Ancillary tests can be helpful in certain situations to confirm the diagnosis or exclude other differential diagnoses. The tests include indocyanine green angiography, fluorescein angiography, optical coherence tomography, ocular ultrasound, and spinal tap to detect cerebrospinal fluid (CSF) lymphomonocytic pleocytosis.[2,5] At present, there are no specific serological tests that are being used in the diagnosis of VKH.[1,2] Diagnostic criteria from the past have been replaced by the International Committee on Nomenclature Revised Diagnostic Criteria.[5] In 2001, the first international workshop on VKH disease recommended new revised diagnostic criteria to help facilitate the diagnosis of VKH disease (Table 1).[5] It includes both exclusion and inclusion criteria and classifies the disease into complete, incomplete, and probable according to the presenting clinical signs.[5,6]

The etiopathogenesis of VKH is still obscured, but selective, autoimmune processes against melanocytes have been hypothesised.[1,7] The role of genetic factors may be vital in the development of VKH. In Japan, extensive studies have been conducted and it was found that *HLA-DRB1*04* had a significant association with VKH disease.[8-11] According to Islam *et al.*, the suballele *HLA-DRB1*0405* showed a significant association with VKH syndrome.[8] The frequency of suballele *HLA-DRB1*0405* was noted to be higher in chronic VKH patients.[8,9] A meta-analysis by Shi *et al.* further confirmed that suballeles *HLA-DRB1*0404*, *0405*, *0410* increased the risk of VKH.[12]

Table 1. Diagnostic criteria for VKH disease[5]

Complete VKH disease (criteria 1 to 5 must be present)
Incomplete VKH disease (criteria 1 to 3 and either 4 or 5 must be present)
Probable VKH (isolated ocular disease; criteria 1 to 3 must be present)

1. No history of penetrating ocular trauma or surgery preceding the initial onset of uveitis.
2. No clinical or laboratory evidence suggestive of other ocular disease entities.
3. Bilateral ocular involvement (a or b must be met, depending on the stage of disease when the patient is examined)
    a. Early manifestations of disease
        I. There must be evidence of a diffuse choroiditis (with or without anterior uveitis, vitreous inflammatory reaction, or optic disc hyperaemia), which may manifest as one of the following:
            i. Focal areas of subretinal fluid, or
            ii. Bullous serous retinal detachments.
        II. With equivocal fundus findings; both of the following must be present as well:
            i. Focal areas of delay in choroidal perfusion, multifocal areas of pinpoint leakage, large placoid areas of hyperfluorescence, pooling within subretinal fluid, and optic nerve staining (listed in order of sequential appearance) by fluorescein angiography and
            ii. Diffuse choroidal thickening, without evidence of posterior scleritis by ultrasonography
    b. Late manifestation of disease
        I. History suggestive of prior presence of findings from 3a, and either both (II) and (III) below, or multiple signs from (III):
        II. Ocular depigmentation (either of the following manifestation is sufficient):
            i. Sunset glow fundus, or
            ii. Sugiura sign
        III. Other ocular signs:
            i. Nummular chorioretinal depigmented scars, or
            ii. Retinal pigment epithelium clumping and/or migration, or
            iii. Recurrent or chronic anterior uveitis
4. Neurological/auditory findings (may have resolved by the time of examination)
    a. Meningismus (malaise, fever, headache, nausea, abdominal pain, stiffness of the neck and back, or a combination of these factors; headache alone is not sufficient to meet definition of meningismus, however), or
    b. Tinnitus, or
    c. Cerebrospinal fluid pleocytosis
5. Integumentary finding (not preceding onset of central nervous system or ocular disease)
    a. Alopecia, or
    b. Poliosis, or
    c. Vitiligo

In Malaysia there has been no previous study on the genetic predisposition of patients with VKH disease. The human leucocyte antigen (HLA) association in VKH patients of Chinese and Indian descent has been previously studied in other countries.[13,14] However, there were no similar studies done among Malay patients with VKH. Therefore, in this study, VKH patients of Malay ethnicity were analysed to determine the frequency and association with *HLA-DRB1*04* and its suballele *HLA-DRB1*0405*. The relationship of suballeles *HLA-DRB1* 0404* and *0410* were also explored. Further observation of the patients' clinical characteristics in terms of disease classification, recurrence, and ocular complications – such as glaucoma, cataract, subretinal fibrosis, and choroidal neovascularisation – in relation to *HLA-DRB1*04* and its suballele *HLA-DRB1*0405* were made.

Materials and methods

Sample size

A study by Islam *et al.* among the Japanese population noted the prevalence of *HLA-DRB1*04* was 93% among patients with VKH and 43.2% among controls. However, only suballele *HLA-DRB1*0405* showed a significant association with VKH syndrome (77.2%) as compared to healthy controls (26.2%).[8] In view of the rarity of the disease, case-control study was chosen as the study design. Based on the research hypotheses, it is to be expected that VKH patients would have a higher rate of *HLA-DRB1*0405*. Therefore, the sample size was calculated using PS power and sample size calculation software (version 3.0; Dupont and Plummer, 2009), based on a study of independent cases and controls with one control per case. Prior data indicated that the probability of exposure among controls was 0.262. If the true probability of exposure among cases was 0.772, then the study needed 14 case patients and 14 control patients to be able to reject the null hypothesis that the exposure rates for cases and controls are equal with probability (power) 0.8. The type I error probability associated with this test of the null hypothesis is 0.05.

Subjects and data collection

A case-control study was conducted from December 2016 to December 2017 among VKH patients attending the Ophthalmology Clinic, Hospital Selayang, Malaysia. Located close to the nation's capital of Kuala Lumpur, it is the referral centre for medical retina subspecialty in Malaysia. A total of 129 patients from all ethnicities were screened from the patient database in the clinic. There were 91 Malay patients with VKH and of these, 14 patients were randomly selected via an excel spreadsheet. Only Malay patients as defined by Article 160 of the Constitution of Malaysia and which could be traced back for at least two generations were eligible for this study.[15]

Patients were diagnosed by typical clinical presentation and according to the revised diagnostic criteria for VKH disease.[5] Ancillary tests, such as optical coherence

tomography, ocular ultrasound, fluorescein angiography, and indocyanine green angiography, were done in certain cases to aid in the diagnosis of VKH. Clinical data of patients, which included disease classification, chronic recurrence, and presence of ocular complications – such as glaucoma, cataract, subretinal fibrosis, and choroidal neovascularisation – were analysed. The course of uveitis was defined according to the Standardization of Uveitis Nomenclature. Chronic disease was characterised by persistent inflammation with relapse occurring less than three months after discontinuation of treatment. Recurrent uveitis was defined as recurring episodes of inflammation after being inactive without treatment for more than three months.[16]

Subjects that fulfilled the eligibility criteria were given a patient information sheet regarding the study prior to recruitment. An interview of the patients was conducted by the investigator using a standardized data extraction form. The form contained questions on demographic data and relevant clinical history. Written, informed consent was obtained prior to commencement of the study. For comparison, 14 randomly selected, unrelated, hospital-based, healthy controls with similar ethnic background, age, and sex-matched were included.

Subjects with any autoimmune disease or diseases that are related to the *HLA-DRB1*04* gene were excluded. *HLA-DRB1*04*-related diseases include rheumatoid arthritis,[17] multiple sclerosis,[18] type 1 diabetes mellitus,[19] lyme disease-induced arthritis,[20] drug-triggered/idiopathic pemphigus vulgaris,[21] pemphigoid gestationis,[22] pemphigus foliaceus,[23] obstructive hypertrophic cardiomyopathy,[24] IgA nephropathy,[25] polymyalgia rheumatica,[26] alopecia areata,[27] systemic lupus erythematosus,[28] polycystic ovary syndrome,[29] and autoimmune hepatitis.[30] This research was approved by the Medical Research and Ethics Committees, Ministry of Health Malaysia, with National Medical Research Registry identification number NMRR-16-1075-29116 [Research ID: 29116]. This study was conducted in accordance with the tenets of the Declaration of Helsinki.

DNA extraction

Each of the subjects had 6 ml of peripheral venous blood sample drawn and placed in a bottle containing ethylenediaminetetraacetic acid (EDTA). The collected samples were processed immediately for HLA typing and were not stored for future research. Genomic DNA was obtained from peripheral blood leukocytes using the commercial QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) from 200 µl of buffy coat according to the manufacturer's instructions. 25-200 ng/µl final concentration of DNA is optimum for HLA typing with an A260/280 ratio of 1.65 to 1.80.[31]

***HLA-DRB1*04* allele-specific typing by polymerase chain reaction sequence-specific primer (PCR-SSP)**

*HLA-DRB1*04* allele-specific typing was done by PCR-SSP, a DNA-based tissue typing method. In contrast with other PCR-based methodologies, the SSP

methodology utilised by One Lambda, Inc., Micro SSP™ DNA typing test, differentiates alleles in the PCR process. The Micro SSP™ DNA typing trays contain dried, pre-optimized primers for PCR amplification of HLA alleles. Perfectly matched oligonucleotide primers are best for amplification of a target sequence by recombinant Taq polymerase. Amplification of target sequences (*i.e.*, a positive result) occurs in precisely matched primer pairs, while there is no amplification in an unmatched primer pair (*i.e.*, a negative result). Purified DNA samples, deoxynucleotide solution mix (Micro SSP™ D-mix), and recombinant Taq polymerase were added to the pre-optimized primer trays for PCR. The primer trays were placed in the thermal cycler using the recommended DNA cycling program. The amplified DNA fragments were then size-separated by 2.5% agarose gel electrophoresis. Visualisation was aided by ethidium bromide staining and ultraviolet light exposure. It was then documented via photography and interpreted accordingly. The results of PCR-SSP were interpreted based on whether particular PCR products were present or absent.[32]

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, version 20; IBM Corp., Armonk, NY, USA). Data was entered into a Microsoft excel worksheet (2013) and transferred to SPSS. Basic characteristics were analysed by descriptive statistics. Independent t-test was used to assess differences between patient and control age. Fisher's Exact test was used to test the association between two categorical variables, with a p-value < 0.05 considered to be of statistical significance.

Results

A total of 14 Malay patients diagnosed with VKH were enrolled in this study, of whom 71.4% were female. Mean age for the patients was 38.9 years, with age ranging from 16 to 61 years old. Fourteen randomly selected, unrelated, healthy control subjects with no antecedent of VKH or any other autoimmune disease were included for comparison, and all individuals were of similar ethnic background, age, and sex-matched. There was no significant difference in age between patients and controls ($p = 0.735$; Table 2).

The frequency of *HLA-DRB1*04* was found to be increased in patients (42.9%) as compared to controls (14.3%); however, this was not statistically significant ($p = 0.209$). The frequency of suballele *HLA-DRB1*0405* was also noted to be higher in patients (42.9%) in comparison to controls (7.1%), but did not reveal any significant association ($p = 0.077$). Suballeles *HLA-DRB1*0404* and *HLA-DRB1*0410* were not detected in either group. Suballele *HLA-DRB1*0401* was noted to be present only in controls (7.1%; Table 3).

Table 2. Patients and controls characteristics

Variable	Value	Patients (n = 14)	Controls (n = 14)	p-valuea
Gender	Male Female	4 (28.6%) 10 (71.4%)	4 (28.6%) 10 (71.4%)	
Age (years)	Mean, range	38.9, 16-61	41, 19-64	0.735

[a] p-values were calculated using the independent t-test.

*Table 3. HLA-DRB1*04* allele and suballeles frequencies in VKH patients and controls

Allele/ Suballele	Patients (n = 14)	Controls (n = 14)	p-valuea
*HLA-DRB1*04*	6 (42.9%)	2 (14.3%)	0.209
*HLA-DRB1*0405*	6 (42.9%)	1 (7.1%)	0.077
*HLA-DRB1*0404*	0 (0.0%)	0 (0.0%)	-
*HLA-DRB1*0410*	0 (0.0%)	0 (0.0%)	-
*HLA-DRB1*0401*	0 (0.0%)	1 (7.1%)	-

HLA-DRB1: major histocompatibility complex, class II, DR beta 1; [a]p-values were calculated using the Fisher's Exact test.

Table 4. Clinical features in *HLA-DRB1*0405*-positive and *HLA-DRB1*0405*-negative VKH patients

Clinical features	VKH patients	
	***HLA-DRB1*0405* positive (n = 6)**	***HLA-DRB1*0405* negative (n = 8)**
Complete VKH Incomplete VKH Probable VKH	0 (0) 4 (66.7%) 2 (33.3%)	0 (0) 4 (50.0%) 4 (50.0%)
Chronic recurrence	5 (83.3%)	4 (50.0%)
Ocular complications: Cataract Glaucoma Subretinal fibrosis Choroidal neovascularisation	 5 (83.3%) 2 (33.3%) 0 (0.0%) 0 (0.0%)	 6 (75.0%) 1 (12.5%) 0 (0.0%) 0 (0.0%)

HLA-DRB1: major histocompatibility complex, class II, DR beta 1.

In VKH patients with *HLA-DRB1*0405* positive, 66.7% had incomplete VKH and 33.3% had probable VKH. In *HLA-DRB1*0405*-negative patients, the frequency of both incomplete and probable VKH was similar (50%). There were no patients with complete VKH noted. The chronic recurrence rate for VKH patients that were positive for *HLA-DRB1*0405* (83.3%) was noted to be higher compared to patients that were negative for *HLA-DRB1*0405* (50%). The frequency of ocular complications, such as cataract and glaucoma, was higher in *HLA-DRB1*0405*-positive patients – cataract (83.3%), glaucoma (33.3%) – vs *HLA-DRB1*0405*-negative patients: cataract (75%), glaucoma (12.5%; Table 4).

Discussion

VKH is a bilateral, granulomatous panuveitis involving melanocyte-containing tissues that is associated with extraocular manifestations. Although the specific cause of VKH syndrome has yet to be ascertained, the possible, suggested pathogenesis is an autoimmune response towards melanocytes in a genetically predisposed person.[1,7] Viral symptoms that precede the onset of the disease could link viral infection as an inciting factor in this autoimmune process.[2,7] Mechanisms that could explain this association are viral molecular mimicry and cross-reactions to melanocytes. Molecular studies utilizing PCR on CSF from patients with VKH revealed the presence of the Epstein-Barr virus.[1] According to Sugita *et al.*, T-cell cross-reactivity between tyrosinase and cytomegalovirus antigen could be involved in the onset of VKH. The data was inconclusive, which requires further studies to identify the triggering factors.[33]

Electron microscope studies of VKH eyes revealed a direct contact between melanocytes and lymphocytes, followed by absence of choroidal melanocytes at the site of contact.[1] Studies in active VKH eyes demonstrated increased activated T lymphocytes in the choroidal inflammatory foci that expressed both early and late T-cell activation markers. During the convalescent stage of VKH, presence of CD4+ and CD8+ cytotoxic T lymphocytes was noted, with lymphocyte infiltrates and loss of choroidal melanocytes. Immunohistochemical evidence has contributed further to the understanding of the autoimmune process in VKH disease. The suggested autoimmune mechanism involved is T-cell mediated, delayed-type hypersensitivity towards uveal melanocytes that abnormally express class II major histocompatibility complex (MHC) antigens.[1,7] In humans, HLA class II is an MHC on chromosome 6p21 that encodes for specific cell-surface molecules on antigen-presenting cells. The CD4+ T-helper cell receptor then interacts with the exogenous antigen presented by HLA molecules, resulting in an immune response that leads to host-tissue destruction.[34]

Various studies have been conducted in other populations and VKH was noted to be associated with several HLA serotypes and genotypes. The linked HLA serotypes

include: *HLA-DQ4* in Japanese;[8] *HLA-DQ7* in Chinese;[13] *HLA-DR1* in Hispanic;[35] *HLA-DR4* in Japanese,[8] Chinese,[13] Hispanic,[35,36] and Italian;[37] and *HLA-DR53* in Japanese.[8] The associated HLA genotypes include: *HLA-DQA1*0301* in Japanese;[8] *HLA-DQB1*0401* in Japanese;[8,11] *HLA-DRB1*0101* in Hispanic;[36] *HLA-DRB1*0404* in Hispanic;[38] *HLA-DRB1*0405* in Japanese,[8,10,11] Hispanic,[38] Saudi Arabian,[39] Brazilian,[40] Korean,[41] and Indian;[14] and *HLA-DRB1*0410* in Indian.[14] Although multiple HLA serotypes and genotypes have been found to be associated with VKH, *HLA-DRB1*04/DR4* and its suballele *HLA-DRB1*0405* in particular were reported in most populations. Therefore, in our study, high-resolution typing via PCR-SSP was limited to *HLA-DRB1*04* alleles.

In our patients (42.9%), the frequency of *HLA-DRB1*04* was higher compared to the control group (14.3%), which suggests a possible association with VKH in our patients. All patients who had *HLA-DRB1*04* detected were positive for only suballele *HLA-DRB1*0405*, with patients (42.9%) vs controls (7.1%; p = 0.077). Although the results were not statistically significant, the association has been proven and well documented in other study populations. Islam *et al.* showed that the prevalence of *HLA-DRB1*04* was 93% among patients with VKH and 43.2% among controls, with a relative risk (RR) of 17.4.[8] It was noted that VKH patients that have the *HLA-DRB1*04* gene either carry suballele *HLA-DRB1*0405* or 0410.[8,10,11] However, by statistical analysis, only *HLA-DRB1*0405* showed a significant association with VKH syndrome (77.2%) compared to healthy controls (26.2%) with an RR of 9.5.[8] Damico *et al.* reinforced the importance of this allele as a predisposition to the development of VKH syndrome. The study showed that there was an increased sensitivity in VKH patients to melanocyte epitopes, and patients with *HLA-DRB1*0405* demonstrated a larger melanocyte-derived peptide repertoire.[42]

Shi *et al.* revealed a pooled odds ratio (OR) of 8.42 for the association of *HLA-DRB1*04* with VKH. This meta-analysis confirmed the association of *HLA-DR4/HLA-DRB1*04* with VKH. It also identified suballeles *HLA-DRB1*0404*, *0405*, and *0410* as risk alleles for VKH, and *HLA-DRB1*0401* as the protective allele. Genotyping of the suballeles *HLA-DRB1*0404*, *0405*, *0410* and *0401* was suggested for patients with VKH.[12] Among our study patients, both *HLA-DRB1*0404* and *0410* were absent, and *HLA-DRB1*0401* was present only in control subjects (7.1%). A systemic review and meta-analysis of 21 studies with 1853 VKH patients showed a different strength of association between VKH and *HLA-DRB1*04*; the diversity was due to different ethnicity, with the highest association among Eastern Asians (OR = 13.69) and the lowest in Indians (OR = 2.09).[12] Different ethnic backgrounds with variable strengths of association could possibly explain the weak association with *HLA-DRB1*04* among Malay patients.

The clinical course of VKH patients that are positive for *HLA-DRB1*0405* was compared with patients negative for *HLA-DRB1*0405*. The frequency of chronic recurrence was higher in *HLA-DRB1*0405*-positive patients (83.3%) than in *HLA-DRB1*0405*-negative patients (50%).Similar findings were noted in a Japanese study, where the frequency of suballele *HLA-DRB1*0405* was increased in chronic VKH

patients (93%) compared to nonchronic patients (56%).[8,9] The presence of ocular complications, such as cataract and glaucoma, was higher in patients positive for this suballele, with 83.3% and 33.3%, respectively. Kim *et al.* revealed that patients with *HLA-DRB1*0405* have more ocular complications than *HLA-DRB1*0405*-negative patients.[41] Although the study subject numbers were not appropriate for statistical analysis, we surmised the possibility of a relation between *HLA-DRB1*0405* and poorer disease outcome.

