CURRENT UNDERSTANDING OF AGE-RELATED MACULAR DEGENERATION

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ABSTRACT

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the elderly population in the developed countries. This review discusses the traditional clinical and histopathological presentation of AMD, epidemiology and genetics component in relation to the current understanding of the vascular nature of the disease. Therapeutic approaches to treat the disease are also included in the review.

Keywords: Age related macular degeneration, Imaging techniques, current treatment, risk factors, inflammation.

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INTRODUCTION

Age-related macular degeneration (AMD) is a degenerative disorder involving not only the central retina macular area but the entire retina as drusen and pigmentary abnormalities are also seen in mid-periphery and peripheral retinal areas. The disease presentation is characterised by the appearance of soft drusen, pigmentary changes in the retinal pigment epithelium (RPE) and geographic atrophy (GA). Then further progression leads into advanced AMD with growth of leaky new blood vessels causing choroidal neovascularization (CNV), the wet AMD form. The new International classification of AMD includes Early AMD where drusen size of 65-125µm are noted with pigmentary abnormalities. Late AMD (indeterminate form) include RPE changes, sub retinal fluid and intra retinal fluid with serious pigmented epithelial detachment with no neovascularisation. Late Dry stage AMD includes Geographic Atrophy stage. Late Wet inactive form includes fibrous scar, retinal tear leading to and final late AMD (Wet active form) includes CNV, Retinal angiomatous proliferation (RAP) and Polypoidal choroidal vasculopathy (PCV) results in loss of central vision. Most of the “Risk factors” which cause AMD include advanced age, sunlight exposure, smoking, high blood pressure, cholesterol, obesity; demographic factors, with higher incidence in females, Caucasian race, and in those with a family history of AMD.
Few of the risk factors are controllable and which reduces progression of the disease. The diagnosis and management of the disease are based on regular eye examination including visual field testing (VFT), fluorescein angiography (FFA), indocyanine green angiography (ICG) and optical coherence tomography (OCT). Optical coherence tomography angiography (OCT-A) is a new non-invasive retinal imaging technique useful for detection of choroidal vascular and its blood flow. This technique is useful in diagnosis of Late Wet AMD. Currently, there is no treatment available for dry AMD or GA but the intake of antioxidants and other dietary supplements has been suggested for the management of dry AMD. Wet AMD treatment involves anti-Vascular endothelial growth factor (VEGF) injections, laser therapy and/or photodynamic therapy but none of these target the underlying cause or halt the progression. New therapeutic approaches are required to target molecular and cellular changes in the disease, preventing deposition of lipofuscin, drusen, reducing oxidative stress, regenerating and protecting RPE cells, inhibiting inflammatory pathways, all of these related to the role of the choroidal vasculature in the disease.

Morphology of Normal Retina
Ophthalmoscopic observation of the posterior segment of the eye allows assessment of the retina up to the ora serrata. The retina is a very thin and transparent tissue of 200 µm thickness in a normal healthy eye. The posterior pole of the eye has a central macular zone which contributes to central vision and is fundamental to the perception of sharp, clear, focused images. Within the macula, the fovea has the highest density of cone photoreceptors diminishing in numbers towards the periphery of the retina; whereas in the periphery of the retina rods are more prominent. Nutrients are delivered to the inner retinal layers through the central retinal artery (CRA) the endothelial cells of which form the retinal blood barrier. The blood–retinal barrier is also formed by the retinal pigment epithelium (RPE) that lies adjacent to photoreceptor cells. The RPE functions includes phagocytosis of the photoreceptor outer segment, absorption of scattered light, vitamin A storage, involvement in the visual cycle, nutrients and ion transport and secretion of growth factors. Posterior to the RPE lies the vascular layered choroid with the anterior layer in contact with RPE being Bruch’s membrane, juxtaposed to which there are three layers of different size blood vessels which form the vascular bed of the choroid. The thinner, fenestrated layer of the choroid is the choriocapillaris which directly supplies nutrients via the RPE to the outer layers of the retina, and is especially important to nurture the central retinal artery-free area of the fovea.

