EVALUATION OF TEARS OXIDATIVE STRESS MARKERS IN MALAY AGE-RELATED MACULAR DEGENERATION PATIENTS

by

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DISCLAIMER

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

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ABSTRAK

Pengenalan:

Tekanan oksidatif dipercayai memainkan peranan dalam patogenesis degenerasi makula yang berkaitan dengan usia (ARMD). Tekanan oksidatif yang meningkat dan pertahanan antioksidan yang terjejas adalah faktor penyumbang kepada pembentukan dan perkembangan ARMD. Oleh itu, pengesanan tahap tekanan oksidatif dalam tisu okular mungkin memberi gambaran tentang peranan tekanan oksidatif dalam patogenesis ARMD.

Objektif:

Objektif kami adalah untuk membandingkan tahap oksidatif, iaitu *glutathione peroxidase*, *catalase* dan *malondialdehyde* dalam air mata di kalangan pesakit Melayu yang mengidap ARMD dan kumpulan kawalan. Kajian ini juga untuk membandingkan tahap purata tanda-tanda tekanan oksidatif antara keterukan ARMD yang berbeza.

Kaedah:

Kajian keratan rentas telah dijalankan antara September 2015 dan November 2017 di kalangan pesakit Melayu dengan diagnosis ARMD awal, ARMD lewat dan kumpulan kawalan. Kajian ini dijalankan di dua klinik mata hospital tertiari di Malaysia; Hospital Universiti Sains Malaysia dan Hospital Raja Permaisuri Bainun. Sampel air mata dikumpul dengan menggunakan kertas Schirmer. Analisis makmal dijalankan untuk menguji tahap *glutathione peroxidase*, *catalase* dan *malondialdehyde* dalam air mata dengan menggunakan komersial kit penanda oksidatif. Analisis statistik dilakukan dengan menggunakan Pakej Statistik untuk Sains Sosial (SPSS Inc Versi 20).

Keputusan:

Seramai 136 pesakit ARMD (ARMD awal: 68 pesakit, ARMD lewat: 68 pesakit) dan 68 pesakit kumpulan kawalan telah terlibat dalam kajian ini. Tahap purata *catalase* dan *glutathione peroxidase* jauh lebih rendah di kalangan pesakit ARMD [1348.97 (109.11) µM dan 453.87 (41.96) U/L masing-masing] berbanding dengan kumpulan kawalan [1453.38 (38.87) µM dan 502.28 (34.29) U/L masing-masing] sebelum ($P<0.001$ dan $P<0.001$) dan selepas ($P=0.001$ and $P<0.001$) diselaraskan untuk umur. Tiada perbezaan yang signifikan untuk tahap purata *malondialdehyde* antara ARMD dan kumpulan kawalan. Tahap purata *catalase* jauh lebih rendah dalam kumpulan ARMD lewat berbanding dengan kumpulan ARMD awal [1309.29 (112.47) µM vs 1388.06 (100.31) µM, $P=0.044$] dan selepas diselaraskan untuk umur ($P=0.029$). Tahap purata *catalase* jauh lebih tinggi dalam kumpulan *idiopathic polypoidal choroidal vasculopathy* (IPCV) jika dibandingkan dengan kumpulan ARMD neovaskular [1393.24 (53.12) µM vs 1267.27 (128.21) µM, $P=0.031$] di kalangan pesakit ARMD lewat dan selepas diselaraskan untuk umur ($P=0.027$).

Kesimpulan:

Kajian ini menunjukkan bahawa kapasiti antioksidan yang tidak mencukupi (tahap *catalase* dan *glutathione peroxidase* yang lebih rendah) mungkin memainkan peranan penting dalam ARMD. Tahap *catalase* menunjukkan perhubungan ketara dengan keterukan ARMD dan IPCV di kalangan pesakit ARMD lewat subjenis neovaskular selepas diselaraskan untuk umur.