Conclusion

Although the association between *HLA-DRB1*04* and its suballele *HLA-DRB1*0405* with VKH patients of Malay descent was found to be statistically insignificant, the increased frequency of this HLA allele gives us an insight into the possible etiopathogenesis of VKH syndrome among the Malay population. As this study is only an observation of a small cohort and not a representation of the Malaysian population, the novel pilot data could be of interest and importance for the direction of future studies.

Acknowledgements

We thank the Director General of Health, Ministry of Health Malaysia, for the permission to publish this study. Our sincere gratitude goes to Dr Zabri bin Kamarudin, the Head of Department, Ophthalmology, Hospital Selayang, for his guidance. We also express our gratitude to Assoc Prof Dr Jemaima Che Hamzah, the Head of Department, Ophthalmology, The National University of Malaysia Medical Centre, for her support. A special thanks to Dr Hjh Siti Zaleha bt Mohd Salleh, the Director, Hospital Selayang, for her advice and assistance and providing us with the necessary facilities to conduct our study. Our thanks to Dr Nor Asiah Muhamad, Institute for Public Health, for her advice during the preparation of this study. Appreciation is also due towards the National University of Malaysia Medical Centre for providing funding through the research grant number FF-2016-170. Lastly, we would like to thank everyone who has contributed to this study.

References

1. Moorthy RS, Inomata H, Rao NA. Vogt-Koyanagi-Harada syndrome. Surv Ophthalmol. 1995;39(4):265-292.
2. Sakata VM, da Silva FT, Hirata CE, de Carvalho JF, Yamamoto JH. Diagnosis and classification of Vogt-Koyanagi-Harada disease. Autoimmun Rev. 2014;13(4-5):550-555.

3. Chee SP, Jap A, Bacsal K. Spectrum of Vogt-Koyanagi-Harada disease in Singapore. Int Ophthalmol. 2007;27(2-3):137-142.
4. Suttorp-Schulten MS, Rothova A. The possible impact of uveitis in blindness: a literature survey. Br J Ophthalmol. 1996;80(9):844-848.
5. Read RW, Holland GN, Rao NA, et al. Revised diagnostic criteria for Vogt-Koyanagi-Harada disease: report of an international committee on nomenclature. Am J Ophthalmol. 2001;131(5):647-652.
6. Rao NA, Sukavatcharin S, Tsai JH. Vogt-Koyanagi-Harada disease diagnostic criteria. Int Ophthalmol. 2007;27(2-3):195-199.
7. Damico FM, Bezerra FT, Silva GC, Gasparin F, Yamamoto JH. New insights into Vogt-Koyanagi-Harada disease. Arq Bras Oftalmol. 2009;72(3):413-420.
8. Islam SM, Numaga J, Fujino Y, et al. HLA Class II genes in Vogt-Koyanagi-Harada disease. Invest Ophthalmol Vis Sci. 1994;35(11):3890-3896.
9. Islam SM, Numaga J, Matsuki K, Fujino Y, Maeda H, Masuda K. Influence of HLA-DRB1 gene variation on the clinical course of Vogt-Koyanagi-Harada disease. Invest Ophthalmol Vis Sci. 1994;35(2):752-756.
10. Shindo Y, Inoko H, Yamamoto T, Ohno S. HLA-DRB1 typing of Vogt-Koyanagi-Harada's disease by PCR-RFLP and the strong association with DRB1*0405 and DRB1*0410. Br J Ophthalmol. 1994;78(3):223-226.
11. Shindo Y, Ohno S, Yamamoto T, Nakamura S, Inoko H. Complete association of the HLA-DRB1*04 and -DQB1*04 alleles with Vogt-Koyanagi-Harada's disease. Hum Immunol. 1994;39(3):169-176.
12. Shi T, Lv W, Zhang L, Chen J, Chen H. Association of HLA-DR4/HLA-DRB1*04 with Vogt-Koyanagi-Harada disease: A systemic review and meta-analysis. Sci Rep. 2014;4:6887.
13. Zhang XY, Wang XM, Hu TS. Profiling human leukocyte antigen in Vogt-Koyanagi-Harada syndrome. Am J Ophthalmol. 1992;113(5):567-572.
14. Tiercy JM, Rathinam SR, Gex-Fabry M, Baglivo E. A shared HLA-DRB1 epitope in DR beta first domain is associated with Vogt-Koyanagi-Harada syndrome in Indian patients. Mol Vis. 2010;16:353-358.
15. Constitution of Malaysia (Reprint 2010). Federal Constitution. 1963 (Article 160).
16. Jabs DA, Nussenblatt RB, Rosenbaum JT. Standardization of uveitis nomenclature (SUN) working group. Standardization of uveitis nomenclature for reporting clinical data. Results of the first international workshop. Am J Ophthalmol. 2005;140(3):509-516.
17. Angelini G, Morozzi G, Delfino L, et al. Analysis of HLA DP, DQ, and DR alleles in adult Italian rheumatoid arthritis patients. Hum Immunol. 1992;34(2):135-141.
18. Laroni A, Calabrese M, Perini P, et al. Multiple sclerosis and autoimmune diseases: Epidemiology and HLA-DR association in North-east Italy. J Neurol. 2006;253(5):636-639.
19. Fernandez-Viña M, Ramirez L, Raskin P, Stastny P. Genes for insulin-dependent diabetes mellitus (IDDM) in the major histocompatibility complex (MHC) of African-Americans. Tissue Antigens. 1993;41(2):57-64.
20. Gross D, Forsthuber T, Tary-Lehmann M, et al. Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis. Science. 1998;281(5377):703-706.
21. Matzner Y, Erlich H, Brautbar C, et al. Identical HLA class II alleles predispose to drug-triggered and idiopathic pemphigus vulgaris. Acta Derm Venereo. 1995;75(1):12-14.

22. Shornick J, Jenkins R, Artlett C, et al. Class II MHC typing in pemphigoid gestationis. Clin Exp Dermatol. 1995;20(2):123-126.
23. Matsuyama M, Hashimoto K, Yamasaki Y, et al. HLA-DR antigens in pemphigus among Japanese. Tissue Antigens. 1981;17(2):238-239.
24. Sakurami T, Ueno Y, Iwaki Y, Park M, Terasaki P, Saji H. HLA-DR specificities among Japanese with several autoimmune diseases. Tissue Antigens. 1982;19(2):129-133.
25. Kashiwabara H, Shishido H, Tomura S, Tuchida H, Miyajima T. Strong association between IgA nephropathy and HLA-DR4 antigen. Kidney Int. 1982;22(4):377-382.
26. Cid MC, Ercilla G, Vilaseca J, et al. Polymyalgia rheumatica: a syndrome associated with HLA-DR4 antigen. Arthritis Rheum. 1988;31(5):678-682.
27. Entz P, Blaumeiser B, Betz RC, et al. Investigation of the HLA-DRB1 locus in alopecia areata. Eur J Dermatol. 2006;16(4):363-367.
28. Galeazzi M, Sebastiani G, Tincani A, et al. HLA class II alleles associations of anticardiolipin antibodies. Lupus. 2000;9(1):47-55.
29. Kaibe M, Takakuwa K, Murakawa H, Ishii K, Tamura M, Tanaka K. Studies on the human leukocyte antigens in patients with polycystic ovary syndrome in a Japanese population-possible susceptibility of HLA-A11 and -DRB1*0403 to patient population with polycystic ovary syndrome. Am J Reprod Immunol. 2006;55(4):301-306.
30. Vázquez-García M, Aláez C, Olivo A, et al. MHC class II sequences of susceptibility and protection in Mexicans with autoimmune hepatitis. J Hepato. 1998;28(6):985-990.
31. DNA Mini and Blood Mini handbook. QIAGEN QIAamp®.
32. Product insert, Micro SSP™ HLA DNA typing trays. One Lambda
33. Sugita S, Takase H, Imai Y, Mochizuki M. Immunopathogenic mechanisms of a melanocyte-specific autoimmune disease: a cross-reaction between tyrosinase peptides and cytomegalovirus antigen. Invest Ophthalmol Vis Sci. 2002; 43(13):1529.
34. Gough SC, Simmonds MJ. The HLA region and autoimmune disease: associations and mechanisms of action. Curr Genomics. 2007;8(7):453-465.
35. Weisz JM, Holland GN, Roer LN, et al. Association between Vogt-Koyanagi-Harada syndrome and HLA-DR1 and -DR4 in Hispanic patients living in southern California. Ophthalmology. 1995;102(7):1012-1015.
36. Alaez C, del Pilar Mora M, Arellanes L, et al. Strong association of HLA class II sequences in Mexicans with Vogt-Koyanagi-Harada's disease. Hum Immunol. 1999;60(9):875-882.
37. Pivetti-Pezzi P, Accorinti M, Colabelli-Gisoldi RA, Pirraglia MP. Vogt-Koyanagi-Harada disease and HLA type in Italian patients. Am J Ophthalmol. 1996;122(6):889-891.
38. Aláez C, Flores-A H, Concha del Río LE, et al. Major histocompatibility complex and strong human leukocyte antigen-DRB1 and gender association with Vogt-Koyanagi-Harada syndrome in Mexican Mestizos. Hum Immunol. 2011;72(12):1198-1203.
39. Iqniebi A, Gaafar A, Sheereen A, et al. HLA-DRB1 among patients with Vogt-Koyanagi-Harada disease in Saudi Arabia. Mol Vis. 2009;15:1876-1880.
40. Goldberg AC, Yamamoto JH, Chiarella JM, et al. *HLA-DRB1*0405* is the predominant allele in Brazilian Patients with Vogt-Koyanagi-Harada disease. Hum Immunol. 1998;59(3):183-188.

41. Kim MH, Seong MC, Kwak NH, et al. Association of HLA with Vogt-Koyanagi-Harada syndrome in Koreans. Am J Ophthalmol. 2000;129(2):173-177.
42. Damico FM, Cunha-Neto E, Goldberg AC, et al. T-cell recognition and cytokine profile induced by melanocyte epitopes in patients with *HLA-DRB1*0405*-positive and -negative Vogt-Koyanagi-Harada uveitis. Invest Ophthalmol Vis Sci. 2005;46(7):2465-2471.

Malaysian Journal of Ophthalmology

© Malaysian journal of Ophthalmology 2019; 2:98-113
Original article

Low density lipoprotein receptor (*LDLR*) gene and ocular manifestation in Malay patients with familial hypercholesterolaemia

Nor Idahriani Muhd Nor[1], Alyaa R. Al-Khateeb[2], Wan Hazabbah Wan Hitam[1], Zilfalil Bin Alwi[3], Liza Sharmini Ahmad Tajudin[1]

[1]Department of Ophthalmology, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia; [2]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia; [3]Department of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia

Abstract

Introduction: Corneal arcus and eyelid xanthelasma are the common ocular findings but not exclusively found in familial hypercholesterolaemia (FH) patients. Low density lipoprotein receptor (*LDLR*) gene is the one of the most common genes investigated in FH. There is no study predicting ocular manifestations with genetic variations of the *LDLR* gene.
Purpose: To associate common ocular manifestations of FH and *LDLR* gene in Malays.
Material and methods: A cross-sectional study involving 50 unrelated Malay patients with FH were recruited. FH was diagnosed based on Dutch Lipid Clinic Network diagnostic criteria. The right eye was examined for eyelid xanthelasma and corneal arcus, while mean retinal nerve fibre layer thickness (RNFL) was assessed using Heidelberg retinal tomography II. Venepuncture was performed and genomic deoxyribonucleotide acid (DNA) was extracted. *LDLR* gene variations were screened using denaturing high-performance liquid chromatography and confirmed through DNA sequencing.

Correspondence: Dr. Liza Sharmini Ahmad Tajudin, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kota Bharu, Kelantan, Malaysia.
E-mail: liza@usm.my; sharminiliz@live.com

Results: Corneal arcus was detected in 86.0% of patients, while eyelid xanthelasma was detected in 2.0% of patients. Mean RNFL thickness was 254.16 µm (SD: 60.67 µm). *LDLR* gene variations were identified in 32 patients (64.0%), including 5 mutations and 9 single nucleotide polymorphisms (SNPs). Two novel mutations were detected: c.1705+117T>G and p.Asp139His. There was significant association between genotype frequency of *LDLR* variations c.940+36G>A, p.Glu201Lys and p.Asp304Asn with FH, p.Glu201Lys with corneal arcus, and p.Cys255Ser and c.1705+117T>G with mean RNFL thickness.
Conclusion: LDLR gene variations were not uncommon in Malay patients with FH. Two novel variations, c.1705+117T>G and p.Asp139His, were identified. *LDLR* gene is a potential predictor genetic marker for corneal arcus in Malay patients with FH. c.1705+117T>G is associated with thinner mean RNFL thickness.

Keywords: corneal arcus, familial hypercholesterolaemia (FH), low density lipoprotein receptor (*LDLR*) gene, retinal nerve fibre layer (RNFL) thickness

Gen Reseptor lipoprotein berkepadatan rendah *(LDLR)* dan manifestasi okular dalam pesakit Melayu yang mengalami hiperkolesterolemia warisan

Abstrak

Pengenalan: Arkus kornea dan xanthelasma kelopak mata adalah penemuan okular biasa tetapi tidak semata-mata dijumpai dalam pesakit hyperkolesterolaemia warisan (FH). Gen reseptor lipoprotein berkepadatan rendah (*LDLR*) adalah salah satu daripada gen yang paling sering biasa disiasat dalam FH. Tiada sebarang kajian yang dibuat bagi meramalkan hubungkait variasi genetik gen LDLR dan manifestasi okular.
Tujuan: Untuk mengaitkan manifestasi okular umum dengan mutasi gen FH dan *LDLR* dalam pesakit FH berbangsa Melayu.
Bahan dan kaedah: Kajian rentas keratan yang melibatkan 50 orang pesakit Melayu yang tiada pertalian keluarga dan mengalami FH telah dijalankan. FH didiagnos berdasarkan kriteria diagnostik Dutch Lipid Clinic Network. Mata kanan diperiksa untuk xanthelasma kelopak mata dan arkus kornea, sementara ketebalan lapisan ketebalan serat saraf retina (RNFL) dinilai menggunakan Heidelberg retinal tomography II. Pengambilan darah vena telah dilakukan dan asid deoxyribonucleotide genomik (DNA) telah diekstrak. Variasi gen *LDLR* ditapis

menggunakan kromatografi cecair prestasi tinggi dan disahkan melalui penjujukan DNA.
Keputusan: Arkus kornea dikesan pada 86.0% pesakit, manakala xanthelasma kelopak dikesan pada 2.0% pesakit. Ketebalan purata RNFL adalah 254.16 μm (SD: 60.67 μm). Variasi gen *LDLR* telah dikenalpasti dalam 32 pesakit (64.0%), termasuk 5 mutasi gen 9 tunggal polimorphisma nukleotida tunggal (SNP). Dua mutasi novel dikesan: c.1705+117T>G dan p.Asp139His. Terdapat hubungan yang signifikan antara kekerapan genotip variasi *LDLR* c.940+36G>A, p.Glu201Lys dan p.Asp304Asn dengan FH, p.Glu201Lys dengan arkus kornea, dan p.Cys255Ser dan c.1705+117T>G dengan min purata ketebalan RNFL.
Kesimpulan: Variasi gen *LDLR* bukanlah tidak lazim dikalangan pesakit FH berbangsa Melayu. Malah dua variasi novel, c.1705+117T>G dan p.Asp139His, telah dikenalpasti. Gen *LDLR* adalah penanda genetik yang berpotensi sebagai peramal pembentukkan arkus kornea dikalangan pesakit Melayu dengan FH. c.1705+117T>G dikaitkan dengan kenipisan purata RNFL.

Kata kunci: arkus kornea, hyperkolesterolaemia warisan (FH), Gen reseptor lipoprotein ketumpatan rendah (*LDLR*), ketebalan lapisan serat saraf retina (RNFL)

Introduction

Familial hypercholesterolaemia (FH), an autosomal dominant disease, is considered a global public health problem due to elevated risk of premature coronary heart disease.[1] FH is characterized by elevated serum cholesterol.[2] A majority of cases are caused by mutations in the low density lipoprotein receptor gene (*LDLR*). Other identified potential causative genes include apolipoprotein B100 and a protease known as proprotein convertase subtilin/kexin type 9 (PCSK9).[3,4]

The *LDLR* gene is located on chromosome 19p13.1-13.3, spanning 45 kb, and contains mature protein of 839 amino acids encoded by 18 exons and 17 introns.[5] It is comprised of six functional domains: the signal sequence (exon 1); ligand binding domain (exon 2 to 6); epidermal growth factor precursor-like domain (exon 7 to 14); O-linked sugar domain (exon 15); transmembrane domain (exon 16 and some of 17); and the cytoplasmic domain (exon 18 and the rest of 17).[5] It has been estimated around ten million people suffer from the disease worldwide.[6] The homozygous type is very rare, affecting only 1 in 1,000,000 of the general population.[6] Phenotypically, this type is characterized by accelerated atherosclerosis at early childhood and early development of coronary heart disease (CHD).[4] The heterozygous type is the most common genetic disorder in Europe and United States, affecting 1 in 500 of the population.[7] Heterozygous type is phenotypically less severe and CHD developed at a later age.[8]

Excess cholesterol leads to deposition of cholesterol in various tissue of the

body including the eye. Eyelid xanthelasma is the most common type of cutaneous xanthoma. However, 25% to 70% of patients with eyelid xanthelasma have been found to have normal levels of cholesterol.[9] The pathogenesis of corneal arcus is not fully understood, but has been postulated to share similarities with the atherosclerotic process.[10] Hypercholesterolaemia has been implicated in the presence of corneal arcus in subjects less than 60 years old.[11] In addition to the anterior segment manifestation, a reduction in the density of the retinal ganglion cells and thickness of the inner nuclear layer and photoreceptors has also been reported in hypercholesterolaemic-induced rabbits.[12]

To date, there have been genetic studies conducted on FH patients in Malaysia.[13-15] However, there is no report on the association of ocular manifestation and genetic variations in Malay patients. Our aim in this study was to screen the *LDLR* gene in Malay FH patients. The potential association between common ocular manifestations such as eyelid xanthelasthma, corneal arcus, and retinal nerve fibre layer (RNFL) thickness with *LDLR* gene was also studied.

Table 1. Dutch Lipid Clinic Network diagnostic criteria[16]

Criteria	Points
Family history	
First degree relative with known premature (men:<55 years; women:<60 years) coronary and vascular disease or	
First degree relative with known LDL-c above 95th percentile	1
First degree relative with with tendinous xanthomata and or arcus cornealis or	
Children aged less than 18 years with LDL-c above the 95th percentile	2
Clinical history	
Patient with premature (men:<55 years; women:<60 years) CHD	2
Patient with premature (men:<55 years; women:<60 years) cerebral or peripheral vascular disease	1
Physical examination	
Tendinous xanthomata	6
Arcus cornealis prior to age 45 years	4
Cholesterol levels (mmol/L)	
LDL-c ≥ 8.5	8
LDL-c 6.5-8.4	5
LDL-c 5.0-6.4	3
LDL-c 4.0-4.9	1
DNA analysis	
Functional mutation in the LDLR gene	8

Methods

Patient recruitment and ocular examination

A cross-sectional study was conducted involving 50 Malay patients with FH seen in the physician clinic, Hospital Universiti Sains Malaysia between June 2011 and April 2012. This study received ethical approval from Research and Ethical Committee, School of Medical Sciences, Universiti Sains Malaysia and was conducted in accordance to Declaration of Helsinki for human research.