Clinical Presentation and Classification of The Disease
AMD is classically referred to as dry and wet. Clinical manifestations define it as non-exudative (dry, atrophic) and exudative (wet or neovascular) (Figure 1). Although different international classifications exist for AMD, there are a few signs common to the classification by stages of the disease. In early and intermediate stages there are: RPE changes, drusen in Bruch’s membrane, hypo and hyperpigmentation at the macula. These signs precede the occurrence of GA. In advanced stages of the disease the choroidal blood vessel region is affected; disciform scarring, neovascularisation and haemorrhages are noted. Changes in the choroid vessel bed may in fact occur much earlier but are difficult to discern clinically in the dry form of the disease.
AMD clinical presentation starts as an accumulation of lipofuscin in the RPE and drusen between the RPE and Bruch's membrane. These are identified as calcified or cholesterol filled round structures, classified as hard and soft drusen. Hard drusen are ≤ 63 µm in size and soft drusen are larger and may be ≥ 125 µm in diameter or in clusters around the central macular area. Soft drusen are indistinct confluent structures, which increase in number with age, and they occur more frequently than hard drusen\(^{(12)}\). GA usually starts in the parafoveal area and progression towards the fovea is noted in late stages of the disease, causing clinically appreciated scotomas. People with GA also tend to develop pigmentary abnormalities, which then further develop into choroidal neovascularization underneath the RPE or entering the sub retinal space by breaching the RPE. A sub retinal haemorrhage is the first clinical sign noted in CNV. A detached RPE and degeneration of photoreceptors leads to disciform scarring, which is formed due to fibroglial reaction stimulated by continuous blood leakage\(^{(13)}\). An Amsler grid is used for identification of metamorphopsia, with a perceived distortion of lines indicating loss of central vision. In neovascular AMD, the deep retinal vasculature is affected; with the development of new vessels outwards into the sensory retina and anastomose of the retinal tissue with the choroidal circulation\(^{(14)}\).

**Imaging Modalities**

Multi modal imaging techniques allow diagnosis of AMD; these include fundus photography/ fundus autofluorescence, optical coherence tomography (OCT), fluorescence angiography (FAF) and...
indocyanine angiography (ICG). These imaging modalities are useful in evaluating the natural course of the disease and for further identifying possible therapeutic treatments. Clinically, spectral domain OCT, allows us to visualize microstructural alterations in the retina and choroid. OCT is a unique technique useful to measure the progression of the disease such as increase in size of drusen and/or GA area. These are confluent areas in histological or OCT analysis that show loss of RPE cells and atrophic patches in photoreceptors regions. Newer imaging technologies used in understanding subcellular changes of the disease in dry and wet stage are FAF and ICG. Advances in imaging the retina have allowed to identify the choroid as contributing tissue to the onset, progression and understanding of disease mechanisms in AM. The most advanced non-invasive angiography technique currently available in clinical practice is OCT angiography (OCT-A) which enables three-dimensional visualization of retina and choroid blood flow. It makes it possible to estimate the size, structure, configuration, and location of the newly formed vessels. OCT-A does not require intravenous dye injection, is free of complications and side effects and allows to identify blood flow particularities of both wet and dry AMD.

**Risk Factors**

Various epidemiological studies have evaluated these factors and the risk for the disease progression although more detailed assessment of risk factors could be useful in understanding etiology, pathogenesis of the disease and to evaluate new treatment therapies of the disease at various stages. Age and family history are the main risk factors for the progression of AMD. Other risk factors associated with the disease are smoking, dietary factors, inflammation, vascular and cardiovascular factors. Studies have also found relationships between UV-B exposure and AMD. Ethnically, the prevalence of AMD is higher in whites than in darkly pigmented races. This thought to be owing to more melanin pigment preventing development of CNV. In contrary, studies in the literature found no association between iris colour and AMD disease progression. A study done in the general population, supports the fact that the presence of melanin pigment in RPE and the macular pigment which is composed of carotenoids lutein and zeaxanthin in eyes with and without AMD has no difference in prognosis of the disease.