ABSTRACT

Introduction:

Oxidative stress has been postulated to play a role in the pathogenesis of age-related macular degeneration (ARMD). The increased oxidative stress and impaired antioxidant defenses are some contributory factor to the initiation and progression of ARMD. Thus, detection of oxidative stress level in ocular tissue may perhaps provide insight into a role of oxidative stress in pathogenesis of ARMD.

Objective:

Our objective was to compare the oxidative stress level, namely glutathione peroxidase, catalase, and malondialdehyde in tears, between Malay ARMD patients and controls. This study also to compare the mean level of oxidative stress markers between different severities of ARMD.

Methods:

A cross sectional study was conducted between September 2015 and November 2017 involving Malay patients with confirmed diagnosis ARMD and controls, attending eye clinic of two tertiary hospitals in Malaysia; Hospital Universiti Sains Malaysia and Hospital Raja Permaisuri Bainun. Tear samples collected by using Schirmer paper. Laboratory analysis was performed to test on glutathione peroxidase, catalase and malondialdehyde level of tears using commercially available oxidative stress markers kits. Statistical analysis was done using Statistical Package for the Social Science (SPSS Inc Version 20).

Results:

A total of 136 ARMD patients (early ARMD: 68 patients, late ARMD: 68 patients) and 68 controls were included into the study. Mean catalase level and glutathione peroxidase level were significantly lower in ARMD patients [1348.97 (109.11) µM and 453.87 (41.96) U/L respectively] as compared to controls [1453.38 (38.87) µM and 502.28 (34.29) U/L respectively] before (P<0.001 and P<0.001) and after adjusted for age (P=0.001 and P<0.001). There was no significant difference for malondialdehyde level between ARMD and controls. Catalase level was significantly lower in late ARMD group as compared to early ARMD group [1309.29 (112.47) µM vs 1388.06 (100.31) µM, P=0.044] and also after adjusted for age (P=0.029). Among the subtypes of neovascular late ARMD, catalase level was significantly higher in idiopathic polypoidal choroidal vasculopathy (IPCV) group compared to neovascular ARMD [1393.24 (53.12) µM vs 1267.27 (128.21) µM, P=0.031] and also after adjusted for age (P=0.027).

Conclusion:

This study showed that insufficient antioxidant capacity (lower catalase and glutathione peroxidase level) may play an important role in pathogenesis of ARMD. Catalase level was significantly related to severity of ARMD and IPCV among the subtypes of neovascular late ARMD after adjusted for age.

CHAPTER 1

INTRODUCTION

1.1 Age-related Macular Degeneration

Age-related macular degeneration (ARMD) is the leading cause of irreversible central vision loss in developed countries. Risk of ARMD increases with age, especially 75 years and older, and in women. With the aging of populations, ARMD will become an increasingly prevalent and important condition worldwide. Estimates vary according to the exact definition of ARMD.

ARMD is the term applied to ageing changes without any other obvious precipitating cause that occur in the central area of the retina (macula) in people aged 55 years and above (Ferris *et al.*, 2013).

1.2 Classification of Age-related Macular Degeneration

There are a number of classification schemes for ARMD. The aim of these schemes is to provide a common nomenclature so that the prevalence of ARMD and its development over time can be compared between different studies often undertaken in widely differing geographical locations.

The main classification schemes share many similar features and are largely based on the Wisconsin Age-Related Maculopathy Grading Scheme (WARMGS) (Klein *et al.*, 1991). This grading system is based on the presence and severity of the characteristic features of ARMD namely drusen, pigmentary irregularities, geographic atrophy and neovascularisation. The WARMGS has been in use for over 2 decades and owing to its complexity and multiple scales attempts have been made to simplify it for use in both research and clinical situations. The first attempt to undertake this was in the mid-nineties when a consensus group met and developed the early age-related maculopathy (ARM) international classification system (Bird *et al.*, 1995). This system attempted to distinguish the early features of macular ageing namely drusen

and pigmentary irregularities from the late features of geographic atrophy and choroidal neovascularization by using the term ARM to signify only early disease. This has now come to be known as early ARMD.

In this study, Wisconsin Age-Related Maculopathy Grading Scheme (WARMGS) was used. This grading system classified ARMD into early ARMD and late ARMD.