Sample size calculation was based on two proportion formula and the expected sample size was 70. However, due to time, financial constraints, and difficulty to find patients with FH who fulfilled the inclusion criteria, only 50 Malay patients were recruited. Diagnosis of FH was based on the Dutch Lipid Network diagnostic criteria (Table 1).[16] According to the criteria, the recruited patients were divided into definite, probable, and possible FH based on the cumulative score. A pedigree chart was drawn for each patient to establish the diagnosis and to exclude potential mixed parentage or any uncertainty. The Malay ethnicity is defined as a person who professes to be a Muslim, habitually speaks the Malay language, and adheres to Malay custom, based on the Malaysian Constitution of 1964.[17] Patients with CHD, history of acute myocardial infarction, percutaneous transluminal coronary angioplasty, and coronary artery bypass surgery were included. Exclusion criteria include secondary hypercholestrolaemia due to diabetes mellitus, hypothyroidism, renal disease, liver disease, and drugs taken (*e.g.*, prednisolone). Those with preexisting ocular disease such as retinopathy or optic neuropathy were also excluded. Written consent was obtained after thorough explanation was given to the patients and family members.

A thorough ocular examination was conducted for both eyes, including external eye examination to identify eyelid xanthelasma. However, for the purpose of analysis, only the right eye was included. Corneal arcus was identified using a slit lamp biomicroscope (Topcon, Japan) with magnification (X10). For the purpose of grading corneal arcus, the peripheral cornea was divided into four quadrants: upper, lower, nasal, and temporal. The grading of severity of corneal arcus was based on the scoring system proposed by Varnek et al. on the densest corneal arcus chosen from one of the four quadrants.[18] Corneal arcus grading was conducted by the primary investigator (NIMN).

Heidelberg Retinal Tomography (HRT) II (Heidelberg Engineering, Germany) was used to evaluate the structural changes of the optic nerve head. The pupil was dilated if the quality of image was poor due to a small pupil. An experienced masked technician was responsible in evaluating the optic nerve head. Mean RNFL thickness was obtained for statistical analysis. Fundus was also examined for any significant retinal change secondary to FH, such as changes in vasculature or signs of ischaemia.

Screening of *LDLR* gene

Venesection was conducted and 3 ml of blood was collected in ethylenediaminetetraacetic acid (EDTA)-containing bottle for genetic analysis. DNA was extracted from the leukocytes using the standard procedure for commercial DNA extraction kit (QIAamp DNA extraction kit, QIAGEN, Germany). A total of 15 primers were designed to cover 18 exons and the promoter region of the *LDLR* gene. The primers were designed from reference sequences (accession no. NT_011295) (Table 2) obtained from the Genebank of the National Centre of Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/Genbank). The primers that covered the promoter region were selected based on previous study (Table 2).[19] Polymerase chain reaction (PCR) was carried out. The PCR products were then run through the denaturing high performance lipid chromatography (dHPLC) to detect the presence of mutation or polymorphism in each amplified exon and promoter of the *LDLR* gene. DNA sequencing was performed on samples with heteroduplexity to determine the specific type of mutations or polymorphisms.

Statistical analysis

Statistical Package for Social Sciences (SPSS) Windows version 19 (2010) was used for analysis. One-way ANOVA was used to determine the association between age, total cholesterol, LDL cholesterol, and mean RNFL thickness with FH diagnostic grouping. Association of genotype frequency of *LDLR* variations with the ocular manifestation of FH was determined using a Fischer exact test. Independent t-test was used to analyse the mean RNFL thickness and *LDLR* variation. $P < 0.05$ was deemed statistically significant.

Results

A total of 50 Malay patients with FH were recruited with a mean age of 46.2 (14.4) years. Male and female FH patients were equally distributed. Nearly half (46%) fulfilled the criteria for possible FH based on the WHO definition. More than half (54%) were hypertensive and 46% already developed ischemic heart disease (IHD) or myocardial infarction (MI). Eyelid xanthelasma was identified in only one patient (2%). The most common ocular manifestation in FH patients was corneal arcus (86%). Based on the severity of corneal arcus, 39.5% were mild, 37.2% moderate, and 23.3% severe. There were no significant retinal findings.

As expected, serum total cholesterol and LDL was highest in the definite FH group (Table 3). There was no significant difference in the presence of corneal arcus and mean RNFL thickness according to the definition of FH. However, there was a higher percentage of severe corneal arcus and thinner mean RNFL in definite FH compared to other groups (Table 3).

Table 2. Primer sequence in LDLR

Primer name	Primer sequence
Promoter region F	5'CAGCTCTTCACCGGAGACCC'3
Promoter region R	5'ACCTGCTGTGTCCTAGCTGG'3
Exon 2 F	5'CTGATTCTGGCGTTGAGAG'3
Exon 2 R	5'GAGGCCAGCGGATCACTT'3
Exon 3 F	5'GAGTGACAGTTCAATCCCTG'3
Exon 3 R	5'GAAGAGGCTTGGTATGAGC'3
Exon 4 F	5'GAGACTTCACACGGTGATG'3
Exon 4 R	5'CCCAGGGACAGGTGATAG'3
Exon 5 F	5'TCTGGTTGTCTCTTCTTGAG'3
Exon 5 R	5'TGCAAGCAGCAAGGCACA'3
Exon 6 F	5'TCAGACACACCTGACCTTC'3
Exon 6 R	5'CCGTGCGAGACTGTCTCA'3
Exon 7 F	5'GTTGTAATGAGCCAAGGTTG'3
Exon 7 R	5'CTCCTAACTGCTTTCAAGCA'3
Exon 8 F	5'TCTCCTGGCTGCCTTCGAA'3
Exon 8 R	5'CTAGGACATATGCAGGCATC'3
Exon 9 F	5'CACTCTTGGTTCCATCGAC'3
Exon 10 R	5'CCACTAACCAGTTCCTGAA'3
Exon 11 F	5'CTTCCAGAATTCGTTGCAC'3
Exon 11 R	5'ACAGACCAAGACCTCATCT'3
Exon 12 F	5'GTTCAGGCTCACATGTGGTT'3
Exon 12 R	5'GTTCATCTTGGCTTGAGTG'3
Exon 15 F	5'TAGGCGCACACCTATGAGAA'3
Exon 15 R	5'GTGAGAGAAGGTCAGCAAGG'3

F: forward primer; R: reverse primer

Table 3. Comparison of demographic, lipid profile, and ocular manifestation according to FH groups

FH (WHO classification)				
Criteria	**Definite N = 12**	**Probable N = 15**	**Possible N = 23**	**p-value****
Age (year)*	42.6 (16.4)	48.1 (13.8)	46.9 (13.9)	0.590a
Sex; n (%) Male Female	 6 (24.0) 6 (24.0)	 11 (44.0) 4 (16.0)	 8 (32.0) 15 (60.0)	0.067b
Total cholesterol (mmol/L)*	7.87 (0.60)	6.28 (0.30)	6.23 (0.27)	0.008a
LDL-c (mmol/L)*	5.85 (0.55)	4.13 (0.32)	4.02 (0.27)	0.002a
Corneal arcus n (%)	9 (20.9)	14 (32.5)	20 (46.5)	0.388c
Severity of corneal arcus n (%) Mild Moderate Severe	 1 (5.8) 3 (18.7) 5 (50.0)	 5 (29.4) 6 (37.5) 3 (30.0)	 11 (64.7) 7 (43.7) 2 (20.0)	0.082c
Mean RNFL thickness (µm)*	246.83 (65.91)	256.13 (53.54)	256.70 (64.52)	0.895a

LDL: low density lipoprotein; RNFL: retinal nerve fibre layer; [a]One-way ANOVA; [b]Chi square test; [c]Fisher's exact test; *Mean (SD); **$p < 0.05$: statistically significant

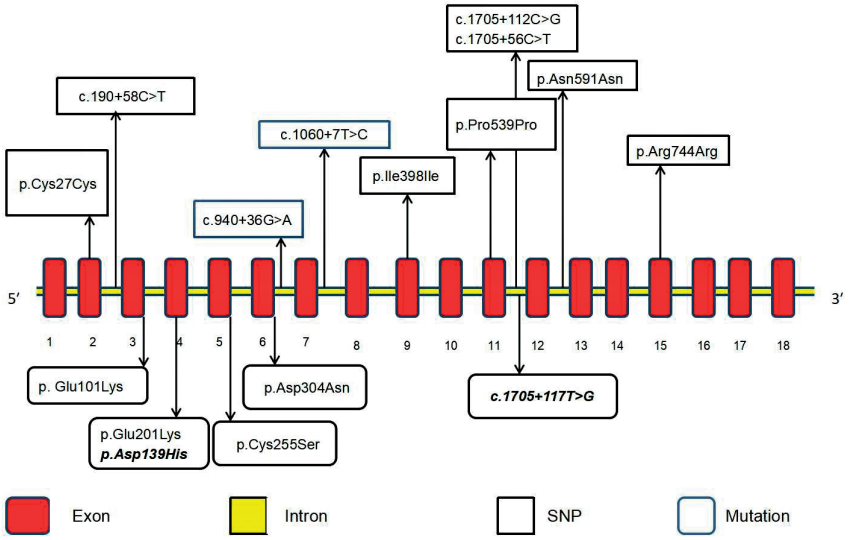


Fig. 1. Identification of LDLR gene variations in Malay patients with FH

LDLR gene variations were detected in 32 patients (64.0%). Eighteen patients had no *LDLR* gene variations. A total of 14 variants of the *LDLR* gene were identified in Malay patients with FH: 5 mutations and 9 single nucleotide polymorphisms (SNPs), including 2 novel mutations, Asp139His in exon 4 and 1705+117T>G in intron 11 (Fig. 1). The mutation type was mainly missense mutations except for one intronic mutation. There were 5 SNPs found in the exon and 4 in the intron (Fig. 1). Asp304Asn was found only in the probable group (Table 4). SNP 940+36GA was exclusively found in the probable group. Glu201Lys increased the possibility of manifestation of corneal arcus ($p = 0.016$) (Table 5). However, there was no significant association with the severity of corneal arcus (Table 5). Mean RNFL thickness was significantly thinner in FH patients with Cys255Ser ($p = 0.044$) and 1705+117TG ($p = 0.020$) (Table 5).

Discussion

Extensive study involving FH patients has been conducted worldwide. Malaysia is a multiracial country compromised of Malays (65%), Chinese (26%), and Indians (8%). There have been several studies conducted in Malaysia involving the main ethnic groups.[13,14] Our study was conducted exclusively among Malays diagnosed with FH. In addition, to the best of our knowledge, this is the only available data on ocular manifestation of FH in Malays.

Xanthomatous deposition on the skin and tendon is the hallmark of homozygous FH, but may not be found in heterozygous FH.[7,8] Deposition in the eye is not mandatory for diagnosis of FH. Corneal arcus (86%) is the most common ocular manifestation in Malays with FH. Our percentage was much higher than reported in Hong Kong (63.5%), Philippines (36.7%), and Finland (16%).[20-22] In fact, it was higher than previously reported in Malaysia involving all major ethnic groups (31.8%).[14] Higher detection of corneal arcus in the current study may be due to more thorough ocular examination using slit lamp biomicroscopy examination. Detection of corneal arcus was essentially based on torchlight examination in previous studies, a process which may easily miss mild and early arcus.[20-22] However, there was no significant association between the severity of corneal arcus and the definition group of FH. Nevertheless, due to a small sample size, our study was still unable to provide a conclusive association between severity of corneal arcus and FH. The small sample size in this study may also contribute to the low detection of xanthelasma.

Previous studies only concentrated on detection of xanthelasma in FH, neglecting the ocular findings of the posterior segment.[20-22] It has been postulated that atherosclerosis diminishes RNFL perfusion, causing acceleration of RNFL damage and reduction of RNFL thickness. This postulation was proven in an animal experimental study.[12] Since FH may cause premature atherosclerosis, it may also affect ocular blood perfusion, particularly to the RNFL.[10,11] Our study was unable to prove this postulation due to the absence of a control group (healthy individuals without

Table 4. Genotype frequency of mutation and SNP found in *LDLR* gene according to FH groups

Variant sequence		FH Groups n (%)			p-value*
		Definite	Probable	Possible	
Mutation					
p.Glu201Lys	GG	9 (20.0)	13 (28.9)	23 (51.1)	**0.029**[a]
	GA	3 (60.0)	2 (40.0)	0 (0.0)	
p.Asp304Asn	GG	12 (26.1)	11 (23.9)	23 (50.0)	**0.008**[a]
	GA	0 (0.0)	4 (100.0)	0 (0.0)	
p.Glu101Lys	GG	11 (22.9)	14 (29.2)	23 (47.9)	0.287[a]
	GA	1 (50.0)	1 (50.0)	0 (0.0)	
p.Asp139His	GG	11 (22.4)	15 (30.6)	23 (46.9)	0.240[a]
	GC	1 (100.0)	0 (0.0)	0 (0.0)	
p.Cys255Ser	TT	10 (20.8)	15 (31.3)	23 (47.9)	0.054[a]
	TA	2 (100.0)	0 (0.0)	0 (0.0)	
c.1705+117T>G	TT	12 (25.0)	15 (31.3)	21 (43.8)	0.493[a]
	TG	0 (0.0)	0 (0.0)	2 (100.0)	
SNPs					
c.940+36G>A	GG	12 (25.5)	12 (25.5)	23 (48.9)	**0.034**[a]
	GA	0 (0.0)	3 (100.0)	0 (0.0)	
c.190+58C>T	CC	11 (22.9)	15 (31.3)	22 (45.8)	0.718[a]
	CT	1 (50.0)	0 (0.0)	1 (50.0)	
p.Cys27Cys	CC	12 (24.5)	15 (30.6)	22 (44.9)	1.000[a]
	CT	0 (0.0)	0 (0.0)	1 (100.0)	
p.Ile398I1e	CC	12 (24.5)	14 (28.6)	23 (46.9)	0.540[a]
	CT	0 (0.0)	1 (0.0)	0 (0.0)	
c.1060+7T>C	TT	12 (24.5)	14 (28.6)	23 (46.9)	0.540[a]
	TC	0 (0.0)	1 (100.0)	0 (0.0)	
p.Pro539Pro	CC	12 (24.5)	15 (30.6)	22 (44.9)	1.000[a]
	CT	0 (0.0)	0 (0.0)	1 (100.0)	
c.1705+56C>T	CC	11 (22.4)	15 (30.6)	23 (46.9)	0.240[a]
	CT	1 (100.0)	0 (0.0)	0 (0.0)	
c.1705+112C>G	CC	12 (24.5)	15 (30.6)	22 (44.9)	1.000[a]
	CG	0 (0.0)	0 (0.0)	1 (100.0)	

Variant sequence		FH Groups n (%)			p-value*
		Definite	Probable	Possible	
p.Asn591Asn	CC	12 (26.1)	14 (30.4)	20 (43.5)	0.676[a]
	CT	0 (0.0)	1 (25.0)	3 (75.0)	
p.Arg744Arg	AA	11 (22.9)	15 (31.2)	22 (45.8)	0.718[a]
	AG	1 (50.0)	0 (0.0)	1 (50.0)	

SNPs: single nucleotide polymorphisms; [a]Fisher's exact test: *p <0.05: statistically significant

Table 5. Genotype frequency of mutation found in *LDLR* gene according to corneal arcus, severity of corneal arcus, and mean RNFL thickness

Variant sequence	Genotype	Corneal arcus		p-value*
		Present N = 43	Absent N = 7	
Mutation				
p.Glu201Lys	GG	41 (91.0)	4 (8.9)	**0.016[a]**
	GA	2 (40.0)	3 (60.0)	
p.Glu101Lys	GG	41 (85.4)	7 (14.6)	0.737[a]
	GA	2 (100.0)	0 (0.0)	
p.Asp139His	GG	42 (85.7)	7 (14.3)	0.860[a]
	GC	1 (100.0)	0 (0.0)	
p.Cys255Ser	TT	41 (85.4)	7 (14.6)	0.737[a]
	TA	2 (100.0)	0 (0.0)	
p.Asp304Asn	GG	40 (87.0)	6 (13.0)	0.464[a]
	GA	3 (75.0)	1 (25.0)	
c.1705+117T>G	TT	42 (87.5)	6 (12.5)	0.263[a]
	TG	1 (50.0)	1 (50.0)	

Variant sequence	Genotype	Severity of corneal arcus			p-value*
		Mild	Moderate	Severe	
p.Glu101Lys	GG	16 (39.0)	16 (39.0)	9 (22.0)	0.699[a]
	GA	1 (50.0)	0 (0.0)	1 (50.0)	
p.Asp139His	GG	17 (40.5)	16 (38.1)	9 (21.4)	0.233[a]
	GC	0 (0.0)	0 (0.0)	1 (100.0)	
p.Glu201Lys	GG	16 (39.0)	15 (36.6)	10 (24.4)	1.000[a]
	GA	1 (50.0)	1 (50.0)	0 (0.0)	
p.Asp304Asn	GG	16 (40.0)	15 (37.5)	9 (22.5)	1.000[a]
	GA	1 (33.3)	1 (33.3)	1 (33.3)	
p.Cys255Ser	TT	17 (41.5)	15 (36.6)	9 (22.0)	0.511[a]
	TA	0 (0.0)	2 (100.0)	0 (0.0)	
c.1705+117T>G	TT	17 (40.5)	15 (35.7)	10 (23.8)	0.605[a]
	TG	0 (0.0)	1 (100.0)	0 (0.0)	
Variant sequence	**Genotype**	**RNFL thickness Mean (SD) (µM)**			**p-value***
p.Cys255Ser	TT	257.67 (59.04)			0.044b
	TA	170.00 (42.43)			
c.1705+117T>G	TT	254.98 (61.80)			**0.020[b]**
	TG	234.50 (7.78)			
p.Glu101Lys	GG	256.08 (61.18)			0.277[b]
	GA	208.00 (2.83)			
p.Asp139His	GG	253.82 (61.25)			0.782[b]
	GC	271.00 (0.00)			
p.Glu201Lys	GG	257.33 (62.30)			0.272[b]
	GA	225.60 (35.60)			
p.Asp304Asn	GG	251.46 (60.55)			0.290[b]
	GA	285.25 (61.08)			

RNFL: retinal nerve fibre layer; [a]Fisher's exact test; [b]Independent t-test; *p <0.05: statistically significant

FH). There was no significant difference in mean RNFL thickness according to the recommended definition group of FH. The thinnest mean RNFL thickness was observed in the definite FH group.

