Targeted nutrition for optimising vitreous health in subjects with symptomatic vitreous degeneration

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This dissertation is submitted for the Degree of *Doctor of Philosophy*

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Declaration

No element of the work described in this Thesis, or the Thesis itself, except where otherwise acknowledged, has been previously submitted for a degree at this or any other institution. The work described in this Thesis has been performed entirely by the author.

Signature _____ Date _____

Dedication

To my dear wife Benny

Acknowledgement

I would like to express my profound gratitude to God Almighty, the most gracious and eternal Father, who has sustained me on this PhD journey. Indeed, "*The Sovereign Lord is my strength; he makes my feet like the feet of a deer, he enables me to tread on the heights*" (Habakkuk 3:19). I have gotten this far because God made it possible.

I would also like to thank two incredible giants who have been more than supervisors (maybe even 'fathers') to me. Professor John Michael Nolan, you are super incredible! You made every bit of life and research in Carriganore exciting. Thanks for your advice, corrections and encouragement all through the years. If I ever had to do this again, I would not think twice in choosing you as my supervisor. Dr. Eugene Ng, thank you for all the support and wise counsel. Thank you for the help with recruitment for the Floater Intervention Study (FLIES). I am fortunate to have had you as my supervisor.

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I would like to thank my family as well – Grandma Monica, Prince, Sandra, Collins, Ernest, Joel, Jeff, Gloria, Daniel, Ella and Lillie. Thank you for all the times you made me laugh and forget about the pressures of PhD. I couldn't have done it without those moments. You guys rock!

A special thank you to Waterford Institute of Technology and ebiga-VISION GmbH for instituting the WIT Co-Fund PhD scholarship that enabled me to pursue my PhD. Thank you Mr. Robert Kuchling for trusting Prof. Nolan to select the right candidate for this incredible project. Thank you to all the volunteers who participated in the FLIES trial; your commitment made this project a success.

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Special thanks to the Maranatha Christian Community Church in Waterford, especially the Worship Team (*you guys are my family*) and the pastoral team (Pastor & Mrs. Dunphy and Pastor & Mrs. van Maarleveld). I have enjoyed my time and service in Maranatha, and I will carry these memories with me forever.

Finally, to *my most precious pearl,* Benny, the amazing woman who has stood by me during this PhD journey; our few years together as a couple have been spent chasing this dream of mine and you have been supportive. Thank you for believing in me, for tagging along and for taking care good of me. This PhD is for your my dear. I love you dearly.

Disclaimer

The author of this thesis is listed as an inventor on the patent application submitted to protect the intellectual property generated from the interventional study that has been reported in this thesis.

Abstract

Purpose: Degeneration of the vitreous, the homogenous gel that fills the posterior segment of the eye, is ubiquitous during life and leads to the entoptic phenomenon, vitreous floaters. Floaters impact negatively on the quality of life and visual function of its sufferers. Unfortunately, most floater sufferers are not treated since the available treatment options, pars plana vitrectomy and laser vitreolysis, are not readily proffered to these patients due to their accompanying potential, sight-threatening complications. It follows from the foregoing that a low-risk yet effective therapy is warranted for the management of symptomatic vitreous degeneration. This PhD thesis reports three main studies (Chapters 3 – 5) which were conducted to: (a) investigate the impact of vitreous degeneration on photopic and mesopic contrast thresholds (Study 1); (b) assess the use of a novel objective methodology for quantifying vitreous opacities as a measure of treatment success in patients who underwent laser vitreolysis for managing symptomatic vitreous floaters (Study 2); and (c) investigate the impact of targeted nutrition with a micronutrient formulation on vitreous health in patients with symptomatic vitreous degeneration (Study 3).