1.2.1 Early Age Related Macular Degeneration

Features of early ARMD include:

- Soft drusen ≥ 63 μm (drusen are discrete lesions consisting of lipids and protein deposited under the retina)
- Areas of increased pigment or hyperpigmentation (in the outer retina or choroid) associated with drusen
- Areas of depigmentation or hypopigmentation of the retinal pigment epithelium (RPE)

1.2.2 Late Age Related Macular Degeneration

Late ARMD is another term used for the late stages namely geographic atrophy or neovascular ARMD.

Geographic atrophy is a sharply demarcated area of partial or complete depigmentation reflecting atrophy of the retinal pigment epithelium (RPE). The margins of the de-pigmented area are usually scalloped and the large choroidal vessels are visible through the atrophic RPE.

Neovascular ARMD is also termed exudative ARMD. In the vast majority of eyes with neovascular disease, new blood vessels that have their origin from the choroid known as

choroidal neovascularization (CNV) are seen. CNV breaches the normal anatomical barrier of Bruch's membrane and invades the subpigment epithelial and or subretinal spaces.

There are three subtypes of neovascular ARMD, namely typical ARMD, idiopathic polypoidal choroidal vasculopathy (IPCV) and retinal angiomatous proliferation. IPCV is considered a variant of neovascular ARMD. It consists of neovascularisation, primarily located within the choroid (Ciardella *et al.*, 2004; Imamura *et al.*, 2010).

1.3 Pathogenesis of Age-related Macular Degeneration

The pathogenesis of ARMD is likely to be multifactorial. It involves a complex interaction of genetic, metabolic, functional and environmental factors, which remains poorly understood (Wiktorowska-Owczarek and Nowak, 2010).

In early ARMD, lipid material accumulates as deposits beneath the RPE and within Bruch's membrane. When focal collections of lipid material are present these are referred to as drusen and can be seen as pale yellow deposits on a clinical examination of the retina. The RPE also undergoes morphological alteration seen clinically as areas of hyperpigmentation and hypopigmentation. Generally drusen and RPE irregularities are not associated with disturbances of central visual function. A proportion of people (12.9 and 17.8% respectively) (Klein *et al.*, 2007) with these early changes will progress to advanced ARMD. When vision loss occurs, it is usually due to the development of geographic atrophy and/or exudative disease.

Late ARMD is either characterized by geographic atrophy or neovascular ARMD. Geographic atrophy is recognized as a sharply defined area in the posterior pole, with atrophy of the RPE, the overlying photoreceptors and the choriocapillaris. The defect in structures allows the

observer to see the larger underlying choroidal vessels (Schmitz-Valckenberg *et al.*, 2011). The progression rate varies, but is relatively slowly progressing over years. As the atrophic area expands, visual function decreases (Holz *et al.*, 2004). Clinically, the geographic atrophy and neovascular ARMD are very different, but these late stages of ARMD are far from exclusive. Individuals with geographic atrophy are in high risk of developing CNV, and patients with neovascular ARMD are in high risk of developing atrophic areas.

In neovascular ARMD, abnormal blood vessels grow underneath the retina. These vessels can leak fluid and blood, which may lead to swelling and damage of the macula. The damage may be rapid and severe, unlike the more gradual course of geographic atrophy. It is possible to have both geographic atrophy and neovascular ARMD in the same eye, and either condition can appear first.

1.4 Oxidative Stress Markers

Numerous studies have shown reactive oxygen species are among the contributing factors to the development of ARMD (Ito *et al.*, 1995; Beatty *et al.*, 2000). The increased oxidative stress and impaired antioxidant defenses are some contributory factor to the initiation and progression of ARMD.

Oxidative stress may cause injury to the RPE, the Bruch's membrane, and the choroid, which are layers in the eye involved in the pathophysiology of ARMD (Anderson *et al.*, 2002; Johnson *et al.*, 2000; Mullins *et al.*, 2000). The retina is particularly susceptible to oxidative stress due to its high concentration of oxygen, its high proportion of polyunsaturated fatty acids, and its exposure to visible light (Beatty *et al.*, 2000). Prior reports have suggested that the retina is susceptible to lipid peroxidation and that this susceptibility also increases with aging in the macular region (Ito *et al.*, 1995).