LDLR gene variations were detected in more than half of our Malay patients with FH (64%). The mutation rate of the *LDLR* gene in our study was higher compared to previous studies in Malaysia (26.7%), the Philippines (20.0%), and Taiwan (9.2%).[14,21,23] Mexicans with FH had a higher rate of mutation (37.0%).[24] The rate of SNP in our study was higher than in Taiwan (6.9%).[23] The different rate of *LDLR* gene variation might be due to the different diagnostic criteria used for FH and the sensitivity and specificity of the methods used for the detection of *LDLR* gene variation.[25,26] For example, Khoo *et al.* used denaturing gradient gel electrophoresis (DGGE) for *LDLR* gene mutation screening.[14] DHPLC was reported to be superior to DGGE.[27]

Missense mutations were the most common mutation. The most common types of *LDLR* gene mutation in this study occurred in exon 4 (6 patients). Glu201Lys was detected in five patients and Asp139His in one patient. As reported in the University College London *LDLR* database, exon 3 and 4 were the so-called 'hot-spot' for *LDLR* variations.[28] The second most common mutation was Asp304Asn in exon 6 (4 patients). A similar mutation was reported in one FH patient in France.[29]

Two novel mutations, Asp139His in exon 4 (accession no: HM853677) and 1705+117T>G in intron 11 (accession no: HQ190924), were identified in our Malay patients with FH. The missense mutation of Asp139His occurred due to substitution of G to C at nucleotide 415, causing the change of aspartate acid to histidine located at 3rd cysteine-rich repeat of ligand binding domain.[30] A change in the amino acid affects the binding of LDL cholesterol to the receptor.[28] Intronic mutation of c.1705+117T>G in intron 11 is another novel mutation. There was limited knowledge on intronic mutation causing disease.[29] Perhaps intronic mutation increases the susceptibility to develop FH.

Apart from the mutation, nine SNPs were also identified in our FH patients. There was a significant difference in genotype frequency of Glu201Lys, Asp304Asn, and 940+36G>A according to the definition of FH. Heterozygous of these mutations and SNP seem to be found exclusively in probable FH patients. Glu201Lys was also found in definite FH patients. *LDLR* is the potential marker for FH in Malays. However, *LDLR* variation was not detected in 34% of our recruited patients. Other genes may be responsible in these patients.

To the best our knowledge, this is the first report on the association of *LDLR* with ocular manifestations in FH patients. Glu201Glu was found to associate with corneal arcus. Glu201Lys was only found in two patients with corneal arcus. Regression of corneal arcus has not yet been reported in humans. Corneal arcus may represent a phenotype marker for exposure to dyslipoprotenaemia and reflex the tissue deposition with atheroma.[31] *LDLR* gene variation has potential as the predictor for ocular manifestation of FH. However, there was no association between *LDLR* variations and corneal arcus severity. There is a possibility of ethnic- or popula-

tion-specific mutation.[30] A larger sample size is needed to conclude this association. There was no significant association of the identified SNPs with corneal arcus.

Asn591Asn was detected in one patient with bilateral xanthelasma who also presented with tendon xanthoma but without history of CHD. It was previously reported that xanthelasma increased the risk of CHD.[23] Reduction of RNFL thickness was seen in hypercholesterolaemic induced animals due to the enhanced activity of nitric oxide synthase that lead to retinal ganglion cell apoptosis.[13] Thinner mean RNFL thickness was found in patients with the novel intronic mutation 1705+117T>G. However, 1705+117TG was found in only two patients. Thus, thinner mean RNFL perhaps occurs by chance and not really in relation to the mutation. For a similar reason, significantly thinner RNFL thickness was also observed in patients with Cys255Ser.

The major weakness of this study is the small sample size, especially for achieving conclusive findings on the association between the *LDLR* gene and FH. The sample size seems to be adequate for genotype association with corneal arcus. Nevertheless, this study provides baseline data for future studies. Identification of potential genetic markers in FH is important in the prevention of mortality and morbidity related complications.

Acknowledgements

We thank the patients who participated in this study. This study was financially supported by eScience Fund grant No. 305/PPSP/6113212, Ministry of Science, Technology and Innovation (MOSTI), Malaysia.

References

1. Marks D, Thorogood M, Neil HAW, Humphries SE. A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia. Atherosclerosis. 2003;168(1):1-14.
2. Yang KC, Su YN, Shew JY, et al. LDLR and ApoB are major genetic causes of autosomal dominant hypercholesterolemia in a taiwanese population. J Formos Med Assoc. 2007; 106(10):799-807.
3. Datta BN, Cole DS, Shortland GJ. Hyperlipidaemia. Paediatr Child Health. 2010;21(2):94-98.
4. Farnier M, Bruckert E. Severe familial hypercholesterolaemia: Current and future management. Arch Cardiovasc Dis. 2012; 105(12):656-665.
5. Sudhof T, Goldstein JL, Brown MS, Russell DW. The LDL receptor gene: a mosaic of exons shared with different proteins. Science. 1985;228(4701):815.
6. Fernando C. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. Atherosclerosis. 2004;173(1):55-68.
7. David S. Guidelines for the diagnosis and management of familial hypercholesterolaemia. Heart Lung Circ. 2007;16(1):25-27.

8. Graham CA, McClean E, Ward AJM, et al. Mutation screening and genotype:phenotype correlation in familial hypercholesterolaemia. Atherosclerosis. 1999;147(2):309-316.
9. Bergman R. The pathogenesis and clinical significance of xanthelasma palpebrarum. J Am Acad Dermatol. 1994;30(2, Part 1):236-242.
10. Fernandez AB, Keyes MJ, Pencina M, D'Agostino R, O'Donnell CJ, Thompson PD. Relation of corneal arcus to cardiovascular disease (from the Framingham Heart Study Data Set). Am J Cardiol. 2009;103(1):64-66.
11. Hoogerbrugge N, Happee C, van Domburg R, Poldermans D, van den Brand M JBM. Corneal arcus: indicator for severity of coronary atherosclerosis? Neth J Med. 1999;55(4):184-187.
12. Shibata M, Sugiyama T, Hoshiga M, et al. Changes in optic nerve head blood flow, visual function, and retinal histology in hypercholesterolemic rabbits. Exp Eye Res. 2011;93(6):818-824.
13. Junit SM, Othman S, Yusof R, Billett MA, Harun F. Ala519Thr Mutation in Exon 11 of LDL Receptor Gene in Members of a Malaysian Family with Hypercholesterolaemia. As Pac J Mol Biol Biotechnol. 2003;11(2):77-82.
14. Khoo K, Van Acker P, Tan H, Deslypere J. Genetic causes of familial hypercholesterolaemia in a Malaysian population. Med J Malaysia. 2000;55(4):409-418.
15. Livy A. Lye SH. Familial hypercholesterolemia in Asia: A Review. OMICS Res. 2011;1(1):22-31.
16. World Health Organization. Familial hypercholesterolaemia (FH): report of a second WHO consultation, Geneva, 4 September 1998, World Health Organization, Geneva. http://whqlib.who.int/hq/1999/WHO_HGN_FH_CONS_99.2.pdf.
17. Groves HE. The Constitution of Malaysia 1964. Malaysia Publications.
18. Varnek L, Schnohr P, Jensen G. Presenile corneal arcus in healthy persons. A possible cardiovascular risk indicator in younger adults. Acta Ophthalmol. 1979; 57(5):755-65.
19. Bodamer OA, Bercovich D, Schlabach M, Ballantyne C, Zoch D, Beaudet AL. Use of denaturing HPLC to provide efficient detection of mutations causing familial hypercholesterolemia. Clin Chem. 2002; 48(11):1913-1918.
20. Hu M, Lan W, Lam CW, Mak YT, Pang CP, Tomlinson B. Heterozygous familial hypercholesterolemia in Hong Kong Chinese. Study of 252 cases. Int J Cardiol. 2013;167(3):762-767.
21. Punzalan FER, Sy RG, Santos RS, Cutiongco EM, et al. Low density lipoprotein-receptor (LDL-R) gene mutations among Filipinos with familial hypercholesterolemia. J Atheroscler Thromb. 2005;12(5):276.
22. Koivisto P, Koivisto U-M, Miettinen TA, Kontula K. Diagnosis of heterozygous familial hypercholesterolemia. DNA analysis complements clinical examination and analysis of serum lipid levels. Arterioscler Thromb Vasc Biol. 1992;12(5):584-592.
23. Yang KC, Su YN, Shew JY, et al. LDLR and ApoB are major genetic causes of autosomal dominant hypercholesterolemia in a Taiwanese population. J Formos Med Assoc. 2007;106 (10):799-807.
24. Robles-Osorio L, Huerta-Zepeda A, Ordoez ML, et al. Genetic heterogeneity of autosomal dominant hypercholesterolemia in Mexico. Arch Med Res. 2006;37(1):102-108.
25. Austin MA, Hutter CM, Zimmern RL, Humphries SE. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. Am J Epidemiol. 2004;160(5):407-420.
26. Watts GF, Sullivan DR, Poplawski N, van Bockxmeer F, et al. Familial hypercholesterolaemia: A model of care for Australasia. Atheroscler Suppl. 2011;12(2):221-263.

27. Meldrum CJ, McPhillips M, Crooks R, et al. A comparison between denaturing gradient gel electrophoresis and denaturing high performance liquid chromatography in detecting mutations in genes associated with hereditary non-polyposis colorectal cancer (HNPCC) and the identifications of 9 new mutations previously unidentified by DGGE. Hered Cancer Clin Pract. 2003;1(1):39-48.
28. Leigh, S, Foster A, Whittall R, Hubbart C, Humphries S. Update and analysis of the University College London low density lipoprotein receptor familial hypercholesterolemia database. Ann Hum Genet. 2008; 72(4):485-498.
29. Amsellem S, Briffaut D, Carrie A, et al. Intronic mutations outside of Alu-repeat-rich domains of the LDL receptor gene are a cause of familial hypercholesterolemia. Hum Genet. 2002;111(6): 501-510.
30. Russell DW, Brown MS, Goldstein JL. Different combinations of cysteine-rich repeats mediate binding of low density lipoprotein receptor to two different proteins. J Biol Chem. 1989; 264(36): 21682-21688.
31. Winder AF. Corneal arcus & prognosis in familial hypercholesterolaemia (FH). Atherosclerosis 1987; 68(3):276.

Malaysian Journal of Ophthalmology

© Malaysian journal of Ophthalmology 2019; 2:114-125
Original article

Ocular biometry and refractive changes post sutureless vitrectomy surgery

Raja Nor Farahiyah Raja Othman[1,2], Zabri Kamaruddin[2], Selva Raja Vengadasalam[2], Mushawiahti Mustapha[1]

[1]Department Ophthalmology, Hospital Canselor Tuanku Muhriz, Kuala Lumpur, Malaysia; [2]Department Ophthalmology, Hospital Selayang, Selangor, Malaysia

Abstract

Introduction: Pars plana vitrectomy (PPV) without endotamponade should not induce significant change to the refractive status of the eye. However, several studies have reported minor refractive changes after plain vitrectomy.
Purpose: To compare the changes in refraction following PPV and to examine the biometry parameters that may affect the refractive change.
Materials and methods: In this prospective cohort study, patients who were listed for 23-gauge 3-port PPV without buckling or silicone oil tamponade were enrolled between December 2015 and September 2017. Autorefraction, keratometry, anterior chamber depth (ACD), and axial length (AL) were measured preoperatively and three months postoperatively.
Results: This study involved 41 eyes from 38 patients. The mean spherical equivalent (SE) before PPV was -1.08 dioptres (D), (standard deviation (SD) 2.18), which changed to a mean of -1.88 D (SD 2.20) postoperatively. The mean SE change was -0.80 D (SD 1.61, 95% confidence interval (CI) -1.31 to 0.30 D, P = 0.003). The median astigmatism before PPV was 0.69 D (Interquartile range (IQR) 0.69 D) reduced to 0.66 D (IQR 0.60 D) after PPV (P = 0.882). Median ACD preoperatively was 3.55 mm (IQR 0.76 mm) and reduced postoperatively to 3.44 mm (IQR 0.67 mm), (P = 0.028). The median AL was 23.36 mm (IQR 1.42 mm) and 23.48 mm (IQR 1.56 mm) before and after PPV, respectively, (P = 0.029). No significant SE change was found between phakic and pseudophakic groups (P = 0.155).

Correspondence: Raja Nor Farahiyah Raja Othman, Dr.Oft (UKM), Department of Ophthalmology, Hospital Canselor Tuanku Muhriz, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Batu 9 Cheras, Wilayah Persekutuan Kuala Lumpur, Malaysia.
E-mail: rajanorfarahiyah@gmail.com

Conclusion: Patients experience myopic shift post plain PPV, possibly due to the reduction of ACD. The ACD tended to be shorter in the pseudophakic group, suggesting that the myopic shift in the phakic group may be a result of the development of nuclear sclerosis cataract.

Keywords: anterior chamber, biometry, refraction, vitrectomy

Biometri okular dan perubahan refraktif selepas pembedahan vitrektomi tanpa jahitan

Abstrak

Pengenalan: Vitrektomi pars plana (PPV) tanpa endotamponad tidak boleh menyebabkan perubahan ketara pada status refraktif mata. Walau bagaimanapun, beberapa kajian telah melaporkan perubahan refraktif kecil selepas vitrektomi biasa.
Tujuan: Untuk membandingkan perubahan dalam refraksi berikutan dari PPV dan untuk mengkaji parameter biometri yang boleh menjejaskan perubahan refraktif.
Bahan dan kaedah: Dalam kajian kohort prospektif ini, pesakit yang disenaraikan untuk PPV 3-port 3-port tanpa troll atau minyak silikon tamponad telah direkrutkan antara Disember 2015 dan September 2017. Autorefraksi, keratometri, kedalaman ruang anterior (ACD), dan paksi panjang (AL) diukur secara pra pembedahan dan tiga bulan selepas pembedahan.
Keputusan: Kajian ini melibatkan 41 mata dari 38 pesakit. Median spherikal equivalent (SE) sebelum PPV adalah -1.08 dioptres (D), (sisihan piawai (SD) 2.18), yang berubah menjadi min -1.88 D (SD 2.20) selepas. Perubahan purata SE ialah -0.80 D (SD 1.61, 95% selang keyakinan (CI) -1.31 hingga 0.30 D, P = 0.003). Adakah spherikal ekuivalen atau keseimbangan spherikal yang betul? Median Astigmatisma sebelum PPV adalah 0.69 D (Julian Interquartile (IQR) 0.69 D) dikurangkan kepada 0.66 D (IQR 0.60 D) selepas PPV (P = 0.882). Median ACD pra-operasi adalah 3.55 mm (IQR 0.76 mm) dan berkurangan selepas operasi ke 3.44 mm (IQR 0.67 mm), (P = 0.028). Median AL adalah 23.36 mm (IQR 1.42 mm) dan 23.48 mm (IQR 1.56 mm) sebelum dan selepas PPV, (P = 0.029). Tiada perubahan SE yang penting didapati di antara kumpulan fakik dan psuedofakik (P = 0.155).
Kesimpulan: Pesakit mengalami enjakan myopik pasca PPV biasa, mungkin disebabkan pengurangan ACD. ACD cenderung lebih pendek dalam kumpulan pseudofakik, yang menunjukkan bahawa enjakan miopik dalam kumpulan fakik mungkin disebabkan oleh perkembangan katarak sklerosis nuklear.

Kata kunci: ruang anterior, biometri, pembiasan, vitrektomi

Introduction

Pars plana vitrectomy (PPV) and scleral buckle surgery are routinely performed for various vitreoretinal pathologies. With advancement of technology, anatomical success rate has improved.[1-3] Scleral buckle surgery and PPV using silicone oil endotamponade are known to cause refractive changes.[2,4,5] Scleral buckle increases axial length (AL) causing a myopic shift post-surgery.[2,4,5]

Theoretically, PPV alone without endotamponade should not induce significant refractive changes to the eye. However, several studies have reported refractive changes between -0.26 D and -1.21 D after plain primary PPV without buckling or silicone oil tamponade.[6-10] Byrne *et al.* reported significant spherical equivalent (SE) changes after 20-gauge PPV in pseudophakic eyes for various diseases.[7] A study in Taiwan described significant changes in refractive status after primary PPV in both phakic and pseudophakic eyes.[8] However, the study was not able to describe the reason for such changes.

There are few studies that report potential reasons for this myopic shift. It could either be due to a reduction of anterior chamber depth (ACD)10-12 or an increase in AL.[3] To the best of our knowledge, this is the first prospective study directly looking at the possible association between refractive changes and various ocular biometry variables.

Materials and methods

Ethics statement

The protocol of this study was approved by the ethics committee of the National Medical Research Registry (NMRR-15-1146-25564 (IIR)) and the Hospital Canselor Tuanku Muhriz, and conformed to the Declaration of Helsinki. Written informed consent was obtained.

Sampling strategy and recruitments

A prospective cohort study was conducted from 1st of December 2015 until 30th of September 2017 involving patients referred to a tertiary eye care center in central Malaysia for various vitreoretinal conditions. Patients listed for vitrectomy were recruited.

Inclusion and exclusion criteria

In pre-existing pseudophakic eyes, an interoperative period greater than six months between cataract operation and PPV was allowed for refractive stabiliza-

tion. Exclusion criteria for the study were patients with concurrent ocular pathology such as glaucoma or corneal disorders, previous ocular surgery, combined buckle surgery, and patients who required silicone oil endotamponade.

Clinical method

The required data was taken a day prior to surgery and three months after the operation. Refraction data was obtained with an autorefractometer (Nidek Autorefractometer, Model AR 600A, Nidek, Japan) and converted to SE. Non-contact optical biometry (IOL Master 500, Carl Zeiss Meditec AG, Germany) was used to study keratometry, ACD, and AL of the globe. The corneal astigmatism was determined by differences in keratometry (dK) along the flattest and the steepest meridian. Calibration of the instrument was done by the optometrist every week for the IOL Master and monthly for the Nidek Autorefractometer.

Vitrectomy was performed under local anaesthesia (retrobulbar and/or peribulbar) by two vitreoretinal surgeons of at least three years' experience. The surgical procedure was done using the standard 23-gauge vitrectomy system. Postoperatively, all patients received routine topical medications: guttae dexamethasone 0.1% and guttae chloramphenicol 0.5% every two hours. Patients were followed up at three months postoperatively and similar measurements were repeated.

Sample size and methods

The sampling method was purposive sampling, also known as selective sampling. It was homogeneous in nature. The sample size used was based on the formula Sample Size for Comparing Means formula, introduced by Wang *et al.*[13] The formula was used to achieve 80% of power with reference to a similar study by Tseng *et al.*[8] (38 eyes plus 20% dropout).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science, version 24.0 (IBM, Armonk, USA) for Windows. Refractive status and biometry components (keratometry, ACD, and AL) were analyzed by paired t-test/independent t-test for normally distributed data and Wilcoxon-Mann-Whitney test for non-normally distributed data, respectively. A P-value < 0.05 was considered statistically significant.