Methods: Study 1 was a case-control study that enrolled an age-matched sample of 115 subjects, comprising 30 subjects with vitreous floaters (cases) and 85 healthy subjects (controls). Best corrected visual acuity (BCVA), flicker thresholds, photopic and mesopic functional contrast thresholds (at 10 cycles per degree) were measured for all participants. Further, the cases were split into cases with (n=12) and without (n=18) posterior vitreous detachment (PVD), and their contrast thresholds were compared with the controls, to determine the effect of PVD on contrast. Study 2 was a retrospective study of 77 patients who underwent Nd:YAG laser vitreolysis and had a minimum follow-up of 3 months. Quantitative vitreous opacity areas, the lack of need to proceed to vitrectomy, patient satisfaction outcomes post vitreolysis, BCVA and intraocular pressure (IOP) were assessed at baseline and all follow-ups. In Study 3, 61 patients with vitreous floaters were randomised to consume daily, the active supplement consisting of 125 mg L-lysine, 40 mg vitamin C, 26.3 mg *Vitis vinifera* extract, 5 mg zinc, and 100 mg *Citrus aurantium* or placebo for 6 months. Subjective change in visual discomfort from floaters, BCVA, letter contrast sensitivity, photopic functional contrast sensitivity with positive and negative contrast polarity, and quantitative vitreous opacity areas were assessed for all participants at baseline and final visits.

Results: In study 1, photopic and mesopic contrast thresholds were lower by 37.4% and 27.5%, respectively, when the cases were compared with the controls ($p=0.028$ and $p<0.001$ for photopic and mesopic contrast thresholds, respectively). Further, photopic and mesopic contrast were lower by 64.0% and 30.3% in cases with PVD compared with controls ($p=0.001$ and $p=0.014$ for photopic and mesopic contrast, respectively). In study 2, there was a significant decrease in vitreous opacity areas (objective treatment success of 89.6%) at the final visit following laser vitreolysis ($p<0.001$). In addition, subjective treatment success reported at 1-month and the last follow-up were 77% and 71%, respectively. There was a lack of need of vitrectomy in 65 eyes. Intra-operative complications recorded included posterior lens injury in one eye and retinal bleed in another eye. For study 3, the active group reported a significant decrease in their visual discomfort from floaters ($p<0.001$), whereas the placebo group had no significant change in their visual discomfort ($p=0.416$) after supplementation. At 6 months, there was a significant decrease in vitreous opacity areas in the active group ($p<0.001$) and an insignificant increase in vitreous opacity areas in the placebo group ($p=0.081$). Also, there

was a significant improvement in photopic functional contrast sensitivity in the active group after supplementation (p=0.047).

Conclusions: Study 1 demonstrates that subjects with vitreous degeneration have diminished photopic and mesopic contrast thresholds compared with controls. This finding highlights the negative impact of vitreous degeneration on the quality of vision. Study 2 reveals that Nd:YAG laser vitreolysis results in both objective and symptomatic improvement in at least two-thirds of patients who undergo the procedure. Vitreous opacity areas quantification can be employed by clinicians as an objective outcome measure for diagnosing, planning and quantifying the treatment outcomes for vitreous floater patients. The findings of Study 3 indicate improvements in vision-related quality of life and visual function of patients suffering from vitreous floaters after supplementation with the active formulation. Notably, these improvements were confirmed by the decrease in vitreous opacity areas in the active group. This targeted dietary intervention should be considered to support patients with symptomatic vitreous degeneration.

Preface

"We are not making science for science. We are making science for the benefit of humanity."

- Françoise Barré-Sinoussi (Nobel Prize Winner, 2008)

Over the past three and half years, I have been devoted to investigating a fragment of an aspect of vision science research focussed on targeted nutrition for optimising the health of the vitreous, the clear gel that fills the posterior segment of the eye. My passion to succeed on this journey was partly fuelled by the potential benefits this piece of research held for humanity, in general, and for vitreous floater patients, in specific. In line with the above-stated quote by Françoise Barré-Sinoussi, my PhD journey has not only been one for my personal development into an independent researcher but also, one that has afforded me the opportunity to undertake science that holds tremendous value for a major underserved population, floater sufferers.

This PhD thesis is, therefore, a compendium of what I have learnt and discovered so far while researching this very important topic. The thesis is organised into six inter-related chapters as illustrated by the flow chart below (Figure 0.1):

Chapter 1 – The vitreous: This chapter summarises the literature related to the broader area of study, the vitreous. It discusses the ultrastructure of the vitreous and highlights the various antioxidant molecules present in the vitreous.

Chapter 2 – Vitreous Degeneration: This chapter summarises the appropriate literature related to the specific area of study, vitreous degeneration. It details the mechanisms responsible for vitreous degeneration, highlighting the role of intravitreal antioxidant depletion or reduction in the degenerative process. It also documents the research questions and objectives that are addressed by this thesis.