Several ocular degenerative disorders have been studied, and the presence of oxidative stress has been demonstrated through markers of lipid peroxidation, the activity of antioxidant enzymes, and the levels of low-molecular-weight antioxidants (Halliwell and Gutteridge, 2015).

There are two type of oxidative stress; reactive oxygen species (e.g. malondialdehyde) and reactive nitrogen species (e.g. nitric oxide). The major antioxidants are superoxide dismutase, catalase and glutathione peroxidase. Among the oxidative stress markers that we used in this study are catalase and glutathione peroxidase (antioxidant enzyme) and malondialdehyde (reactive oxygen species).

1.4.1 Malondialdehyde

Malondialdehyde is a three-carbon, low-molecular weight aldehyde that can be produced by different mechanisms. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde.

A malondialdehyde formation route is described in Figure 1. The target of reactive species is the carbon-carbon double bond of polyunsaturated fatty acids (I). This double bond weakens the carbon-hydrogen bond, allowing easy abstraction of the hydrogen by a free radical. Then, a free radical can abstract the hydrogen atom and a lipid free radical is formed (II), which suffers oxidation generating a peroxyl radical (III). The peroxyl radical can react with other polyunsaturated fatty acids, abstracting an electron and producing a lipid hydroperoxide (IV) and another lipid free radical. This process can be propagated continually in a chain reaction. The lipid hydroperoxide is unstable and its fragmentation yields products such as malondialdehyde (V) and 4-hydroxy-2-nonenal (Grotto *et al.*, 2009).

Malondialdehyde is a reactive aldehyde and is one of the many *reactive electrophile species* that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products (ALE), in analogy to advanced glycation end-products (AGE) (Farmer and Davoine, 2007). The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism.

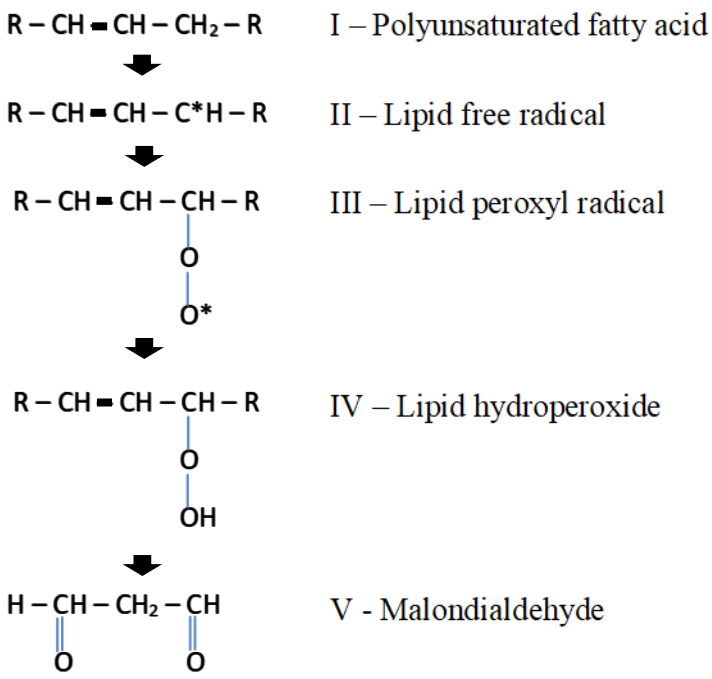

Figure 1. Schematic steps of malondialdehyde formation from polyunsaturated fatty acids

1.4.2 Glutathione Peroxidase

Glutathione peroxidase is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative stress. It provides a mechanism for detoxification of peroxides in living cells. This reaction plays a crucial role in protecting cells from damage by free radicals, which are formed by peroxide decomposition.

Lipid component of the cell are especially susceptible to reactions with free radicals, resulting in lipid peroxidation.