Results

Eighty-eight eyes were eligible for the study. Five cases were rescheduled or cancelled for various non-ocular reasons. Fourteen eyes had intraoperative silicone oil tamponade and another 28 patients were lost at follow up. Finally, a total of 41

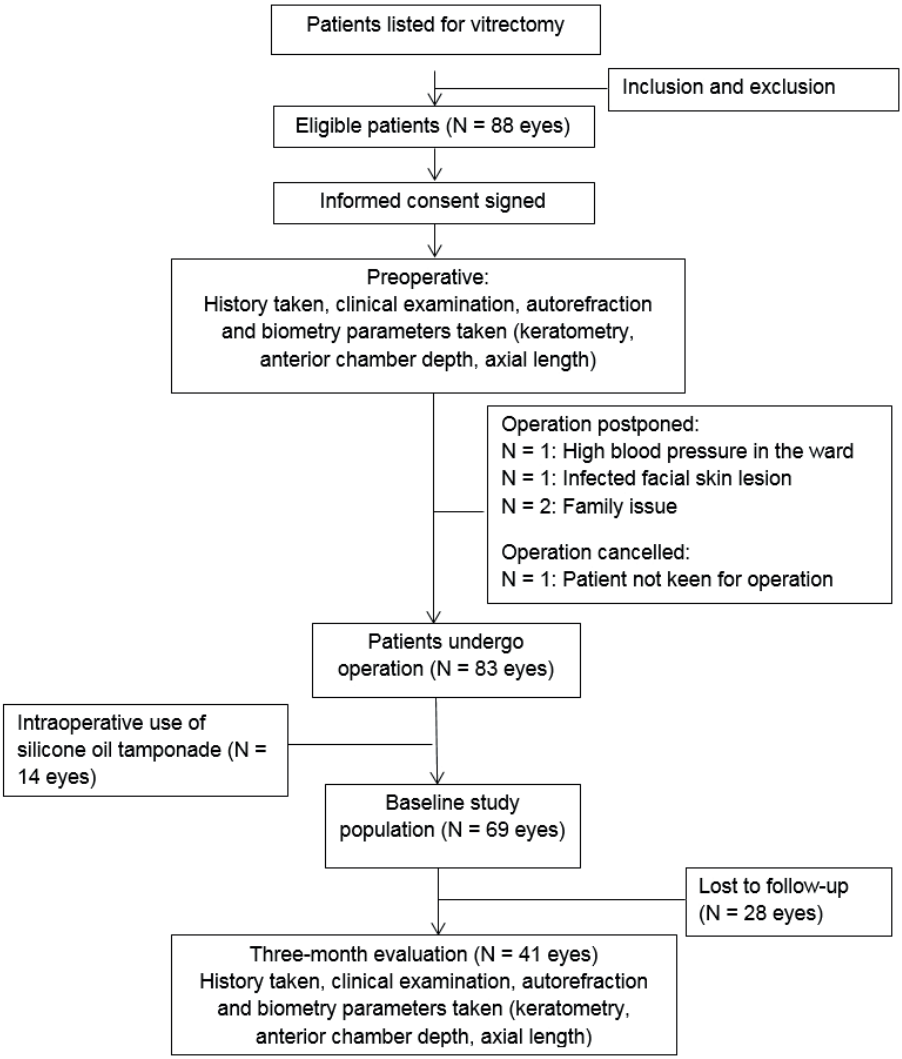


Fig. 1. Flow chart of the study

eyes from 38 patients were recruited for the study (Fig. 1).

Mean age was 53.21 (Standard deviation [SD] 13.84) years, ranging from 22 to 76 years old. The majority were more than 40 years of age (78.9%) (Table 1). There were 32 phakic (78%) and 9 pseudophakic (22%) eyes. Indications for vitrectomy were advanced diabetic eye disease (56.1%), epiretinal membrane (17.1%), vitreous haemorrhage (12.2%), macular hole (9.8%), and rhegmatogenous retinal detachment (4.9%). Gas tamponade (air, sulfur hexafluoride, and perfluoropropane) was used in 37 eyes (90.2%). Preoperative intraocular pressure (IOP) ranged from 6 to 20 mmHg, while the postoperative IOP ranged from 10 to 22 mmHg.

Refractive changes

Mean SE prior to vitrectomy was -1.08 ± 2.18 D. Postoperatively, mean SE was -1.88 ± 2.20 D (P = 0.003). Myopic shift was more significant for the phakic group compared to the pseudophakic patients. (Table 2). Refractive changes were significant for SE (P = 0.002) but not for astigmatism (P = 0.722) in the phakic group. Whereas in pseudophakic eyes, both SE (P = 0.808) and astigmatism changes (P = 0.624) were not significant. SE changes between age group, gender, disease category, and with the use of gas tamponade were not significant.

Ocular biometry changes

ACD reduced significantly (P = 0.028) after surgery. There was no significant correlation between SE and the measured ocular parameters. Therefore, no further multivariate analysis was done.

Discussion

In our study, PPV without scleral buckle/silicone oil endotamponade resulted in an overall myopic shift of -0.80 D. Tseng *et al.* described similar changes in their study.[8] They concluded that myopic changes were possibly due to changes in the position of the macula/fovea postoperatively. Preoperative thickening due to macular oedema or traction may induce hyperopia. Postoperatively, surgical correction improved the anatomical configuration of the fovea. Hence, the surgical correction resulted in a myopic shift. However, the study was done retrospectively and lacked biometry information to support their findings.[8] We found that myopic shift was more prominent in phakic compared to pseudophakic eyes. This could be due to changes of refractive compound within the crystalline lens or progression of cataract post-surgery. Although Byrne *et al.* reported similar myopic changes among pseudophakic eyes,[7] we did not find the myopic shift value to be significant among our pseudophakic subjects. This could be due to the small number of pseudophakic eyes in our series.

Table 1. Demographic data

Demographic	**N (%)**
Age	Mean 53.21 (SD 13.84)
< 40	8 (21.1)
> 40	30 (78.9)
Gender	
Male	24 (63.2)
Female	14 (36.8)
Ethnicity	
Malay	18 (47.4)
Chinese	11 (28.9)
Indian	9 (23.7)
Laterality	
Right	15 (36.6)
Left	26 (63.4)
Indication for PPV	
Epiretinal membrane (ERM)	7 (17.1)
Macula hole (MH)	4 (9.8)
Mild vitreous haemorrhage	5 (12.2)
Rhegmatogenous retinal detachment (RRD)	2 (4.9)
Advanced diabetic eye disease (ADED)	23 (56.1)
Lens status	
Phakic	32 (78.0)
Pseudophakic	9 (22.0)
Tamponade agents used	
No tamponade	4 (9.8)
Gas	37 (90.2)
Air	23 (56.1)
Sulphur hexafluoride (SF6)	2 (14.3)
Perfluoropropane (C3F8)	12 (85.7)

PPV: pars plana vitrectomy; N: number of participants; SD: standard deviation

Table 2. Refractive changes before and after vitrectomy

Biometry parameters	Groups (N)	Before PPV Mean (SD)	After PPV Mean (SD)	P-value
SE#	Phakic (32)	-1.09 (2.40)	-2.08 (2.41)	**0.002**
	Pseudophakic (9)	-1.04 (1.17)	-1.16 (0.95)	0.808
	Overall (41)	-1.08 (2.18)	-1.88 (2.20)	0.003
dK* (astigmatism)	Phakic (32)	0.61 (0.84)	0.63 (0.67)	0.722
	Pseudophakic (9)	0.71 (0.33)	0.77 (0.26)	0.624
	Overall (41)	0.69 (0.69)	0.66 (0.60)	0.882
ACD*	Phakic (32)	3.39 (0.56)	3.34 (0.68)	0.216
	Pseudophakic (9)	4.86 (1.09)	3.94 (0.92)	**0.043**
	Overall (41)	3.55 (0.76)	3.44 (0.67)	**0.028**
AL*	Phakic, (32)	23.50 (2.06)	23.59 (1.95)	0.058
	Pseudophakic, (9)	22.98 (1.33)	23.38 (1.08)	0.183
	Overall, (41)	23.36 (1.42)	23.48 (1.56)	**0.029**

N: number of eyes; PPV: pars plana vitrectomy; SD: standard deviation; SE: spherical equivalent (diopters); dK: difference of keratometry, K2 - K1 (astigmatism; diopters); ACD: anterior chamber depth (mm); AL: axial length (mm); #paired t-test; *Wilcoxon signed rank test (Median (IQR)).

Anterior chamber depth

We reported a significant reduction of ACD post vitrectomy. Similar findings were described by Seo *et al.*[11] The study postulated reduction of zonule-vitreous adherence after surgery causing loosening of the zonules. This potentially resulted in anterior displacement of the lens-zonule diaphragm.[11] Suzuki *et al.* also described the possibility of more severe capsular contraction in post-vitrectomized eyes, which might result in the anterior shifting phenomena.[19] These postulations support our findings of prominent shallowing of ACD among pseudophakic eyes. ACD changes in phakic eyes were not significant; this could be due to better preservation of the peripheral vitreous around the zonules. Hence, less changes occur among these subjects.

Calik *et al.* studied the anterior segment parameters using a Pentacam Scheimpflug camera. The group reported reduction of ACD was related to the occurrence of supraciliary effusion early post-surgery.20 Unfortunately, their observation was only up to one month. Surgery potentially resulted in destruction of the blood-aqueous barrier, causing anatomical changes around the ciliary body. This could be another reason for the decreases in ACD and anterior chamber angular width.[21]

Axial length

Our study showed significant changes in overall AL. Brazitikos *et al.* found a small increment in the AL (0.1 mm) after vitrectomy.[3] Their reasoning for this result was scleral stretching and thinning after surgery.[22] However, other studies did not find significant changes in AL post retinal surgery.[10,11] Leydolt *et al.* reported IOP of 24 mmHg may cause a significant increment in AL of 0.023 mm.[23] The highest IOP in our study was documented as 20 mmHg preoperatively and 22 mmHg postoperatively. Therefore, it was unlikely for the IOP to contribute to the AL changes in our group of patients.

Astigmatism

Among our samples, astigmatism remained the same before and after surgery. This is consistent with previous studies, which described the presence of transient astigmatism. Measurement returned to baseline value at one to four months post-vitrectomy.[14-16] Vitrectomy induced a significant steepening in relation to sutured trocar entry ports.[15] After three months, the changes in scleral elasticity or loosening of the sutures allowed the cornea to flatten in the same meridian,[14] contributing to nonsignificant changes in astigmatism. Even with the usage of a 20-gauge cannula system, corneal topographic changes decreased at 3 months after surgery.[14]

Myopic shift

Our study suggests reduced ACD and increased AL contribute to the overall myopic

changes in our cases. However, reduction of ACD was insignificant among phakic eyes. These findings suggest myopic shifts in phakic eyes were not due to the changes in ocular biometry, but more likely due to the development of nuclear sclerosis cataract, which is very common post-surgery. Okamoto and colleagues reported myopic progression three months after vitrectomy was due to the development of nuclear sclerosis cataract.[24] At the same time, the development of cataract in post-vitrectomized eyes was significantly related to the patients' age.[24]

Further subgroup analysis of our small number of pseudophakic patients showed less significant changes in myopic shift despite decreasing ACD. Although the number of eyes was small, it may still suggest that the effect of anterior shifting of the lens-zonule diaphragm is negligible in pseudophakic patients.

Limitations of the study and study benefits

Limitations of our study were mainly due to the small number of pseudophakic eyes and lack of cataract progression monitoring. We did not look at changes of the crystalline lens as that was not part of the ocular biometry. Despite this limitation, we were able to provide important information on the biometric changes for small gauge vitrectomy surgery. To the best of our knowledge, this is the first study on refractive changes following 23-gauge PPV surgery among the Malaysian population.

Conclusion

In conclusion, there were significant myopic shifts in patients undergoing sutureless vitrectomy surgery among phakic eyes. Ocular biometry was more stable for phakic eyes compared to pseudophakic patients. The myopic shifts in phakic eyes were likely due to the refractive changes of the crystalline lens itself rather than the changes in biometry. Interestingly, despite shallower ACD post-surgery among pseudophakic eyes, myopic shift was not apparent.

Recommendation

Longer follow-up and larger number of pseudophakic eyes to be studied.

Acknowledgements

I would like to acknowledge the contribution made by Professor Dr. Mae-Lynn Catherine Bastion from Universiti Kebangsaan Malaysia and Associate Professor

Dr. Nilo Vincent DG. Flor Cruz from the Philippines who examined and edited the manuscript.

References

1. Sun Q, Sun T, Xu Y, et al. Primary vitrectomy versus scleral buckling for the treatment of rhegmatogenous retinal detachment: a meta-analysis of randomized controlled clinical trials. Curr Eye Res. 2012;37(6):492-499
2. Sharma YR, Karunanithi S, Azad RV, et al. Functional and anatomic outcome of scleral buckling versus primary vitrectomy in pseudophakic retinal detachment. Acta Ophthalmol Scand. 2005;83(3):293-297.
3. Brazitikos PD, Androudi S, Christen WG, et al. Primary pars versus scleral buckle surgery for the treatment of pseudophakic retinal detachment: a randomized clinical trial. Retina. 2005;25(8):957-964.
4. Stangos AN, Petropoulos IK, Brozou CG, et al. Pars-alone vs vitrectomy with scleral buckling for primary rhegmatogenous pseudophakic retinal detachment. Am J Ophthalmol. 2004;138(6):952-958.
5. Habibabadi HF, Hashemi H, Jalali KH, et al. Refractive outcome of silicone oil removal and intraocular lens implantation using laser interferometry. Retina. 2005;25(2):162-166.
6. Shioya M, Ogino N, Shinjo U. Change in postoperative refractive error when vitrectomy is added to intraocular lens implantation. J Cataract Refract Surg. 1997;23(8):1217-1220.
7. Byrne S, Ng J, Hildreth A, et al. Refractive change following pseudophakic vitrectomy. BMC Ophthalmol. 2008;8(1):19.
8. Tseng P-C, Woung L-C, Tseng G-L, et al. Refractive change after pars. Taiwan J Ophthalmol. 2012;2(1):18-21.
9. Hamoudi H, Kofod M, La Cour M. Refractive change after vitrectomy for epiretinal membrane in pseudophakic eyes. Acta Ophthalmol. 2013;91(5):434-436.
10. Huang C, Zhang T, Liu J, Tan R, Ji Q. Changes in axial length and anterior chamber depth after rhegmatogenous retinal detachment repair. J Clin Exp Ophthalmol. 2014;5(6):377.
11. Seo S, Seong MC, Lim HW, et al. The changes of anterior chamber depth, axial length, refractive errors after combined vitrectomy. J Korean Ophthalmol Soc. 2013;54(7):1032-1037.
12. Ünsal E, Eltutar K. Evaluation of the changes in anterior segment morphology with ultrasound biomicroscopy after vitrectomy without use of tamponade in pseudophakic eyes. Open J Ophthalmol. 2016;6(4):210-220.
13. Wang H, Chow SC. Sample size calculation for comparing means. In: D'Agostino RB, Sullivan L, Massaro J, ed. Wiley Encyclopedia of Clinical Trials. Hoboken, NJ: John Wiley and Sons, Inc; 2007.
14. Weinberger D, Lichter H, Loya N, et al. Corneal topographic changes after retinal and vitreous surgery. Ophthalmology. 1999;106(8):1521-1524.
15. Wirbelauer C, Hoerauf H, Roider J, et al. Corneal shape changes after pars. Graefes Arch Clin Exp Ophthalmol. 1998;236(11):822-828.
16. Mohamed AA, Abdrabbo M. Corneal topographic changes following trans-conjunctival 20 gauge sutureless vitrectomy (TC20V). Clin Ophthalmol. 2012;6:565-569.

17. Bullimore MA, Fusaro RE, Adams CW. The repeatability of automated and clinician refraction. Optom Vis Sci. 1998;75(8):617-622.
18. Allen PM, Radhakrishnan H, O'Leary DJ. Repeatability and validity of the power refractor and the Nidek AR600-A in an adult population with healthy eyes. Optom Vis Sci. 2003;80(3):245-251.
19. Suzuki Y, Sakuraba T, Mizutani H, Matsuhashi H, Nakazawa M. Postoperative refractive error after simultaneous vitrectomy and cataract surgery. Ophthalmic Surgery, Lasers and Imaging Retina. 2000;31(4):271-5.
20. Çalık B, Öztürk M, Serdaroğulları H, et al. Evaluation of anterior segment parameters using pentacam in silicone oil-injected patients after pars. Indian J Ophthalmol. 2013;61(11):621-625.
21. Park SP, Ahn JK, Lee GH. Morphologic changes in the anterior segment after phacovitrectomy for proliferative diabetic retinopathy. J Cataract Refract Surg. 2009;35(5):868-873.
22. Jeoung JW, Chung H, Yu HG. Factors influencing refractive outcomes after combined phacoemulsification and pars: results of a prospective study. J Cataract Refrac Surg. 2007;33(1):108-114.
23. Leydolt C, Findl O, Drexler W. Effects of change in intraocular pressure on axial eye length and lens position. Eye (Lond.). 2008;22(5):657-661.
24. Okamoto Y, Okamoto F, Hiraoka T, et al. Refractive changes after lens-sparing vitrectomy for rhegmatogenous retinal detachment. Am J Ophthalmol. 2014;158(3):544-549.

Malaysian Journal of Ophthalmology

© Malaysian journal of Ophthalmology 2019; 2:126-132
Brief Reports

A report on the postoperative outcome of MyIOL603YP implantation in Hospital Melaka

Khavigpriyaa Kalaichelvam, Lai Yin Peng, Norfadzillah Abdul Jalil, Raja Norliza Raja Omar

Department of Ophthalmology, Hospital Melaka, Ministry of Health, Malaysia

Abstract

This study reports the experience and outcomes of a new monofocal intraocular lens (IOL) by the name of MyIOL603YP. The performance of MyIOL603YP over a 2-month follow-up period from June to December 2016 was assessed.

Twenty-nine patients, one eye from each patient, were recruited by convenience sampling method and were evaluated prospectively. Patients who were eligible to receive welfare IOLs were chosen. Detailed, preoperative examination was done, and the surgical procedure was the same for all, which was phacoemulsification with MyIOL603YP implantation with random surgeon selection. Intraoperative complications were recorded by the surgeon. Postoperative examination was done during follow-up at one week and two months post cataract operation.

Results were obtained from 22 eyes (7 defaulted). Twenty eyes (84.7%) achieved an unaided postoperative visual acuity of 6/12 or better. There were no intraoperative or postoperative complications. There was no reported inflammation two months after cataract surgery with implantation of MyIOL603YP and no posterior capsule opacification was reported.

Keywords: acrylic, hydrophobic, monofocal, MyIOL603YP, preloaded, performance, visual outcomes

Correspondence: Khavigpriyaa Kalaichelvam, Department of Ophthalmology, Hospital Melaka, Jalan Mufti Haji Khalil, 75400 Melaka, Malaysia. E-mail: priya8702@yahoo.com

Laporan mengenai hasil pasca pembedahan implantasi MyIOL603YP di Hospital Melaka

Abstrak

Kajian ini melaporkan pengalaman dan hasil pembedahan katarak setelah implantasi kanta intraokular (IOL) monofokal rekaan baru yang bernama MyIOL603YP. Prestasi MyIOL603YP dalam tempoh susulan 2 bulan dari Jun hingga Disember 2016 telah dinilai.

Dua puluh sembilan pesakit, satu mata dari setiap pesakit, telah diambil oleh kaedah pensampelan mudah dan dinilai secara prospektif. Pesakit yang layak menerima IOL kebajikan dipilih. Pemeriksaan terperinci, pra pembedahan dilakukan, dan prosedur pembedahan adalah sama untuk semua, yang merupakan fakoemulsifikasi dengan implantasi MyIOL603YP dengan pemilihan pakar bedah secara rawak. Komplikasi intraoperatif direkodkan oleh pakar bedah. Pemeriksaan pasca pembedahan dilakukan semasa susulan pada satu minggu dan dua bulan selepas pembedahan katarak.

Keputusan diperoleh daripada 22 mata (7 gagal). Dua puluh mata (84.7%) mencapai tahap ketajaman penglihatan tanpa refraksi selepas pembedahan 6/12 atau lebih baik. Tiada komplikasi semasa atauselepas pembedahan direkodkan. Tidak ada keradangan yang dilaporkan dua bulan selepas pembedahan katarak dengan implantasi MyIOL603YP dan tiada kekeruhan pada kapsul posterior dilaporkan.