Chapter 3 – Vitreous degeneration compromises photopic and mesopic contrast sensitivity: This chapter discusses the impact of vitreous degeneration on photopic and mesopic contrast sensitivity in a case-control study. This study was necessitated because, although previous literature has revealed a significant reduction in mesopic contrast with vitreous degeneration, no evidence exists regarding the impact of vitreous degeneration on photopic contrast. As the visual effects of vitreous degeneration are more pronounced against bright backgrounds, this study was warranted to contribute knowledge on the impact of vitreous degeneration on photopic contrast sensitivity.

Chapter 4 – Ultra-widefield infrared imaging of vitreous opacities: This chapter discusses the use of a novel objective methodology for quantifying vitreous opacities as a measure of treatment success in patients who underwent laser vitreolysis for managing symptomatic vitreous floaters. The relevance of this work is that it contributes knowledge to the strategies for objective quantification of vitreous opacities for vitreous research. Developed as part of this PhD thesis, this methodology was then utilised as an outcome measure in the principal experiment of this thesis as explained below.

Chapter 5 – Dietary intervention with a targeted micronutrient formulation optimises vitreous health in patients with symptomatic vitreous degeneration: This chapter reports the findings of an interventional study, the Floater Intervention Study (FLIES), which was the principal experiment for this PhD project. FLIES was designed to investigate the impact of 6-month supplementation with a formulation of antioxidant and antiglycation micronutrients on the visual disturbance, visual function, and objective vitreous imaging parameters in patients with symptomatic vitreous degeneration.

Chapter 6 – Conclusions and Future Recommendations: This chapter summarises the relevance of the findings of this PhD thesis to the research area and offers recommendations that would be beneficial for future research.