**Cardiovascular Factors**

The most important pathogenetic factors associated with the prognosis of AMD are vascular factors. Histological evidence shows that the vascular walls of the larger choroidal blood vessels are thicker in AMD than in normal age matched human tissues. In fact, accumulation of drusen in Bruch’s membrane, and atrophy of RPE layer is due to dysfunction of the choriocapillaris. The vascular model of AMD shows that a combination of elevated choriocapillary pressure, breaks in Bruch’s membrane, and secretion of VEGF causes CNV. Furthermore, in AMD donor tissues, the density of the choriocapillaris is halved in RPE atrophic areas, when dry and wet forms of AMD are compared to normal age matched eyes. Studies in literature also shows that with aging lipid deposition is seen in the walls of systemic arteries and apolipoprotein-B lipid deposition is seen in the sclera and the Bruch’s membrane of the choroid in AMD patient. Increased choroidal vascular resistance, resulting in elevated choriocapillary pressure and development of sub-retinal drusen deposits, as well as decreased choroidal blood flow is lately noted in AMD patients. The lumen diameter of the choriocapillaris underneath the RPE atrophy is not significantly affected. In patients with GA, the presentation of the disease in both eyes may differ, with one eye presenting with GA and the other eye with CNV. In both, however, the choriocapillaris can be reduced in area which leads to hypoxia of the atrophic regions. Some epidemiological studies suggest there is an association between systemic hypertension, cardiovascular diseases and risk of developing AMD.
Angiotensin II has been shown to be elevated in animal studies of systemic hypertension. This molecule triggers expression of VEGF factors. In human donor tissues, the expression of VEGF and its isoforms is elevated in AMD eyes compared to normal age match control eyes. There are many other studies showing the link between hypertension and risk of AMD, suggesting that the vasculature nature of AMD has ample support by a diversity of studies.

Blood vessel changes are associated with pathogenesis and progression of AMD. Through histological evidence it is noted that in early stages of AMD a number of non-functional retinal capillaries with thicker vascular walls are detected compared to normal aged retina. Formation of drusen and RPE atrophy is due to dysfunction in choriocapillaris. The increased resistance to the blood flow in the choroid may also contribute to lipofuscin deposition and drusen formation. There is also constriction of blood vessels with increased resistance to blood flow noted in cardiovascular diseases and hypertension. Population based studies provide evidence that these risk factors are associated with AMD. Furthermore, a study using human donor tissues showed that in late stage AMD there is 50% reduction in choriocapillaris at the RPE atrophic zones compared to normal control eyes. The surviving choriocapillaris are constricted in the areas below RPE atrophy. Nitric oxide is a vasodilator molecule present in blood vessels. It is mainly located on endothelial cells and perivascular nitrogenic neurons. Low production of NO is seen in AMD probably contributing to the constriction of choriocapillaris. Although there is a reduction in choriocapillaris area, the number of choriocapillaris remains the same. Angiogenesis factors also play a key role in progression and development of wet stage of AMD. In the dry stage of AMD, the unaffected RPE area is associated with development of CNV. In GA, hypoxia leads to up regulation of VEGF produced by RPE cells despite a reduction of choriocapillaris being noted. A study done on human AMD donor tissues shows that VEGF isoforms are absent in normal age matched human donor tissues. Animal studies also support the fact that up regulation of VEGF in the RPE layer leads to CNV and blood vessel leakage.

Smoking

In addition to age and family history, cigarette smoking is considered an important well-known risk factor associated with development of AMD. Various epidemiological studies have shown the association between smoking and the risk of AMD. The increased risk of AMD prognosis is noted in both prior and current smokers and has a direct relationship with prognosis of the disease. The antioxidant protective mechanism is compromised by smoking, which in turn progresses the disease AMD. It is also noted that although AMD affects females more than males, male smokers are more prone to the risk of developing AMD than female smokers. Animal studies support the fact that nicotine stimulates neovascularization by increasing production of VEGF and endothelial cell proliferation.

Light Exposure

A variety of studies support the fact that UV exposure and light damage leads to AMD. Although one study done in human suggested that the light damage is limited to only central and superior regions of the retina, the mechanism of photo activation leads to decrease in the flow of ions in RPE before retinal damage. Animal studies demonstrated that intense continuous exposure to light causes alterations in the retinal metabolic function. Photo-oxidative damage causes an elevation of cations in the retina which in turn affects photoreceptor functionality similar to AMD. At the cellular level reactivity includes alterations in Müller cells gene expression with increased expression of vimentin and glutamine synthetase. Study done on relationship between increase in the blood flow at macula with temperature changes induced through light-generated heat in humans suggests that choroidal vasculature changes occur in retinal diseases which are un-noted.