The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water, thus preventing formation of free radicals. The main reaction that glutathione peroxidase catalyzes is:

$$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$$

where GSH represents reduced monomeric glutathione, and GS–SG represents glutathione disulfide. The mechanism involves oxidation of the selenol of a selenocysteine residue by hydrogen peroxide. This process gives the derivative with a selenenic acid (RSeOH) group. The selenenic acid is then converted back to the selenol by a two step process that begins with reaction with GSH to form the GS-SeR and water. A second GSH molecule reduces the GS-SeR intermediate back to the selenol, releasing GS-SG as the by-product.

A simplified representation is shown below (Bhabak and Mugesh, 2010):

$$RSeH + H_2O_2 \rightarrow RSeOH + H_2O$$

$$RSeOH + GSH \rightarrow GS\text{-}SeR + H_2O$$

$$GS\text{-}SeR + GSH \rightarrow GS\text{-}SG + RseH$$

1.4.3 Catalase

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al.*, 2004). It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species.

Hydrogen peroxide is a harmful byproduct of many normal metabolic processes; to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules (Gaetani *et al.*, 1996).

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long (Boon *et al.*, 2007). It contains four iron-containing heme groups that allow the enzyme to react with the hydrogen peroxide. Catalase catalyze the oxidation, by hydrogen peroxide, of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols.

1.5 Detection of Oxidative Stress in Ocular Tissue

Metabolism in the eye is of increasing interest because the organ is highly susceptible to damage by sunlight, oxygen, various chemicals and pollutants. Oxidative stress mechanism in ocular tissues has been associated with a wide variety of disease such as ocular surface disease, cataract, glaucoma, uveitis, retinopathies and ARMD.

It is generally believed that ARMD is caused by numerous biochemical, immunogenic, and environmental factors (Brantley *et al.*, 2012; Delcourt *et al.*, 1999; Ding *et al.*, 2009). The most recent studies point to the key role of oxidative stress in the pathogenesis of ARMD (Brantley *et al.*, 2012; Khandhadia *et al.*, 2014; Tokarz *et al.*, 2013). Since oxidative stress involves almost all other assumptive pathogeneses and almost all risk factors for ARMD, it could be crucial for the initiation and progression of the disease. Excessive generation of free radicals and other reactive oxygen species and imbalance between their generation and the possibility

of their degradation by the antioxidant defense system seem to be the most responsible factor in the development of ARMD (Tokarz *et al.*, 2013).

The retina, particularly macula, is the ideal environment for the generation of reactive oxidative species due to the high oxygen consummation (because of its high metabolic activity) (Yu and Cringle, 2001), lifelong exposure to light irradiation (Youssef *et al.*, 2011), and abundance of photosensitizers in photoreceptors and RPE cells.

The retina possesses a substantial number of antioxidants in the photoreceptor and RPE cells (especially in the area of the macula) (Tokarz *et al.*, 2013). The consequences of oxidative damage on photoreceptors and RPE cells are severe because they are non replicating (postmitotic) cells and must survive a lifetime of oxidative insults (Khandhadia *et al.*, 2014). The disorder occurs when the antioxidant system can no longer compensate the cumulative oxidative damage. Antioxidant defense includes enzymes: superoxide dismutase, glutathione peroxidase, and catalase; nonenzymatic antioxidants (as glutathione, uric acid, albumin, and bilirubin); and the antioxidant micronutrients (vitamin C, vitamin E, and carotenoids) (Curran-Celentano *et al.*, 2001; Halliwell and Gutteridge, 2015).