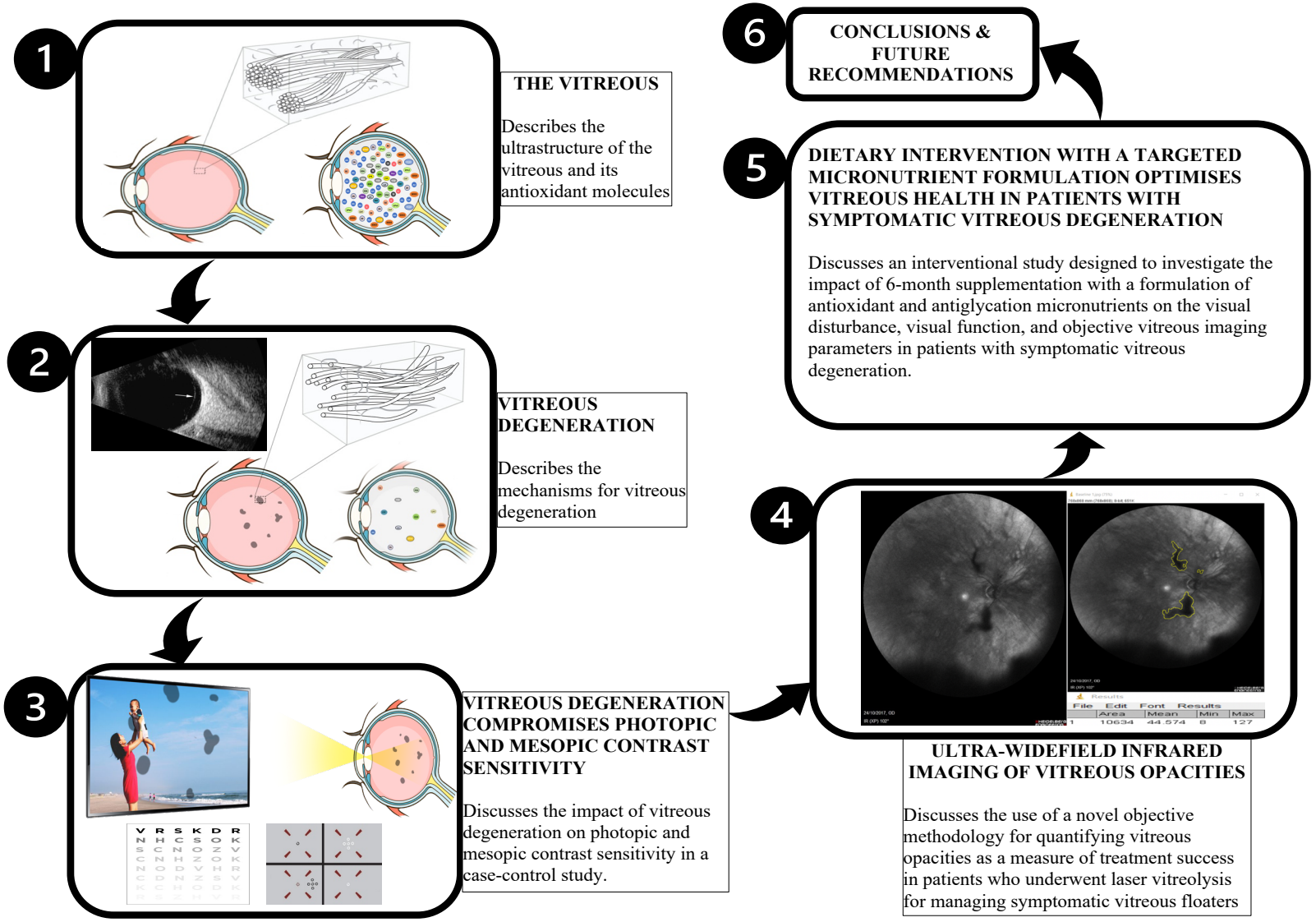

Figure 0.1: *Flow chart of the thesis.*

I am hopeful that this thesis will spark research interests that will culminate in further investigations into targeted nutrition for vitreous health, as this work provides foundational evidence that sets the stage for further lines of questioning and experimentation into this fairly new area of research. But most importantly, I hope that you, the reader, will find this monograph very insightful and informative to read as I have found it fulfilling to study and write.

"Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning."

- Albert Einstein (1916)

Waterford, Ireland Emmanuel Ankamah, 2021

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LIST OF ABBREVIATIONS IN ALPHABETICAL ORDER

Abbreviation	Definition
2D	Two-dimensional
3D	Three-dimensional
AA	Ascorbic acid
AGE	Advanced glycation end-products
AMD	Age-related macular degeneration
ANCOVA	One-way analysis of co-variance
APVD	Anomalous Posterior Vitreous Detachment
ASRS	American Society of Retina Specialist
AVOT	Advanced Vision and Optometric Test
BCVA	Best corrected visual acuity
BEAVRS	British and Eire Vitreoretinal Surgeons
BMI	Body mass index
CONSORT	Consolidated Standards of Reporting Trials
CS	Contrast sensitivity
DME	Diabetic macular oedema
DR	Diabetic retinopathy
DUE	Digital uniformity equaliser
DVH	Diabetic vitreous haemorrhage
ED	Eales' disease
ETDRS	Early Treatment Diabetic Retinopathy Study
EVI	Enhanced vitreous imaging
FACIT	Fibril-associated collagen with interrupted triple helices
FAD	Flavin adenine dinucleotide
FCS	Functional contrast sensitivity
FLIES	Floater Intervention Study
FMN	Flavin mononucleotide
GAG	Glycosaminoglycan
GPX	Glutathione peroxidase

GSH	Reduced glutathione
GSSH	Oxidised glutathione
H_2O_2	Hydrogen peroxide
HA	Hyaluronan
HAS	Hyaluronan synthesising enzymes
HSA	Human serum albumin
ICC	Intraclass correlation coefficient
ILM	Inner limiting membrane
IOP	Intraocular pressure
IR	Infrared
IR (XP)	Cross-polarised infrared reflectance
LCD	Liquid-crystal display
LOD	Lens optical density
MALDI-TOF	Matrix -assisted laser desorption ionisation time of flight
MAR	Minimum angle of resolution
MMP	Matrix metalloproteinase
MoCA	Montreal Cognitive Assessment
OCT	Optical coherence tomography
OD	Oculus Dexter (Right Eye)
OS	Oculus Sinister (Left Eye)
OSI	Objective Scatter Index
PDR	Proliferative diabetic retinopathy
PEDF	Pigment Epithelium-Derived Factor
POM	Primary outcome measure
PROM	Patient-reported outcome measure
PVC	Posterior vitreous cortex
PVD	Posterior Vitreous Detachment
PVR	Proliferative vitreoretinopathy
QUS	Quantitative ultrasound
RCT	Randomised controlled trial