Dietary Factors

Except for the AREDS study that assessed the intake of antioxidants, multi vitamins, beta carotenes, carotenoids, retinol and minerals, systematic reviews and various epidemiological studies have found no relationship between intake of the dietary supplements such as zinc, antioxidants, lutein and xanthophylls, and progression of AMD.
Observational human studies and animal studies suggest that there is no relationship between omega 3 fatty acid consumption and progression of AMD disease. A few studies have also suggested that there is no relationship between medications such as angiotensin, a converting enzyme or cholesterol lowering medication in progression of AMD. In contrast another study done in the United Kingdom supports the fact that cholesterol lowering medications such as statin, lowers the risk of prognosis of AMD as there is an anti-inflammatory effect located at Bruch’s membrane.

Oxidative Stress

Oxidative stress is caused by the overbalance of free radicals such as reactive oxygen species (ROS) or reactive nitrogen species, and is one of the major factors involved in the aging process. ROS are generated under physiological conditions, including normal cellular activities such as NADPH-dependent membrane-bound enzymes, mitochondrial metabolism, and other intercellular oxidases. Cells possess antioxidant enzymes such as superoxide dismutase and glutathione peroxidase which are useful in removal of ROS from cells. Exposure to light leads to oxidative stress in the RPE cell layer, leading to the formation of lipofuscin, an age-related pigment found in different age-related diseases. Increase in vascular endothelial cell dysfunction is caused by oxidative stress which plays a key role the pathophysiology of several vascular diseases and disorders. Nitric oxide is another cytotoxic molecule involved in vascular development and associated with pathogenesis of AMD. Reductions in the antioxidant defence system and increased oxidative stress may play a role in the pathogenesis of AMD.

Role of Inflammation

The first step of the immune defence development in a retinal injury is to activate macrophages, leukocytes and other phagocytes. Macrophages and T cells recognise the oxidative modified lipids. In the eye, enhanced activity of retinal glial cells indicates an inflammatory response before damage to retinal structures. Animal studies support the fact that astrocytes are enhanced in activity after light damage and there is immunohistochemical evidence for increased staining of glial acidic fibrillary protein (GFAP) in the nerve fibre layer which is a noted inflammatory response. In the inflammatory response process, the most important immune cells involved are glial cells and molecularly the complement system. The inflammatory residue composition in drusen provides evidence that complement factor H, are highly involved in this event.

The accumulation of lipofuscin at Bruch’s membrane leads to drusen formation. The undigested residual waste leads to RPE dysfunction and compromises their lysosomal function. Histochemical evidence proves that drusen is a mixture or accumulation of proteins, lipids, lipoproteins, complementary factors (such as C1q, C3a and C5a), complementary regulators (such as complement factor H, clusterin, vitronectin), immunoglobulins (IgG), amyloid β, phospholipids, cholesterol and apolipoproteins B & E. This above process of formation of drusen describes only a part of the inflammatory events happening in AMD, a multifactorial disease in which progression is based on other events such as stress, the para-inflammatory response, environmental and genetical factors. Studies have shown that genetic variations of several complement genes such as complement regulator Factor H, central complement component C3, Factor B, C2 are the risk factors involved in the progression of the disease AMD.

This process is also noted at the protein level, accumulation of waste metabolites in formation of drusen, in the subretinal space, and in capillaries of the choroid. Every complement pathway forms a membrane attack complex, which contributes to drusen formation found in the RPE–choroid interface. Complement factor H plays a key role in forming an alternative pathway by binding with C3b. The other gene associated with complement pathway is C1 with the immunohistochemical evidence indicating that in wet AMD, these C1 proteins are found. The C3 complement pathway is also found in wet AMD, especially in conjunction with choroidal neovascularization. Deposition of these membranes, found in C1 and C3 can be removed surgically. Elevated plasma levels of these proteins are found in AMD patients with C3 and C5 being associated complement factors. The C5 factor is associated with expression of interleukins. These interleukins are responsible for apoptosis and cellular dysfunction of RPE.
The complement pathway illustrated in Figure 2 highlights the different components found in the eye.