1.5.1 Tears Film

Ocular surface of eye is as an inert barrier against infection. Tear film is a complex mixture consisting of protein, enzymes, lipids, electrolytes and metabolites. Oxygen radical formation has been measured before in human tears by using reagent of xanthine, xanthine oxidase, human milk lactoferrin, bovin serum albumin, superoxide dismutase and catalase (Kuizenga *et al.*, 1987). Study by Kuizenga et al. (1987) described that human tears, under the in-vitro conditions do not contain detectable scavengers of superoxide (O^{2-}) and hydrogen peroxide (H_2O_2) but can inhibit OH* formation. They postulated that human tears played an important role in protecting the outer parts of the eye against OH*-induced tissue damage. Besides,

literature review proved that other type of antioxidants such as cysteine, glutathione, urate and ascorbic acid presence in human tears (Frei, 1991). However, ascorbic acid was proved to be a good antioxidant by completely against peroxidative damage by lipid peroxidation (Choy *et al.*, 2001). The ascorbic acid concentration and total antioxidant capacity, ferric reducing ability of plasma (FRAP) were 3-4 folds higher in plasma than tears (Choy *et al.*, 2003). There were limited literature reviews on the normal level of antioxidants in aqueous humour and tears.

1.5.2 Collection of Tears

There was a cross-sectional study done on 383 adult patients seeking for primary health care to evaluate whether tears could be used as tool for health screening (Quah *et al.*, 2014). In this study, the pain score was elicited and compared between tear collection using schirmer strips, previous experience of antecubital venous puncture and finger prick test. They found that the pain score for schirmer tear collection was significantly lower than pain score for antecubital venous puncture. In addition, 70% agreed for their tears being collected to screen for eye problems (Quah *et al.*, 2014). Therefore, to collect the human tear by using schirmer strip is less invasive and less painful as compared to plasma collection.

Glass capillary tube is another method for tears collection. The capillary tube is rested in the lateral tears meniscus and minimizes contact with bulbar conjunctiva (Lam *et al.*, 2014). Tears are immediately collected by capillary tube. The average protein concentration obtained by the microcapillary tube showed the values was lower than the tears collection by schirmer strip (Farias *et al.*, 2013). However, Choy *et al* suggested that capillary tube for human tears collection was less invasive as compared with schirmer strip (Choy *et al.*, 2001). Human tears are relatively easier to be collected simultaneously from eyes by using schirmer' strip and it's convenient as well.

Rationale of the study

Oxidative stress has been postulated to play a role in the pathogenesis of ARMD. Previous studies evaluating the antioxidant levels or oxidative stress in the serum of ARMD patients have reported conflicting results (Mrowicka *et al.*, 2017; Nath *et al.*, 2017; Plestina-Borjan *et al.*, 2015). This may be due to the fact that serum levels of oxidative stress may be affected by the presence of systemic illness (Haskins *et al.*, 2003; Keaney *et al.*, 2003; Mezzetti *et al.*, 1996; Shah *et al.*, 2014). Identification of the serum oxidative stress markers may be difficult due to the relatively small size of affected tissue such as retina and amount of the biomarker released in the large circulating blood volume (Nath *et al.*, 2017). Thus, identification and quantification of serum oxidative stress marker in ocular disease may needs highly sensitive assay systems.

Quantification of oxidative stress level in the ocular tissue, i.e. the aqueous or vitreous, may be more representative of ARMD. This is because oxidative stress levels in vitreous or aqueous more proximity to the retinal itself and better reflecting the pathophysiological process. However, measurement of aqueous or vitreous antioxidant levels is relatively invasive, as it requires intracameral or intravitreal tapping, with all the antecedent risks of surgery complication. Thus, aqueous and vitreous collection is only ethically possible if performed during surgical intervention such as cataract and vitreoretinal surgery. Study by Atalla et al 1987 showed that abundance of antioxidant enzymes were found in epithelial structures of the eye, such as cornea epithelium, ciliary epithelium, lens epithelium and retinal pigment epithelium (Atalla et al., 1987). Antioxidant level in tears may be a reflection of the antioxidant level in cornea epithelium because cornea epithelium is directly in contact with tears and is continuously debrided into tears due to aging process. Similarly, antioxidant level in vitreous may be closely similar to antioxidant level in retina. Increased oxidative stress has been reported in the vitreous fluid in proliferative diabetic retinopathy patients, and oxidative stress

has been speculated to be related to retinal cell damage in such patients (Mancino et al., 2011). Thus, reduced antioxidant capacity in tears may reflect the reduced antioxidant capacity in vitreous as well.