ROI	Region of interest
ROS	Reactive oxygen species
RPE	Retinal pigment epithelium
RRD	Rhegmatogenous retinal detachment
SOD	Superoxide dismutase
SD-OCT	Spectral Domain OCT
SS-OCT	Swept Source OCT
VA	Visual acuity
VAR	Visual acuity rating
VISC	Vitreous Infusion suction cutter
VOD	Vitreous optical density
VOR	Vitreous opacification ratio
YAG	Yttrium aluminum garnet

Chapter 1

THE VITREOUS

"Look at vitreous and not just through it"

- Professor Jerry Sebag

1.1 A CASE FOR THE VITREOUS BODY

The past 5 decades have witnessed significant advancements in research into the vitreous, which is the clear gel that fills the posterior segment of the eye, the space bordered by the posterior lens surface and the inner limiting membrane (ILM) of the retina. Evidence from these years of research has defined what is our current understanding of the vitreous. Essentially, what has become a general consensus among vitreous researchers is succinctly captured in a quote by Prof. J. Sebag, an authority in vitreous research, that admonishes "*look at vitreous and not just through it."*[1] What this quote suggests is that, it has become imperative for eye care professionals to critically observe the vitreous while examining their patients and for scientists to consolidate efforts at enhancing our understanding of the vitreous in healthy and diseased states. We have come to that place where vision should no longer be explained without the contribution of the vitreous.

Principally, vision relies on the coordinated roles played by various structures of the visual system, from the tear film on the ocular surface to the visual centres within the brain. Visual perception commences with sensory information organisation, the process by which the highly specialised neurosensory retina of the eye captures photons from the environment and converts them into neural signals for visual processing.[2] Concurrently, the eye is exposed to exogenous injury-precipitating factors including visible light, ultraviolet light, ionising radiation, and environmental toxins; as well as endogenous stress-inducing influences, generated by the mitochondria within ocular tissues during the eye's physiological functions.[3] These endogenous and exogenous oxidants produce unstable reactive oxygen species (ROS) characterised by one or two unpaired electrons within their external orbit.[4]

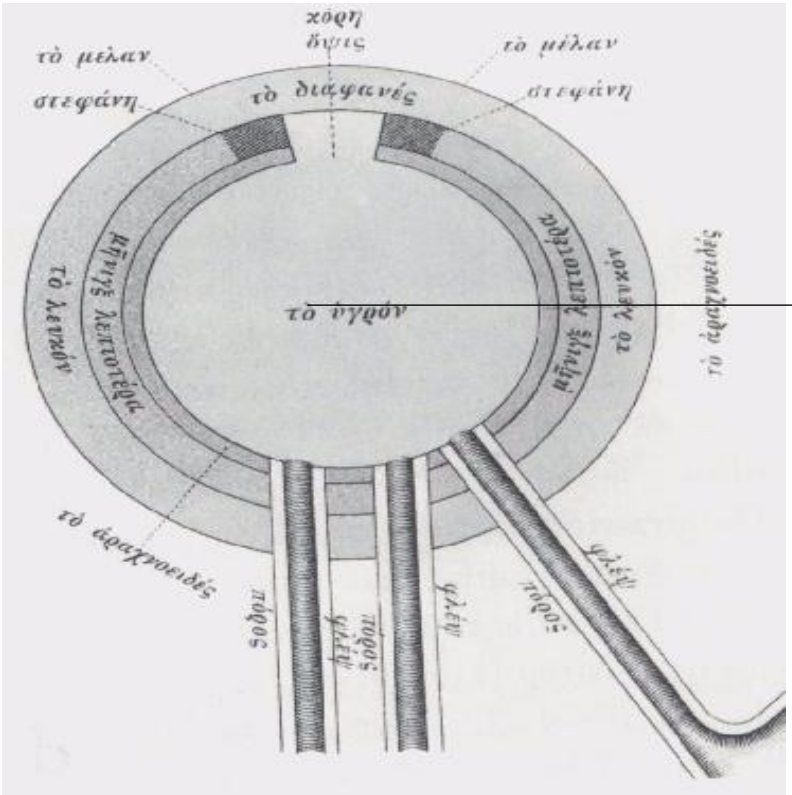
While normal concentrations of ROS act as a physiological response to stress and are an integral part of normal ocular metabolic activity, excess levels could be debilitating to the eye.[5] To remain functional, the eye is replete with an assembly of antioxidants (i.e., substances that, when present in low concentrations compared to that of an oxidisable substrate, significantly delay or inhibit the oxidation of the substrate) by which it mitigates the damaging effects of ROS. [6] Over production of ROS beyond the counteracting ability of the eye's antioxidant system can cause ocular tissues to be overwhelmed, a phenomenon referred to as oxidative stress.[7] The pathological cascade following oxidative stress are ocular physiologic dysfunction, ocular tissue death and consequently, ocular degenerative disorders.[8]