Figure 2: Summary of the complement pathway in the eye.

The complex complement system with several activated pathways are illustrated in the above image. The risk of progression of the AMD disease, is associated with the activation of the complement factor H.

The residential glial cells in the retina are microglia and macroglia\(^{35}\). The retinal macroglia has two main cellular components; Müller cells and astrocytes. Studies have suggested that microglial cells are involved in an immune defence mechanism in AMD and they enlarge and change their shape to an amoeboid form suggestive of a wound healing mechanism. The removal of debris is done by these cells which relocate from the outer retina to the sub retinal space. The recollection of these cells triggers a pro-inflammatory response, which produces cytokines, chemokines, complement receptors and chemokine receptors. CX3CR1 is one example of a chemokine receptor located on microglia in AMD; this protein binds with chemokine ligand 1 (CX3CL1) and it is involved in leukocyte adhesion and migration. Many cytokines, chemokines, calcium ions, ATP, nitric oxide can also increase with microglial activity. Some neurotransmitters such as GABAergic decrease microglial activity whereas glutamatergic neurotransmission increases microglial activity\(^{36}\).

The microglia, astrocytes and Müller cells play a key role in pathogenies of AMD. These residential glial cells provide support and protection of retinal neurons by supplying nutrients, removing waste and playing a role in phagocytotic mechanisms. GFAP is an astrocyte marker which labels that cell type in the nerve fiber and ganglion cell layers of the retina. Previous studies suggest that an increased labelling of GFAP is also noted in Müller cells in AMD human donor tissues\(^{37}\). These astrocytes are increased in number in the ganglion cell layer due to oxidative stress and these cells are found as resident cells in the outer retina and vitreous chamber of young donor tissues. The mechanism by which these cells become enlarged is suggestive of phagocytic activity and maintenance of blood retinal barrier. Neurotoxic factors such as Nitric Oxide (NO), are released by the activation of these glial cells, which are involved in neurodegenerative diseases.
Vascular Factors In AMD

Current Management Of AMD

AMD has a complicated pathophysiology and multiple risk factors associated with its onset and progression makes it a difficult eye condition to manage. Through the course of the pathology there are no persistent molecular targets that can be targeted for treatment of the disease. In the dry form of AMD, the formation of drusen is considered a hallmark sign, associated with RPE degeneration. The initial management of dry AMD is with increase of dietary supplements consisting of leafy vegetables, Vitamin A, C, E, Zinc and carotenoids\(^{(38)}\). In contrast, in the wet stage of the disease, RPE atrophy, haemorrhages, CNV and fibrosis concentrated in the macular area leads to vision loss. In both dry and wet forms photoreceptor layer loss is noted. Current therapeutic treatment options available are only for the wet form of AMD and the dry form remains untreatable. These include options such as photodynamic therapy (PDT), injecting anti–angiogenic agents (anti VEGF therapy) and laser photocoagulation. In addition to these established approaches, potential new investigations suggest replacement of retinal layers and RPE stem cell therapy. In all cases the treatment remains focused on the signs rather than the cause and the late stage of disease\(^{(39)}\).

Anti – Angiogenic Agents

Anti angiogenic agents are the primary therapies for the CNV present in the wet form of AMD. This therapy targets vascular endothelial growth factor (VEGF) and its isoforms. Pegaptanib sodium is an agent which interferes with RNA molecules and targets releasing VEGF, particularly VEGF-A\(^{(40)}\). Other specific agents, which act on VEGF-A, are bevacizumab, ranibizumab and aflibercept. These are all effective in reducing leakage in CNV. In addition to these antibodies, corticosteroids used in treatment of CNV include triamcinolone acetonide\(^{(41)}\) and the wet AMD anti-inflammatory pathway may be reduced using prostaglandins and leukotrienes\(^{(42)}\). Apart from cortisone, anecortave acetate reduces progression of CNV. Many studies however, indicated that anti–VEGF treatment may be a better choice than corticosteroids and/or cortisone usage. Long-term profile studies have noted variations in the response to the anti–VEGF treatment based on age and genetic profile. Based on the extent of lesions, clinicians may choose to perform an anti–angiogenic treatment and/ or photodynamic therapy (PDT) to improve vision\(^{(43)}\).