Antioxidant levels in tears have been shown to reflect that of the aqueous fluid. Study conducted by Horwath-Winter et al (2009) to determine uric acid concentrations in tear fluid and aqueous humour and their contributions to the antioxidative potential of these eye fluids. This study showed that uric acid was found in quite similar amounts in tear fluid and aqueous humour (Horwath‐Winter *et al.*, 2009). Detection of antioxidant levels in tears may thus be a less invasive, safe and reliable method of assessing the oxidative stress level.

There is a strong biological correlation between oxidative stress, and progression of ARMD. Previous studies have assessed the concentrations of antioxidants such as ascorbic acid, catalase, and superoxide dismutase in tears of normal patients and other diseases, but there have been no previous studies on oxidative stress levels in the tears of ARMD patients (Choy *et al.*, 2000; Gus *et al.*, 2006; Jee *et al.*, 2014). Among ARMD patients, the levels of glutathione peroxidase, catalase and malondialdehyde have been measured only in the serum plasma (Mrowicka *et al.*, 2017; Nath *et al.*, 2017; Plestina-Borjan *et al.*, 2015). Glutathione peroxidase and catalase are common antioxidants in the human body, while malondialdehyde is an end product of lipid peroxidation. However, no study has assessed the levels of these antioxidants in the tears of ARMD patients or healthy controls. Thus, our study aims to quantify glutathione peroxidase, catalase and malondialdehyde level in the tears of ARMD patients, which may help in developing a predictable biomarker for strategizing therapeutic modalities based on the underlying pathology.

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CHAPTER 2

OBJECTIVES OF THE STUDY

2.0 STUDY OBJECTIVES

2.1 General Objectives

To evaluate the tears oxidative stress markers in Malay ARMD patients.

2.2 Specific Objectives

2.2.1 To compare the mean level of tears oxidative stress markers (catalase, malondialdehyde and glutathione peroxidase) between ARMD patients and control group.

2.2.2 To compare the mean level of oxidative stress markers between the severity of ARMD (early and late).

2.2.3 To compare the mean level of oxidative stress markers between the subtypes of neovascular late ARMD (neovascular ARMD and IPCV) among late ARMD.

CHAPTER 3

MANUSCRIPT

EVALUATION OF TEARS OXIDATIVE STRESS MARKERS IN MALAY AGE-RELATED MACULAR DEGENERATION PATIENTS

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3.1 ABSTRACT

Background:

Oxidative stress has been postulated to play a role in the pathogenesis of age-related macular degeneration (ARMD). Thus, detection of oxidative stress level in ocular tissue may perhaps provide insight into a role of oxidative stress in pathogenesis of ARMD. The aim of our study was to evaluate the level of oxidative stress markers, namely glutathione peroxidase, catalase, and malondialdehyde in tears between ARMD patients and controls.

Methods:

A cross sectional study was conducted between September 2015 and November 2017 involving Malay patients with confirmed diagnosis ARMD and controls, attending eye clinic of two tertiary hospitals in Malaysia; Hospital Universiti Sains Malaysia and Hospital Raja Permaisuri Bainun. Tear samples collected by using Schirmer paper. Laboratory analysis was performed to test on glutathione peroxidase, catalase and malondialdehyde level of tears using commercially available oxidative stress markers kits. Statistical analysis was done using Statistical Package for the Social Science (SPSS Inc Version 20).

Results:

A total of 136 ARMD patients (early ARMD: 68 patients, late ARMD: 68 patients) and 68 controls were included into the study. Mean catalase level and glutathione peroxidase level were significantly lower in ARMD patients [1348.97 (109.11) µM and 453.87 (41.96) U/L respectively] as compared to controls [1453.38 (38.87) µM and 502.28 (34.29) U/L respectively] before ($P<0.001$ and $P<0.001$) and after adjusted for age ($P=0.001$ and $P<0.001$). There was no significant difference for malondialdehyde level between ARMD and controls. Catalase level was significantly lower in late ARMD group as compared to early ARMD group