Kata kunci: akrilik, hidrofobik, monofokal, MyIOL603YP, pramuat, prestasi, hasil visual

Introduction

Cataract is defined as opacity within the clear lens of the eye. It is the major factor contributing to reversible blindness worldwide.[1] It affects around 18 million people around the globe; this number accounts for 51% of the population.[2] Based on the Malaysian National Eye Survey II 2014 population, cataract is the main cause of blindness (58%) and low vision (68%). Ministry of Health Malaysia (MOH) reported more than a two-fold raise in the number of cataract surgeries being performed from 2002 to 2011. Most MOH hospitals perform around 500 to 1000 cataract surgeries a year.[2] Recently, there has also been a shift in preference in the type of cataract surgery performed, from extracapsular cataract extraction (ECCE) to phacoemulsification. This popular method, which accounts for more than 50% of cataract surgeries, brings a parallel change to the preferred intraocular lens (IOL) type and materials used.[2]

With the new advancement of technology, new IOLs are being created with different designs and additional features. Biocompatibility of an IOL is affected by a few factors, mainly patient factors, surgeon factors, and the IOL itself.[3] The design and material of the IOL plays a major role in influencing the outcome, such as post-operative inflammation and posterior capsule opacification (PCO), which occurs in up to 50% of cases.[4] Nishi *et al.* found that lenses with square edges inhibited lens epithelial cell migration and PCO formation.[5] A study comparing different materials used with the same lens design supports the theory that hydrophobic acrylic IOLs lead to significantly less PCO in a two-year follow-up period compared to hydrophilic acrylic IOLs.[6] As for postoperative inflammation, an IOL with excellent biocompatibility, such as an acrylic lens, incites lower rates of cellular deposits, thus reducing inflammation.[7]

On the other hand, manufacturing details concerning folding, implantation, and affordability of the lens also are important factors to consider. In higher income countries, phacoemulsification followed by implantation of a foldable IOL is the procedure of choice. It provides better visual outcomes and less astigmatism due to the small incision.[8] However, a randomized controlled trial showed that implanting an inexpensive, rigid IOL after phacoemulsification gives comparable visual outcomes to the more expensive foldable IOL, providing surgeons are equally proficient at sclero-corneal tunnel and clear-corneal incisions.[9]

We report the initial experience and outcomes of the newly manufactured IOL, MyIOL603YP. It is a yellow, aspheric, equiconvex, hydrophobic, preloaded lens with a square-edge design and zero-degree angulation. It is made of acrylic material with an overall diameter of 12.5 mm and an optic diameter of 6.0 mm. The incision size is 2.4 mm. Its power ranges from 5.0 D to 30.0 D with 0.5 increments. Its refractive index is 1.4933 and the A-constant is 118.0/118.3. It can be implanted in the sulcus.

Materials and Methods

This is a prospective study to evaluate visual outcomes after cataract surgery with implantation of the MYIOL603YP over a two-month follow-up period from June to December 2016 in Hospital Melaka. Hospital Melaka is a secondary hospital situated on the West coast of Peninsular Malaysia.

This study enrolled 29 patients, 1 eye from each patient. Demographic information, pre- and postoperative visual acuity, diagnosis of unoperated eye, comorbidities in the operated eye, intra- and postoperative complications, postoperative inflammation, and postoperative formation of PCO were recorded.

Adult patients with visually significant cataracts were chosen via convenience sampling. Patients who were eligible to receive welfare IOL were chosen after being assessed by the social welfare department. Preoperative examination included vision, refraction, keratometry, slit-lamp biomicroscopy, tonometry, and detailed

fundus examination. Informed consent was obtained from all patients undergoing cataract surgery.

Biometry calculation was done using immersion (Alcon, Fort Worth, TX, USA) and IOL Master (Zeiss, Oberkochen, Germany). The surgical procedure was the same for all, which was phacoemulsification with MyIOL603YP implantation with random surgeon selection. Any intraoperative complications, specifically IOL-related complications, were recorded by the surgeon. Postoperative examination included vision testing, refraction, slit-lamp biomicroscopy, tonometry, and fundus examination. Follow-up was done at one week and two months post cataract operation. Subjects were evaluated for visual acuity and any postoperative complications during each follow up.

The performance endpoints were best spectacle-corrected visual acuity (BSCVA) and intraoperative and/or postoperative IOL-related complications. Results of the performance endpoints following implantation of MyIOL603YP were compared to the United States Food and Drug Administration (FDA) grid values of a historical population.[10] This study protocol was registered with the National Medical Research Register.

Results

Out of the 29 eyes that were recruited, 7 patients defaulted follow up; results were obtained from 22 eyes. Their ages ranged from 48-83 years with a mean age of 63 years. Of these 22 patients, 4 (18.2%) were male, and 18 (81.8%) were female. There

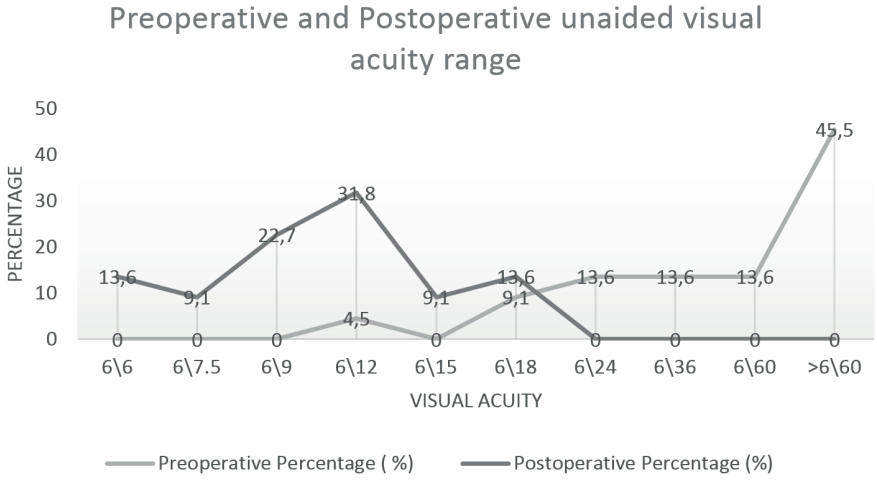


Fig. 1. Preoperative and Postoperative unaided visual acuity range

Table 1. Postoperative best spectacle-corrected visual acuity by age group

Age group	Total eyes	6/12 or better		
		Number of eyes	Distribution (%)	FDA grid (%)
< 60 years old	8	8	100.0	98.5
60-69 years old	9	8	88.9	96.5
70-79 years old	3	3	100.0	97.5
> 80 years old	2	1	50.0	94.8
Total	22	20	84.7	96.7

were six Malay, five Chinese, ten Indian, and one Eurasian patient. Preoperative vision of recruited patients ranged from 6/12 to worse than 6/60, with the major percentage being from vision worse than 6/60. Of the eyes recruited, 77% achieved unaided postoperative visual acuity of 6/12 or better (Fig. 1). Postoperative BSCVA was analysed by age group and compared with the FDA grid values of a historic population. Patients from age group < 60 years old and 70-79 years old yielded very satisfactory results. Two eyes did not achieve 6/12 or better vision postoperatively (Table 1). There were no intra- or postoperative complications. This results in a satisfactory outcome considering the small sample size of this study. There was no reported inflammation two months after cataract surgery with implantation of MyIOL603YP. PCO was not reported in any of the studied eyes at two months post cataract surgery with MyIOL603YP implantation.

Discussion

Cataract surgery is widely perceived to be a safe and successful procedure. Better visual outcomes and lower complication rates were reported with phacoemulsification compared to ECCE.[11]

In this study, it was found that the MyIOL603YP IOL presents satisfactory visual outcomes. This result was compared to the FDA grid values. The FDA has established the BSCVA values and adverse-event rates for a historical control population subjected to IOL implantation.

Considering the small study sample size in comparison to the clinical studies that yielded the FDA grid results, the visual outcomes exceed the FDA grid results for BSCVA in the age group < 60 years old and 70-79 years old. One patient from age group 60-69 years old had a BSCVA of 6/15 due to an underlying epiretinal membrane, and 1 patient from age group > 80 years old had a BSCVA of 6/15 due to ocular surface disorder. BSCVA in pooled subjects was 6/12 or better in 84.7% of eyes. This is lower than the expected value of FDA grid percentage, which is 96.7%.

However, a UK national cataract survey resulted in 86% with vision 6/12 or better.[12] According to the Swedish National Cataract Register, cataract surgery resulted in 84% with vision 6/12 or better.[13] There were no reported intra- or postoperative complications. This also exceeds the FDA grid values.

IOL biocompatibility can be divided into uveal (inflammatory cell attachment) and capsular (anterior capsular fibrosis, PCO). As for postoperative inflammation, only 1 patient had acute, postoperative inflammation of cells 2+ (mild) 1 week post surgery. Acute, postoperative inflammation is defined as an anterior chamber reaction equivalent to, or more than, 2+ cells, and flare or fibrin formation in the anterior chamber.[14] This later resolved at the two-month follow up with regular steroid therapy.

It was also observed that there was an absence of PCO until two months post operation. Wainsztein *et al.* observed in their study that mature cataracts had a significantly lower tendency to produce PCO than other cataract types.[15] Spalton, in his review article, demonstrated that PCO is the commonest complication of cataract surgery, occurring in up to 50% of patients 2-3 years after surgery.[16] Hydrophilic materials are generally considered to have a better uveal biocompatibility profile in comparison to hydrophobic materials, but with present IOL designs the latter may have a better performance in preventing PCO.[17,18]

Overall, this study indicates that MyIOL603YP preloaded, hydrophobic, acrylic IOL is generally efficacious and relatively safe based on an outcome observed at two months post operation.[1]

References

1. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. Br J Ophthalmol. 2012;96:614-618.
2. Lee MY, Goh PP, Salowi MA, Adnan TH, Ismail M. The Malaysian cataract surgery registry: cataract surgery practice pattern. Asia Pac J Ophthalmol (Phila). 2014;3(6):343-347.
3. Huang Q, Cheng GP, Chiu K, Wang GQ. Surface modification of intraocular lenses. Chin Med J (Engl). 2016;129(2):206-214.
4. Apple DJ, Solomon KD, Tetz MR, et al. Posterior capsule opacification. Surv Ophthalmol. 1992;37:73-116.
5. Nishi O, Nishi K, Wickström K. Preventing lens epithelial cell migration using intraocular lenses with sharp rectangular edges. J Cataract Refract Surg. 2000;26:1543-9.
6. Li Y, Wang J, Chen Z, Tang X. Effect of hydrophobic acrylic versus hydrophilic acrylic intraocular lens on posterior capsule opacification: meta-analysis. PLoS One. 2013;8(11):e77864.
7. Ravalico G , Baccara F, Lovisato A, Tognetto D. Postoperative cellular reaction on various intraocular lens materials. Ophthalmology. 1997;104(7):1084-1091.
8. Riaz Y, Mehta JS, Wormald R, et al. Surgical interventions for age-related cataract. Cochrane Database Syst Rev. 2006. doi:10.1002/14651858.CD001323.pub2.

9. Hennig A, Singh S, Winter I, Yorston D. Can phaco be a cost-effective solution to cataract blindness? Costs and outcomes in Nepal. Eye (Lond). 2010;24(6).
10. Stark WJ, Worthen DM, Holladay JT, et al. The FDA report on intraocular lenses. Aust J of Ophthalmol. 1984;12(1):61-69.
11. de Silva SR, Riaz Y, Evans JR. Phacoemulsification with posterior chamber intraocular lens versus extracapsular cataract extraction (ECCE) with posterior chamber intraocular lens for age-related cataract. Cochrane Database Syst Rev. 2014. doi:10.1002/14651858.CD008812.pub2.
12. Desai P, Minassian DC, Reidy A. National cataract surgery survey 1997-8: a report of the results of the clinical outcomes. Br J Ophthalmol. 1999;83:1336-1340.
13. Lundstrom M, Stenevi U, Thorburn W. The Swedish national cataract register: A 9-year review. Acta Ophthalmol Scand. 2002;80:248-257.
14. Mohammadpour M, Jafarinasab MR, Javadi MA. Outcomes of acute postoperative inflammation after cataract surgery. Eur J Ophthalmol. 2007;17(1):20-8.
15. Argento C, Nunez E, Wainsztein R. Incidence of postoperative posterior capsular opacification with types of senile cataracts. J Cataract Refract Surg. 1992;18(6):586-8.
16. Spalton DJ. Posterior capsular opacification after cataract surgery. Eye (Lond). 1999;(Pt 3b):489-492.
17. Hayashi K, Hayashi H. Posterior capsule opacification after implantation of a hydrogel intraocular lens. Br J Ophthalmol. 2004;88(2):182-5.
18. Hollick EJ, Spalton DJ, Ursell PG, Meacock WR, Barman SA, Boyce JF. Posterior capsular opacification with hydrogel, polymethylmethacrylate, and silicone intraocular lenses: two-year results of a randomized prospective trial. Am J Ophthalmol. 2000;129(5):577-84.

Malaysian Journal of Ophthalmology

© Malaysian journal of Ophthalmology 2019; 2:133-138
Case Report

Sweet disaster: use of honey-based eye drops as an alternative treatment for vernal keratoconjunctivitis

Kenneth Teow Kheng Leong[1,3], Rebecca Jennifer Mary Louis[1], Lai Yin Peng[2], Rohanah Alias[1], Safinaz Mohd Khialdin[3]

[1]Department of Ophthalmology, Hospital Kuala Lumpur, Kuala Lumpur, Malaysia; [2]Department of Ophthalmology, Hospital Sungai Buloh, Jalan Hospital, Selangor, Malaysia; [3]Department of Ophthalmology, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Abstract

A 16-year-old boy with underlying bronchial asthma, vernal keratoconjunctivitis, and bilateral eye steroid-induced glaucoma presented with right eye itchiness, redness, and progressive painful blurring of vision for the past 3 weeks. His mother had been treating him with honey-based eye drops purchased from an unregistered source/traditional healer. On presentation his right visual acuity was counting fingers, the cornea was hazy, and a small central stromal abscess with a large endothelial plaque was seen in the anterior chamber. He was treated for right eye fungal keratitis and subsequently admitted for intensive treatment. He showed marked improvement and was discharged. His final vision on follow-up was 6/15.

Keywords: Vernal keratoconjunctivitis, corneal ulcer, fungal, honey

Correspondence: Dr. Kenneth Teow Kheng Leong, Department of Ophthalmology, Hospital Kuala Lumpur, Kuala Lumpur, Malaysia.
Email: kennethteow@yahoo.com

Bencana akibat manis: penggunaan ubat mata berasaskan madu sebagai rawatan alternatif untuk keratokonjunktivitis vernal

Abstrak

Seorang budak lelaki berusia 16 tahun yang mempunyai latarbelakang penyakit asma bronkus, keratoconjunktivitis vernal, dan glaukoma yang disebabkan oleh steroid dirujuk dengan masalah gatal mata, kemerahan, dan kabur penglihatan serta sakit yang semakin teruk selama 3 minggu. Ibu beliau telah merawatnya dengan ubat titisan mata berasaskan madu yang dibeli dari sumber yang tidak berdaftar/pengamal perubatan tradisional. Ketajaman penglihatan pada kali pertama di hospital ialah menghitung jari, dan kornea kabur, dan nanah pada pusat stromal yang kecil dengan plak endothelial besar dilihat di ruang anterior. Dia dirawat untuk keratitis kulat mata kanan dan seterusnya dimasukkan ke wad untuk rawatan intensif. Beliau menunjukkan peningkatan yang ketara dan dibenarkan pulang. Ketajaman penglihatan terakhirnya pada tarikh susulan ialah 6/15.

Kata kunci: Keratoconjunctivitis Vernal, ulser kornea, kulat, madu

Introduction

Vernal keratoconjunctivitis (VKC) is a seasonally occurring allergic eye disease that typically affects young males. The common signs and symptoms are itching, photophobia, burning, tearing, giant papillae, superficial keratitis, and conjunctival hyperaemia. These patients frequently have a family or medical history of atopic diseases, such as asthma, rhinitis, and eczema.[1] Due to the chronicity of the disease and multiple relapses, some patients seek alternative treatment such as herbal and traditional medications to remedy their condition.

Objective

To report a case of fungal keratitis with underlying VKC.

Case description

A 16-year-old boy with underlying bronchial asthma, VKC, and bilateral eye steroid-induced glaucoma presented with right eye itchiness, redness, and progressive painful blurring of vision for the past 3 weeks. Further history revealed that his mother had been treating him with honey-based eye drops purchased from an unregistered source/traditional healer for the past month.

On examination, the right visual acuity was counting fingers. Anterior segment examination showed a hazy cornea, a small central stromal infiltrate, and a large endothelial plaque measuring 4 mm (H) x 2 mm (W) (Fig. 1). The anterior chamber activity was undetermined; however, it was deep and there was no satellite lesion or hypopyon seen. Corneal scraping was performed but the result was negative.

He was clinically diagnosed with right eye fungal keratitis and admitted for intensive eye drops. Guttae Fluconazole 0.02%, Guttae Natamycin 5%, Guttae Moxifloxacin 0.5%, and Guttae Gentamycin 0.9% were started hourly for the right eye. He was also treated with oral Fluconazole 200 mg daily and responded well after a week of treatment. The endothelial plaque had resolved and the stromal infiltrate was smaller. He was discharged with topical eye drops and reviewed a week later in clinic (Fig. 2). Topical eye drops were tapered down during each subsequent outpatient visit and his best vision had improved to 6/15.

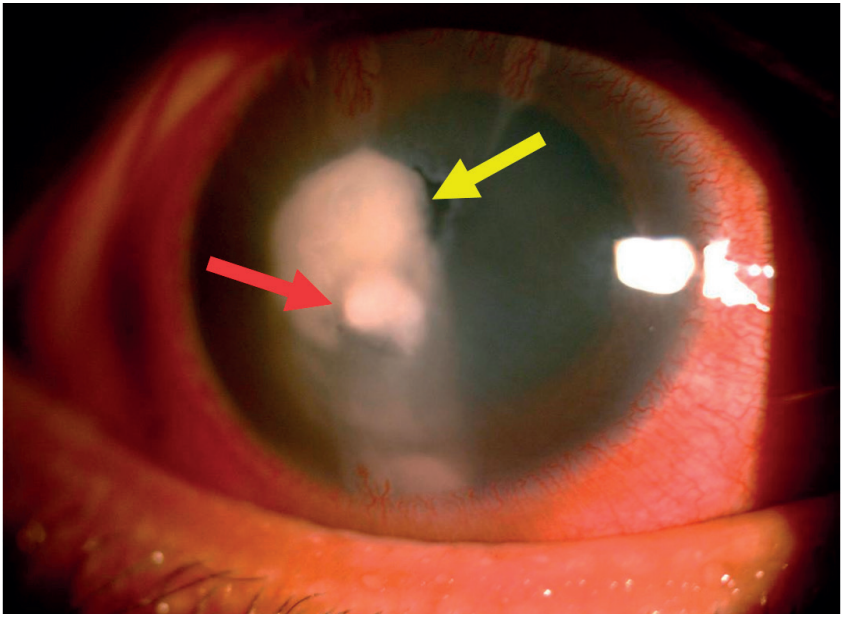


Fig. 1. Shows a fungal-like corneal ulcer with a small central stromal abscess (*red arrow*) and a large endothelial plaque in the anterior chamber (*yellow arrow*).