Emerging evidence suggests a relationship between decreased intraocular antioxidant capacity and the onset of ocular diseases such as endothelial Fuch's dystrophy, cataract, age-related macular degeneration (AMD), and diabetic retinopathy (DR).[3, 9] Also, aging, nutritional imbalance, toxins, and infections deplete intraocular antioxidants, necessitating a constant supply of antioxidants via diets or supplementation.[10] It is thus not surprising to observe recent strides in research focused on the application of antioxidants as plausible therapeutic and prophylactic agents in the management of ocular disease.[11, 12]

While the antioxidant molecules within some ocular structures including the aqueous, cornea, crystalline lens, and retina have been duly explored and discussed, there is a paucity of information regarding the antioxidant capacity of the vitreous.[3, 13] Coupled to that, evidence points towards oxidative stress and reduction in vitreous antioxidant capacity as precursors for age-related vitreous degeneration and subsequent vireo-retinopathies.[14] Thus, adequate levels of intravitreal antioxidants may be protective against vitreous degeneration, possibly preventing and even improving vision degrading myodesopsia (or symptomatic vitreous floaters), the clinically significant entoptic phenomena that can result from advanced vitreous degeneration. Given the apparent importance, there is a need to discuss the vitreous as well as profile its antioxidant molecules in a bid to understand their role in vitreous health, degeneration and vitreo-retinopathies.

1.2 HISTORICAL BACKGROUND OF THE VITREOUS

The earliest record of the vitreous dates back to Aristotle (384 - 322 BCE) who, in his *De sensu et sensibilibus,* described the vitreous as follows: “It is true that the eye consists of water, but it has the power of vision not because it is water, but because it is transparent’. He added, “ It is natural that what is within the eye should consist of water; for water is transparent.”[15] Aristotle believed that the vitreous (what he referred to as water) was only present within the eye to ensure optical transparency (Figure 1.1).



The ‘water’ of the eye

Figure 1.1: *Schematic drawing of the eye as conceived by Aristotle, showing the 'water' of the eye*

Galen, a second century A.D. scientist, is credited as the one who coined the term 'vitreous humour.' Drawing insights from Aristotle's writings as well as the work of anatomists who dissected in Alexandria, including Rufus of Ephesus, Galen was able to describe the retina, cornea, iris, uvea, tear ducts, and eyelids, as well as the two fluids he called the vitreous and aqueous humours (Figure 1.2). The term 'vitreous humour' was coined from two Latin words, *vitreum*, which translates to 'glassy' and *humour* which means 'fluid.'[16] In line with this, Galen described the vitreous as "a substance that was thicker and clearer than blood, which had the appearance of molten glass and was colorless."[17] Galen, however, provided no functions to the vitreous humour except its role in ocular transparency similar to Aristotle's philosophy.