Photodynamic Therapy

PDT was the primary treatment option in the late 1990s but it is for the most part replaced by pharmacological treatments. In monotherapy, though, it remains a strong treatment modality for the wet form of AMD\(^{(44)}\). This method involves Verteporfin, an intravenous photosensitive dye, activated by infrared light. Verteporfin activates singlet oxygen species which damages endothelium and accumulates on neovascular membranes in wet AMD. Lesion size with CNV extending into the fovea and/or leading to visual loss affecting quality of life of an individual are indications for PDT\(^{(45)}\). Usually, 6mg/m² of verteoprofin is infused for 10minutes. The lesion size and location may change the energy, intensity and dosage of the dye. The results of a study in New Zealand suggest that this treatment is very effective and 70% of patients treated with this therapy avoided moderate visual impairment in first one year of the treatment\(^{(46)}\).

Laser Photocoagulation

Laser photocoagulation is an effective argon laser treatment, for treating wet AMD. Argon Laser is useful in closure of newly formed blood vessels in CNV. Studies suggested that it prevents severe visual impairment, especially in eyes with extra foveolar and juxta foveolar choroidal neovascularization. The disadvantage of the treatment is that it causes visual field spots\(^{(47)}\).

Future Managements

Stem Cell Therapy and Cell Replacement

Stem cell therapy is an explorative new route for treating blinding retinal conditions including AMD, Retinitis Pigmentosa, and Stargadt's macular dystrophy. Stem cell therapy may play a key role in treating GA\(^{(48)}\). The principle differences are their cell source, the age of donor, whether they are clinically graded and whether they are useful for multiple recipients. Cell reprogramming affects the immune privilege of donors
and survival of RPE and photoreceptor cells will be based upon long-term survival of grafted cells. Several animal studies supports this therapy. But in humans a better understanding of their role in treating this required assisted technology and adaptation of modern imaging technology\(^{49}\).

**Genetic Approaches**

Clinical trials have indicated that CNV growth is arrested by intravitreal transfer of pigment-epithelium–derived factor The COBLAT clinical trial showed that intravitreal administration of bevasiranib also inhibited CNV growth\(^{50}\). Genetic approaches are useful in treating later stages of the disease but could potentially treat earlier stages once the true underlying causes of AMD are fully elucidated. Numerous clinical trials and ongoing research has developed interventions which are useful to alleviate later stages of the disease. Literature review also reveals that glatiramer acetate may decrease the size of the drusen, retinal transplantation can be successful, fenretidine acts to decrease the size of GA and incidence of CNV, carotenoids and ω-3 fatty acids may decrease the progression of GA. Sildenafil increases choroidal thickness and retinal vascular flow. However, these pharmaceutical treatments do not improve vision. A therapy for treating early stages of AMD is absent although current molecular targets identified for potential management of AMD are useful in better understanding the disease. The literature and recent experiments in animal and donor tissues suggest loss of vasculature homeostasis is a significant triggering cause. Future scope of research is to understand how one progresses to the other (or not), the causative factors for the different stages AMD, and an ongoing need for therapeutic treatments for both forms of the disease (dry and wet AMD). It is increasingly recognised that current mainstream treatments really only provide a five year delay in progression, with most patients then falling back below baseline of visual acuity.

**CONCLUSION**

AMD is a global disease that leads to substantial vision loss and significantly affects the quality of life in the elderly population. The underlying causes of the disease and its pathophysiology still need to be understood. The current treatment strategies and imaging modalities are useful to track the progression of the disease, but not for treating or curing the permanent visual damage. There is no much proven research for treating the early/dry stages of the disease AMD. The current treatment strategies are more useful for stopping the leakage and growth of the new blood vessels in the late/wet stages of AMD. New clinical trials are underway for investigating the novel methods for the treatment of the early stages of AMD which will reduce the global impact of visual burden.

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