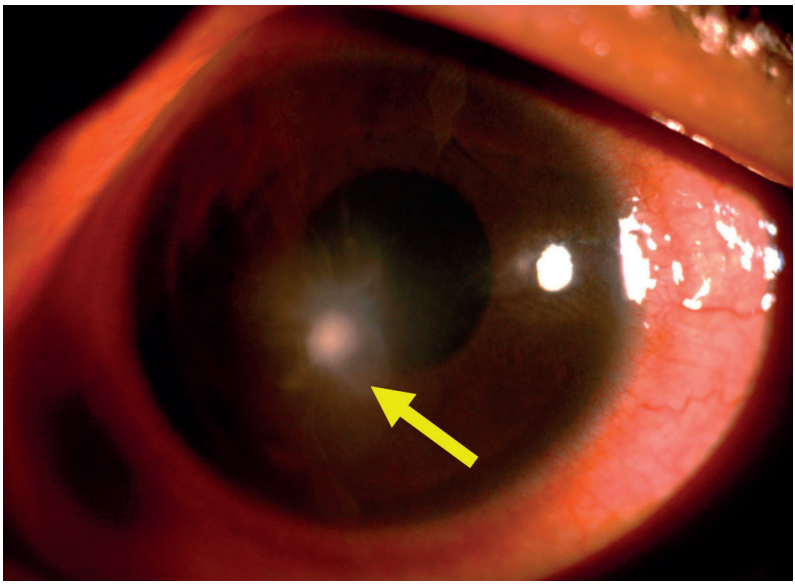


Fig. 2. Shows a healing corneal ulcer with mild scarring (*yellow arrow*).

Discussion

In VKC the therapeutic options are mainly topical eye drops, and they are selected based on the severity of the disease.[2] A vicious cycle of inflammation occurs as a result of reciprocal interactions between the conjunctiva and cornea, which results in damage to the corneal epithelium and stoma, leading to the formation of shield ulcers and plaques, infectious keratitis, keratoconus, scarring, and limbal stem cell deficiency.[3]

The aim of VKC treatment is to remove all possible allergens and suppress allergic inflammatory responses in the eye with the use of topical antihistamine and mast cell stabilizers. Corticosteroids are most effective; however, a quick tapering and use of low-absorption corticosteroids is preferred to avoid secondary development of glaucoma. Alternatively, immunomodulators such as cyclosporine may be considered as a steroid sparring option.[1] The long-term prognosis for patients is generally good; however, 6% of patients develop corneal damage, cataract, or glaucoma.[2]

This article explores the use of honey in traditional eye treatment. Is it a practice that should be discouraged or are there medicinal values and scientific benefits? Recent studies explored the use of honey as a complimentary treatment for corneal wounds and even VKC. A double-blinded study on patients with VKC showed a significant reduction in redness and limbal papillae following the application of the

honey drops when compared to placebo control group.[4] Widely researched are the Manuka and Tualang honeys among others, and they have been found to possess high antimicrobial and wound healing abilities.[5,6]

The antimicrobial activity is attributed to the presence of hydrogen peroxide producing enzymes, acidic pH of 3.2 - 4.5, high osmolarity, and phytochemicals, such as flavonoids and polyphenols, which may function as antioxidants.[5,7] Apart from this, honey maintains a moist wound environment, and its high viscosity helps form a protective barrier to prevent infection.[5] It also exhibits anti-biofilm, anti-inflammatory, and immunomodulatory properties.[5,8]

Corneal fibroblasts migrate and synthesize new extracellular matrix, while myofibroblasts are essential for wound contraction during healing. A study showed that growing corneal fibroblasts in honey-enriched media promotes faster migration and wound closure compared to controls.[6] Hydrogen peroxide provides adequate oxygen and nutrients to the injured tissue through angiogenesis.[6] Honey has been reported to lessen wound pH, reduce protease activity, increase fibroblast activity, and elevate oxygen release, which in turn facilitates wound healing.[6] Honey was also shown to reduce the expression of alpha smooth muscle actin (α-SMA) and increase the expression of lumican. This indicates potential reduction of corneal scarring and increased corneal transparency.[6]

Despite the benefits of honey, many studies have found a range of contaminants in honey, such as pesticides, heavy metals, microorganisms (bacteria and fungus), and many more.[7,9] Therefore, the application of honey as a topical eye drop should be thoroughly sterilized and processed to remove unwanted and harmful contaminants.

This patient most likely developed a shield ulcer due to poorly controlled VKC, subsequently complicated by a fungal infection derived from the homemade honey eye drops. The diagnosis of a fungal corneal ulcer was made on the basis of the characteristic appearance of the fungal infection. Corneal scraping is an important diagnostic tool; however, it may not always be positive and culture and sensitivity testing may take up to three weeks to yield any confirmation.[10] Therefore, good clinical judgment and prompt treatment are essential for a good visual outcome.

Conclusion

Honey-based eye drops have shown positive and promising results as an alternative or complementary treatment for corneal wounds, ulcers, and even VKC in the future. However, patients should be cautious when instilling unsterilized, honey-based eye drops from unapproved sources, otherwise it may do more harm than good.

References

1. Kraus CL. Vernal keratoconjunctivitis. External/Anterior Segment. [American Academy of Ophthalmology]. April 28, 2016. Available at: https://www.aao.org/disease-review/vernal-keratoconjunctivitis-5. Accessed May 8, 2019.
2. Bonini S, Coassin M, Aronni S, Lambiase A. Vernal keratoconjunctivitis. Eye (Lond). 2004;18(4):345–51.
3. Solomon A. Corneal complications of vernal keratoconjunctivitis. Curr Opin Allergy Clin Immunol. 2015;15(5):489–494.
4. Salehi A, Jabarzare S, Neurmohamadi M, Kheiri S, Rafieian-Kopaei M. A double-blind clinical trial on the efficacy of honey drop in vernal keratoconjunctivitis. Evid Based Complement Alternat Med. 2014;2014.
5. Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed. 2011;1(2):154–160.
6. Abd Ghafar N, Ker-Woon C, Hui CK, Mohd Yusof YA, Wan Ngah WZ. Acacia honey accelerates in vitro corneal ulcer wound healing model. BMC Complement Altern Med. 2016;16:259.
7. Olaitan PB, Adeleke OE, Ola IO. Honey: a reservoir for microorganisms and an inhibitory agent for microbes. Afr Health Sci. 2007;7(3):159–165.
8. Majtanova N, Vodrazkova E, Kurilova V, et al. Complementary treatment of contact lens-induced corneal ulcer using honey: a case report. Cont Lens Anterior Eye. 2015;38(1):61–63.
9. Al-Waili N, Salom K, Al-Ghamdi A, Ansari MJ. Antibiotic, pesticide, and microbial contaminants of honey: human health hazards. Scientific World Journal. 2012;2012.
10. Said DG, Otri M, Miri A, Kailasanathan A, Khatib T, Dua HS. The challenge of fungal keratitis. Br J Ophthalmol. 2011;95(12):1623–1624.

Malaysian Journal of Ophthalmology

© Malaysian journal of Ophthalmology 2019; 1:139-144
Case Report

Bilateral optic perineuritis associated with p-ANCA vasculitis

Ivan Cheng En Yoo, Yong Meng Hsien, Mushawiahti Binti Mustapha, Jemaima Che Hamzah

Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Cheras, Wilayah Persekutuan Kuala Lumpur, Malaysia

Abstract

A 69-year-old Chinese woman with underlying perinuclear anti-neutrophil cytoplasmic antibody associated with vasculitis (p-ANCA vasculitis) with no previous history of ocular involvement experienced one week of blurred vision in both eyes associated with pain. Ophthalmological evaluation demonstrated severe visual loss in both eyes, with pale optic discs but without other signs of ocular vasculitis. Magnetic resonance imaging (MRI) of the brain and orbit revealed bilateral enhancement of the optic nerve sheath with classical tram-track and doughnut signs. Intravenous methylprednisolone was given for five days and marked improvement of vision was seen.

In patients with p-ANCA vasculitis, bilateral optic perineuritis (OPN) is uncommon but can be one of the treatable causes to be considered, with good response to prompt steroid treatment.

Keywords: Bilateral optic perineuritis (OPN), Perinuclear anti-neutrophil cytoplasmic antibody associated vasculitis (p-ANCA vasculitis)

Correspondence: Dr. Yong Meng Hsien, Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000, Kuala Lumpur. E-mail: yongmenghsien@ppukm.ukm.edu.my

Perineuritis optik pada kedua-dua mata yang dikaitkan dengan vasculitis p-ANCA

Abstrak

Seorang wanita Cina berusia 69tahun yang telah didiagnosakan mendapat vaskulitis akibat antibodi perinuklear anti-neutrofil sitoplasma (vaskulitis p-ANCA) tanpa sebarang masalah okular sebelumnya,mengalami masalah penglihatan kedua-dua mata menjadi kabur dan kesakitan. Pemeriksaan oftalmologi menunjukkan kehilangan penglihtan yang teruk pada kedua-dua belah mata, dengan cakera optik yang pucat tetapi tanpa tanda-tanda vaskulitis okular yang lain. Pencitraan resonans magnetik (MRI) otak dan orbit mendedahkan peningkatan sarung saraf optik dengan tanda klasik; trek tram dan donat. Metiprednisolone intravena diberikan selama lima hari dan keadaan pulih dengan baik.

Kejadian perineuritis optik (OPN) pada kedua-dua belah mata adalah tidak lazim pada pesakit p-ANCA vaskulitis. Tetapi ianya boleh menjadi salah satu penyebab OPN yang boleh dirawat, dan bertindakbalas dengan baik terhadap rawatan steroid.

Kata kunci: perineuritis optik (OPN), perinuklear anti-neutrophil antibodi sitoplasmik yang berkaitan dengan vaskulitis (p-ANCA vaskulitis)

Introduction

Optic perineuritis (OPN) is a rare inflammatory disorder of the optic nerve sheath. It can be either isolated and idiopathic or manifested as part of a systemic inflammatory disease. Perinuclear anti-neutrophil cytoplasmic antibody associated vasculitis (p-ANCA vasculitis) on the other hand is a systemic inflammatory disease which mainly affects the small blood vessels. Common ocular manifestations are scleritis, uveitis, peripheral ulcerative keratitis, and other ocular inflammatory disease. We report a case of bilateral OPN associated with p-ANCA that responded well to systemic corticosteroids treatment.

Case presentation

A 69-year-old Chinese woman with underlying p-ANCA vasculitis without previous ocular involvement experienced blurring of vision in both eyes (OU) associated with eye pain for one week. Ophthalmological examination showed visual acuity (VA) of hand movement in the right eye (OD) and no perception of light in the left eye (OS) with sluggish pupil reflexes OU. Fundus examination revealed pale optic discs OU

with no inflammatory changes over the vessels, retina, or choroid. Ocular motility was normal, no ptosis or proptosis noted. Anterior segment examination was unremarkable with pseudophakic lens OU.

This lady was diagnosed with p-ANCA vasculitis in 2014 when she presented with peripheral neuropathy with erythematous papules on bilateral lower limbs. Blood tests showed raised erythrocyte sedimentation rate (ESR) and were p-ANCA positive. Skin punch biopsy was done and revealed leukocytoclastic vasculitis. Nerve conduction study showed diffuse axonal sensorimotor polyneuropathy. She was on oral prednisolone 40 mg once daily, commenced in April 2014, and azathioprine since May 2014. Her disease achieved remission in November 2014. However, she had multiple relapses and was having a disease flare-up during this presentation.

Laboratory findings were as follows: white blood cell (WBC; 14.2 x 109 g/L), ESR (120 mm/hour) and c-reactive protein (CRP; 25 mg/L) were raised. Serology for Treponema pallidum, antinuclear antibodies, C3/C4 complement level, anti double-stranded DNA, and chest X-ray with Mantoux test were all normal. Magnetic resonance imaging (MRI) of the brain and orbit showed that there was evidence of bilateral optic nerve sheath enhancement with classical “tram-track” (Fig. 1) and “doughnut” (Fig. 2) signs indicating active inflammation of the optic nerve sheath, without the involvement of other brain structures.

Fundus fluorescein angiography (FFA) was performed and showed no positive finding (Fig. 3). There was no FFA evidence of optic disc or posterior segment inflammation or anterior ischemic optic neuropathy. Subsequently, the patient was subjected to visual evoked potentials. Amplitudes of P100 were reduced OU, with OS (1.8 μV) more affected than OD (4.1 μV), which corresponds to the degree of visual impairment noted in respective eyes.

Diagnosis of bilateral OPN was made in view of the classical findings in the optic nerve imaging and negative FFA. The patient was co-managed with her neurologist for her recurrent systemic vasculitis leading to her OPN. Treatment with intravenous steroids was administered (methylprednisolone 250 mg QID for 5 days), then replaced with oral prednisolone 40 mg once daily for 1 month, then tapered by 5 mg every 2 weeks. Her VA improved during the first few days of intravenous steroid treatment and maintained at 6/18 OD and 6/36 OS in the subsequent follow-up. Despite the improvement in vision, her pale optic discs OU remained the same, suggesting it was an acute on chronic attack even though the patient was unable to recall any previous episode.

Discussion

OPN is a rare orbital inflammatory disorder which can be secondary to systemic inflammatory disease, in the case of our patient p-ANCA vasculitis. p-ANCA vasculitis

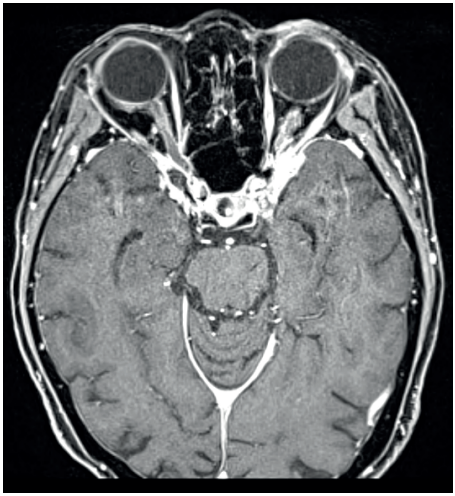


Fig. 1. "tram-track" sign (axial view; MRI T1W post-contrast).

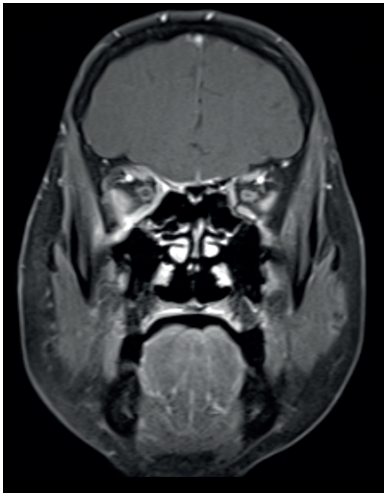


Fig. 2. "doughnut" sign (coronal view; MRI T1W post-contrast).

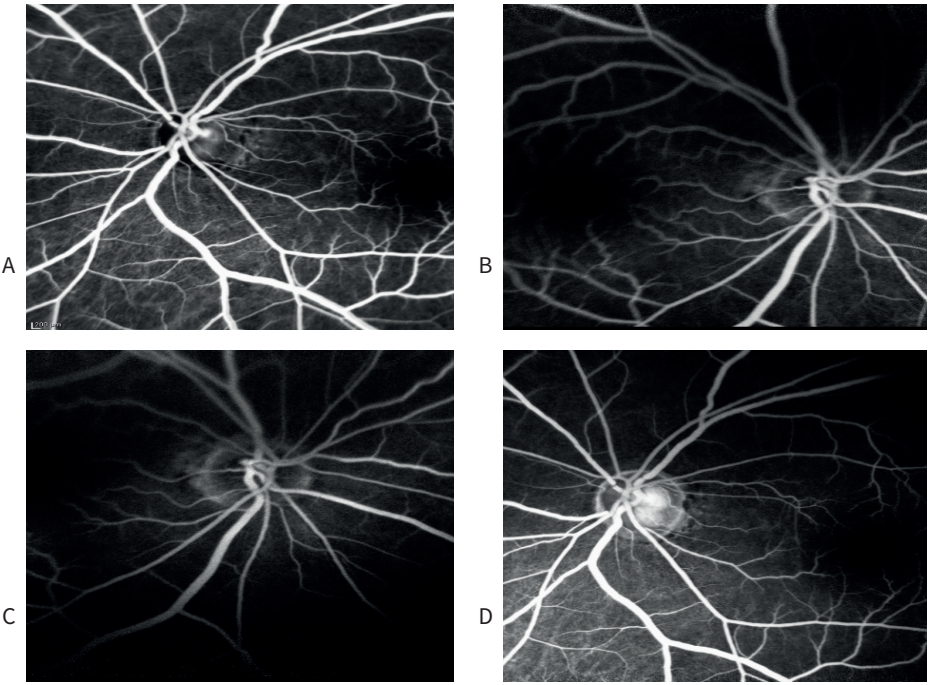


Fig. 3. Normal FFA OU. (*A*) RE at 1 min; (*B*) RE at 6 min; (*C*) LE at 1 min; (*D*) LE at 6 min.

is a systemic inflammatory disease which mainly affects the small blood vessels. There are two previous studies looking at the ocular manifestations of patients with systemic p-ANCA vasculitis (four cases each), which reported that systemic vasculitis predominantly affects the vascular components of the ocular tissues, *e.g.* scleritis, retinal vein occlusion, choroiditis, and ischemic optic neuropathy, in their case series. OPN on the other hand was not well documented as a potential complication of systemic vasculitis.[1,2] In cases of OPN, the optic nerve sheath is the main target of the inflammatory response, and reduction of VA is secondary to optic nerve compression by the inflamed and thickened optic nerve sheath.[3]

Subjects suffering from OPN commonly present with acute or subacute monocular visual loss, with eye pain exacerbated by ocular movement.[4] Bilateral visual loss is uncommon in OPN but should not be a surprise in cases of systemic involvement as in our patient. Visual field involvement in OPN can be variable; however, it tends to spare the central vision.[5] Brain and orbit MRI typically demonstrates pathological enhancement around the optic nerve with classical signs such as the "tram-track" sign on axial view and the "doughnut" sign on coronal view, and both are best depicted on MRI T1 post gadolinium.

It is important to investigate for other more common causes with similar presentation. Intracranial pathology with raised intracranial pressure or compressive optic neuropathy should be ruled out with brain and orbit MRI. Ischemic optic neuropathy usually presents as a painless condition without optic nerve sheath inflammation. Optic neuritis should be considered when an inflammatory process is involved, but optic neuritis commonly involves a younger age group, whereas OPN can be manifested in a broad age range.

The treatment option for both OPN and systemic vasculitis is a high dose and longer duration of corticosteroid, which can reduce the inflammation, minimize risk of recurrence, and prevent relapses.[6] The Optic Neuritis Treatment Trial demonstrated that intravenous pulse steroid may not affect the visual outcome in cases of optic neuritis. In contrast, in cases of OPN a delay in steroid treatment may cause irreversible visual loss.[7]

Conclusion

It is important to accurately diagnose OPN and initiate treatment early as there may be recurrence of visual loss, and in some cases irreversible loss of vision, unless treated with corticosteroids. OPN can be secondary to a systemic inflammatory disorder, in this case p-ANCA vasculitis. MRI plays a vital role in the diagnostic workup and subsequent management.

References

1. Matsuo T. Eye manifestations in patients with perinuclear antineutrophil cytoplasmic antibody-associated vasculitis: case series and literature review. Jpn J Ophthalmol. 2007;51(2):131-138.
2. Dandekar SS, Narendran NN, Edmunds B, Graham EM. Ocular involvement in systemic vasculitis associated with perinuclear antineutrophil cytoplasmic antibodies. Arch Ophthalmol. 2004;122(5):786-787.
3. Morotti A, Liberini P, Padovani A. Bilateral optic perineuritis as the presenting feature of giant cell arteritis. BMJ Case Rep. 2013. doi: 10.1136/bcr-2012-007959
4. Purvin V, Kawasaki A, Jacobson DM. Optic perineuritis: clinical and radiographic features. Arch Ophthalmol. 2001;119(9):1299-1306.
5. Hykin PG, Spalton DJ. Bilateral perineuritis of the optic nerves. J Neurol Neurosurg Psychiatry. 1991;54(4):375-376.
6. Tatsugawa M, Noma H, Mimura T, Funatsu H. High-dose steroid therapy for idiopathic optic perineuritis: a case series. J Med Case Rep. 2010;4:404.
7. Lai HW, Yang N, Ho CK. Optic perineuritis: an important differential diagnosis of optic neuritis. HK J Ophthalmol. 2013;17(1):11-17.

Malaysian Journal of Ophthalmology

© Malaysian journal of Ophthalmology 2019; 2:145-151
Case Report

The right eye abducens nerve palsy as a cranial neuropathy of dengue fever: The benefit of corticosteroids in an unusual dengue sequela

Mohd Khairul Bin Abd Majid, Umi Kalthum Md Noh, Safinaz Md Khialdin

Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Jalan Yaacob Latif, Kuala Lumpur, Malaysia

Abstract

Dengue fever is very common in tropical climate countries and the number of reported cases in Malaysia shows an increasing trend recently, according to the Malaysian Clinical Practice Guidelines. Although dengue fever is common, cranial nerve mononeuropathy is a very rare manifestation in relation to other neurological-associated syndromes. We report a rare case of cranial mononeuropathy of dengue fever in Malaysia and highlight the option of steroid usage as an alternative treatment to hasten the neurological recovery. The patient, a 25-year-old healthy policeman, presented with symptomatic viral fever, which was serologically confirmed as dengue fever. He developed acute-onset binocular diplopia, which was secondary to right eye isolated abducens nerve palsy during the critical phase of dengue fever. His visual acuity was 6/6 in both eyes with slightly restricted abduction of the right eye, consistent with right abducens nerve palsy, which was confirmed with a Hess test. There was corresponding diplopia over the right paracentral visual field. Urgent contrasted brain imaging was done, which ruled out the life-threatening intracranial pathology; therefore, a diagnosis of possible subclinical inflammatory changes causing sixth nerve palsy was made. Subsequently, he was treated with intravenous methylprednisolone 500 mg daily for 3 days and regained full extraocular muscle movement after 1 week. Oral steroid was not initiated. In conclusion, although the isolated unilateral cranial mononeurop-

Correspondence: Mohd Khairul Bin Abd Majid, Department of Ophthalmology, University Kebangsaan Malaysia Medical Centre (UKMMC), Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
E-mail: kerol_mkm@yahoo.com

athy may improve spontaneously within a certain period of time, a short course of systemic corticosteroids may be considered to hasten the recovery, as it has a favourable outcome.

Keywords: Dengue fever, abducens nerve palsy, sixth nerve palsy

Lumpuh saraf abdusen mata kanan sebagai neuropati kranial akibat demam denggi: Manfaat kortikosteroid dalam komplikasi denggi yang luar biasa

Abstrak

Demam denggi sangat biasa to sering terjadi di negara-negara iklim tropika dan jumlah kes dilaporkan di Malaysia menunjukkan peningkatan trend baru-baru ini, mengikut Garis Panduan Amalan Klinikal Malaysia. Demam denggi adalah penyakit yang sering ditemui tetapi mononeuropati saraf kranial adalah manifestasi yang jarang berlaku dikalangan pesakit denggi jika dibandingkan dengan manifestasi neurologi yang lain. Kami melaporkan kes mononeuropati kranial pada pesakit demam denggi di Malaysia dan menonjolkan penggunaan steroid sebagai rawatan alternatif untuk mempercepatkan pemulihan saraf. Pesakit, seorang anggota polis yang sihat berusia 25 tahun, mengalami gejala demam viral, yang disahkan secara serologi sebagai demam denggi. Dia kemudiannya mengalami diplopia binokular akut, yang merupakan gejala sekunder untuk to akibat kelumpuhan lumpuh saraf abdusen mata kanan, pada fasa kritikal demam denggi. Ketajaman visual to penglihatannya 6/6 pada kedua-dua mata dengan pergerakan mata yang sedikit terhad, konsisten dengan lumpuh saraf abdusen mata kanan, yang telah disahkan dengan ujian Hess. Terdapat diplopia yang sama di atas bidang visual paracentral to parasentral. Imbasan otak menggunakan kontras yang dilakukan secara kecemasan menolak kemungkinan terdapat patologi intrakranial yang mengancam nyawa. Diagnosis kelumpuhan saraf keenam disebabkan keradangan secara subklinikal telah dibuat. Seterusnya, ia dirawat dengan metilprednisolon intravena 500 mg setiap hari selama 3 hari dan pemulihan sepenuhnya pergerakan otot ekstraokular diperolehi selepas 1 minggu. Steroid oral tidak dimulakan. Kesimpulannya, walaupun mononeuropati kranial unilateral adalah kes terpencil tetapi boleh berlaku secara spontan dalam tempoh tertentu, kortikosteroid sistemik pada jangka pendek mungkin dipertimbangkan untuk mempercepatkan pemulihan, kerana ia menunjukkan hasil yangmemuaskan.

Kata kunci: Demam denggi, lumpuh saraf abdusens, lumpuh saraf kranial keenam

Introduction

Dengue fever is very common in tropical climate countries including Malaysia, where the number of reported dengue fever and dengue haemorrhagic fever cases shows an increasing trend recently, according to Malaysian Clinical Practice Guidelines.[1] Dengue-related ocular manifestations are more confined towards posterior segment involvement, which consist of maculopathy, retinal oedema, retinal haemorrhages, optic neuropathy, and vitritis.[2] Although dengue fever is common in tropical countries, cranial nerve mononeuropathy is a very rare manifestation in relation to other neurological-associated syndromes.[3]

Dengue-related ocular findings may cause visual disturbances, such as reduced vision, metamorphopsia, or cranial neuropathies involved in ocular muscle movements. We report a rare case of abducens nerve palsy secondary to dengue fever, which resulted in disabling diplopia. We highlight the importance of ruling out intracranial pathology and initiation of systemic corticosteroids to hasten the neurological recovery.

Case Report

A 25-year-old healthy policeman presented with a 3-day history of fever associated with maculopapular rash, vomiting, and abdominal pain. Clinically he was treated for dengue fever with warning signs, which was diagnosed in the febrile phase of dengue fever. Both dengue non-structural protein 1 (NS-1) antigen and dengue-specific immunoglobulin (IgG) results were positive. Subsequently, on day seven of the illness, he was noticed to have symptomatic acute-onset binocular diplopia associated with a slight pain on eye movement on right lateral gaze. There were no other ocular symptoms.

On examination, he had maculopapular rashes over the face, thorax, and both upper and lower limbs. Otherwise his vital signs were stable. His visual acuity was 6/6 in both eyes with slightly restricted abduction of the right eye, consistent with right sixth cranial nerve palsy, confirmed with a Hess test (Fig. 1). There was corresponding diplopia over the right paracentral visual field (Fig. 2). Relative afferent pupillary defect was negative and other ocular examinations, including dilated fundus assessment, were unremarkable. His ocular investigation included optical coherence tomography of both maculae, which was normal (Fig. 3).

The initial laboratory investigations revealed haemoconcentration, with haematocrit of 46% along with low white cell (2.6×10^9/L) and platelet (219×10^9/L) counts. His liver enzyme alanine transaminase (ALT) was elevated as high as

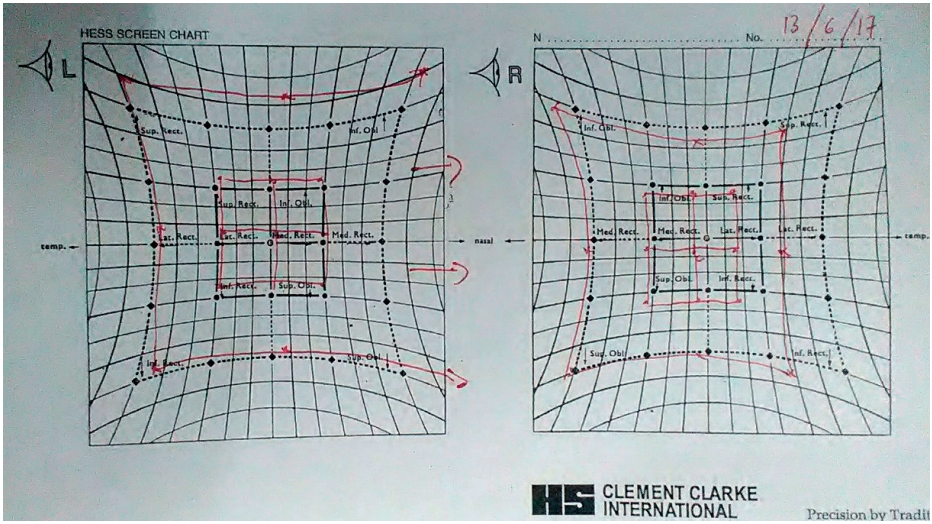


Fig. 1. Hess chart showing right lateral rectus underaction and left medial rectus overaction suggestive for right lateral rectus palsy on day seven of illness.

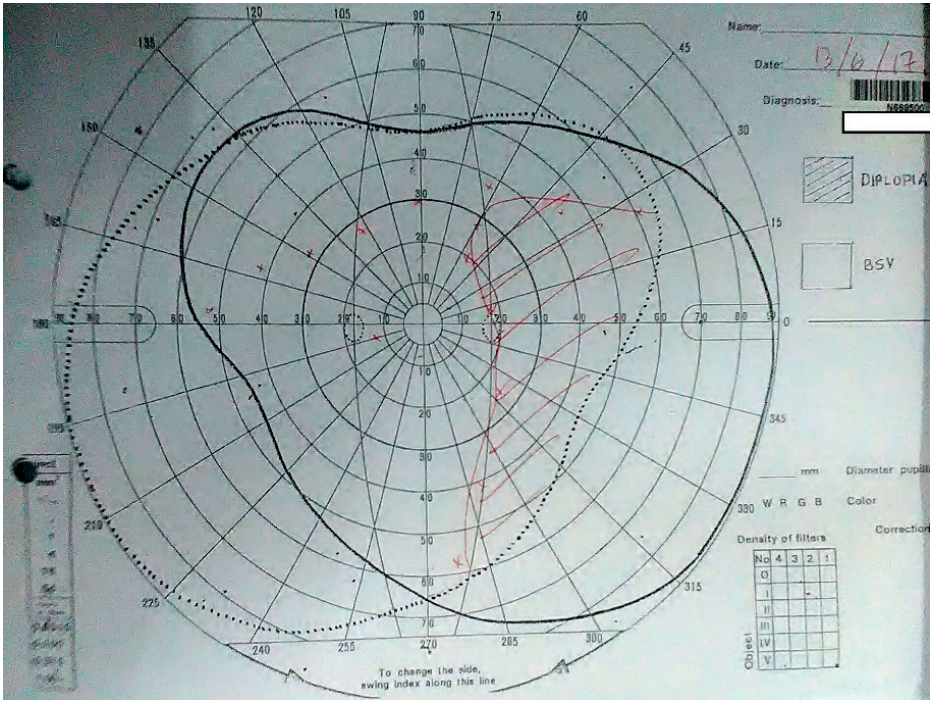


Fig. 2. Binocular single vision (BSV) showed diplopia over the right paracentral visual field.

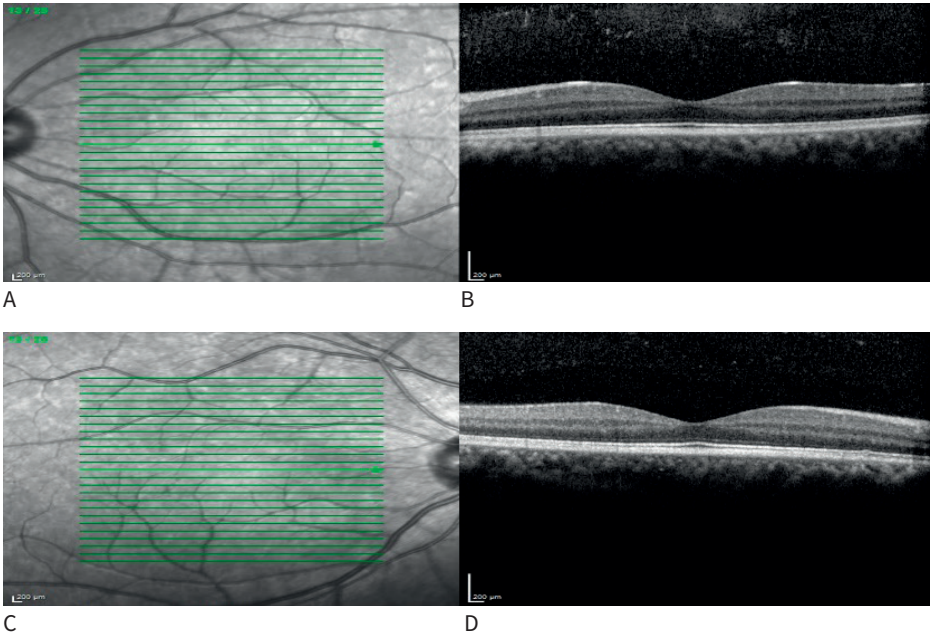


Fig. 3. Normal optical coherence tomography (OCT) of macula (OU). (*A*) OCT of left eye; (*B*) OCT macula, left eye fovea contour preserved; (*C*) OCT of right eye; and (*D*) OCT macula, right eye fovea contour preserved.

138 U/L, which indicates liver transaminitis secondary to dengue fever. His lowest white cell count was 2.6 x 10^9/L (febrile phase), his highest haematocrit was 53.3% (critical phase), and his lowest platelet count was 150 x 10^9/L (critical phase).

His renal profile and chest radiograph were normal. Contrast-enhanced computed tomography of the brain was performed to investigate the sudden development of abducens nerve palsy; the results of the test were normal.

The patient was managed by a multidisciplinary team during admission. He received initial dengue fever supportive treatment; intravenous fluid replacement was given to maintain his haemodynamic status. Pertaining to his ocular symptoms, a short course of intravenous methylprednisolone 500 mg daily for 3 days was initiated after the febrile phase of illness, to expedite his neurological recovery. Subsequently, he was discharged with improving diplopia after completion of the intravenous methylprednisolone course, and no transition to oral steroid was given. A week later, the patient was reviewed at the eye clinic with fully resolved diplopia and full eye movement.

Discussion

The prevalence of neurological-associated complications of dengue fever is between 0.5% and 5.4% in South East Asians.[2] The incident of paralytic squint secondary to abducens nerve palsy is still rare,[3] particularly in Malaysia; there has been only one case involving isolated right cranial nerve palsy, which was reported in 2016. In a previous literature review, other immune-mediated syndromes associated with dengue fever, including myelitis, acute disseminated encephalomyelitis, Guillain–Barré syndrome, and myositis, have been reported.[2]

The exact underlying pathomechanism is still uncertain, as the dengue virus has not been isolated in the cerebrospinal fluid of affected patients. The pathogenesis of ocular manifestation of dengue fever is believed to be due to cell-mediated immunity.[2] Francisco Javier *et al.*[4] reported that post-infectious syndromes, including mononeuropathy, have been linked to immune-related neurological syndromes. Dengue virus (DENV) infection is postulated to trigger cytokine overproduction, resulting in immune-mediated endothelial dysfunction, subsequently causing demyelinating types of conduction defects.

The dengue virus-2 (DENV-2) and dengue virus-3 (DENV-3) serotypes are the virus strains commonly associated with neurological manifestation.[2] Previous case reports of cranial mononeuropathy by Shivanthan *et al.*[3] and Mazliha *et al.*[5] do not describe any visual disturbances. Our patient presented with a sudden onset of binocular diplopia with no blurring of vision. Thus, we postulate that cranial mononeuropathy tends to occur in isolation.

Most of the reported cases that have been treated with intravenous methylprednisolone or oral prednisolone had a favourable outcome. Our patient, who was a previously healthy young man, presented with acute symptomatic binocular diplopia during the critical phase of dengue fever. Intravenous corticosteroid was initiated to expedite the recovery of the mononeuropathy. Clinically, we noticed the patient had a faster response and recovered early, which was comparable to previously reported cases by Shivanthan *et al.*[3] and Mazliha *et al.*;[5] their case reports showed symptoms only recovered after one and three months, respectively, without any steroid commencement. Even though the corticosteroid could reduce the underlying inflammatory process, we were not able to determine the exact factors that contributed to the response.

The prognosis for cranial mononeuropathy due to dengue fever is good, both if treated conservatively or by intravenous corticosteroid. Kristine *et al.*[6] concluded that the usage of steroids to expedite the recovery, as well as to prevent further structural damage, could justify the treatment choice unless it is contraindicated. It is important to be cautious when using high doses of steroids in acute viraemia in the febrile phase of dengue haemorrhagic fever and dengue shock syndrome, due to the possible risk of worsening of the dengue illness. This is because there is the possibility of a rise in viral replication as a result of the immunosuppressive

properties of steroids.[7] In our reported case, the intravenous corticosteroid was only initiated after the febrile phase of the illness with no other clinical contraindication, as to balance the benefit of the outcome.

In conclusion, isolated unilateral cranial mononeuropathy may improve spontaneously within a certain period of time. Monitoring of the disease activity is mandatory, with an option of systemic corticosteroid to be considered, as it has a favourable outcome.

References

1. Clinical practice guidelines: Management of dengue infection in adults. 3rd ed. Putrajaya, Malaysia: Malaysia Health Technology Assessment Section; 2015.
2. Carod-Artal FJ, Wichmann O, Farrar J, Gascón J. Neurological complications of dengue virus infection. Lancet Neurol. 2013;12(9):906-919.
3. Shivanthan MC, Ratnayake EC, Wijesiriwardena BC, Somaratna KC, Gamagedara LK. Paralytic squint due to abducens nerve palsy: a rare consequence of dengue fever. BMC Infect Dis. 2012;12:156.
4. Carod-Artal FJ. Neurological manifestations of dengue viral infection. Res Rep Trop Med. 2014;5:95-104.
5. Mazliha M, Boo YL, Chin PW. Isolated unilateral sixth cranial nerve palsy: A rare presentation of dengue fever. Malays Fam Physician. 2016;11(1);25-26.
6. Bacsal KE, Chee SP, Cheng CL, Flores JV. Dengue-associated maculopathy. Arch Ophthalmol. 2007;125(4):501-510.
7. Kularatne SA, Walathara C, Mahindawansa SI, et al. Efficacy of low dose dexamethasone in severe thrombocytopenia caused by dengue fever: a placebo controlled study. Postgrad Med J. 2009;85(1008):525-529.

www.myjo.org

Malaysian Journal of Ophthalmology