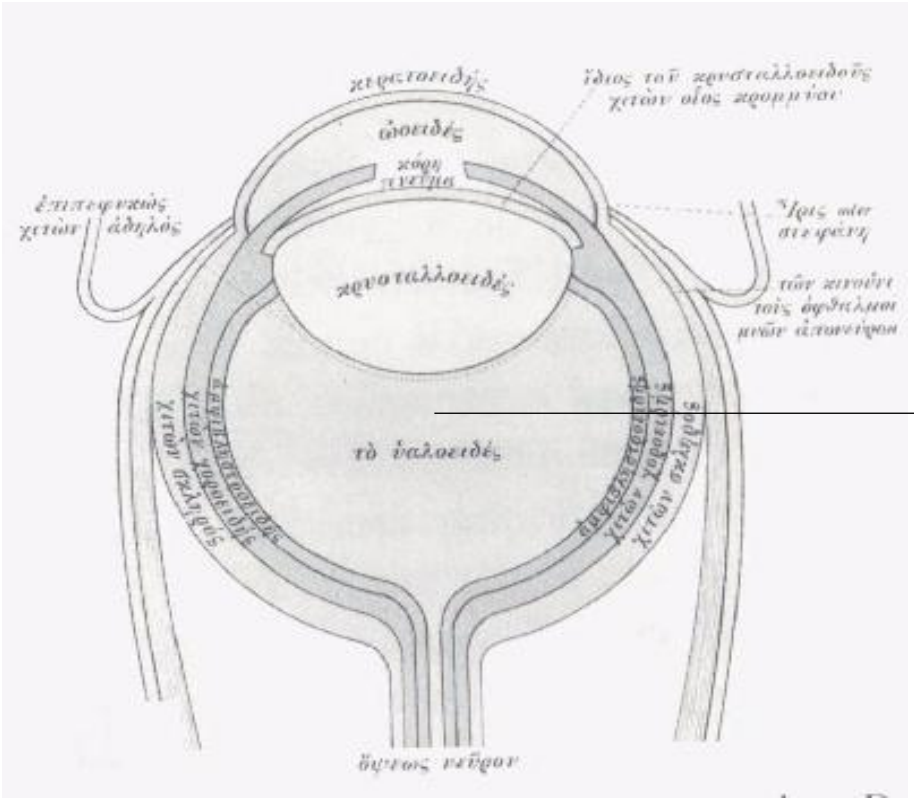


Figure 1.2: *Schematic diagram of the eye by Galen, showing the vitreous humour.*

In the ninth century A.D., Hunain Ibn Ishaq (809 - 877AD) provided the first systematic ophthalmology documentation of the vitreous in his two books, *The book of the ten treatises on the eye* and *Book of the Questions on the Eye*.[18] Hunain reiterated Galen's philosophies that, "the crystalline humour itself is the principal instrument of vision. The principal and greatest usefulness [of the retina], is to perceive the alterations of the crystalline humour and in addition to convey and transmit nutriment to **the vitreous humour**."[15, 19]

In later years, Averroes (1126 – 98) of Cordova, lawyer, physician, and philosopher, described the perspectives of Aristotle and Galen in his *Epitome of Parva Naturalia* as follows: "We maintain that the air, by means of light, receives the forms of objects first and then conveys them to the external coat of the eye, and the external coat conveys them to the remaining coats, until the movement reaches the innermost coat behind which the common sense is located, and the latter perceives the form of the object. In the middle of these coats lies the crystalline coat [the lens], which is like a mirror, partaking equally of the nature of air and of the nature of water. This coat, therefore, receives the forms from the air, since it is like a mirror, and it conveys them to the water, because its nature partakes equally of the two properties of air and water. As for the water, which Aristotle says, lies behind the crystalline humour, that is the one which Galen calls the **vitreous humour**, as I believe. This is the innermost of all the curtains of the eye, and through it, the common sense can perceive the form. After

the common sense perceives it, it conveys it to the *informans*, which is the imaginative faculty."[20]

Up until the early years of the 20th century, there was a dearth of accurate information on the vitreous or vitreous surgery in the ophthalmic literature and the original ideas by Galen and Aristotle were still the widely appreciated perspectives on the vitreous. Vitreous was "considered simply an optical pathway by optical specialists, a space filler that maintains pressure to keep the eye inflated by glaucoma specialists, an environment for inflammation/infection by cataract surgeons, and a possible impediment before the retina by retinal surgeons."[1] In 1892, Frank Ebenezer Miller published a book titled "*Diseases of the Eye, Ear, Throat, and Nose*" and described the vitreous as follows: "It is a transparent, jelly-like mass that occupies the large posterior chamber of the globe known as vitreous chamber. It is composed of very fine fibrillae which enclose a gelatinous fluid in their meshes."[21] He further indicated the following statements about vitreous as part of cataract surgery: "It sometimes happens that the lens, instead of escaping, is pressed backward into the vitreous chamber. When this occurs the wire loop may be passed into the vitreous chamber, the lens engaged, and withdrawn. Escape of vitreous may occur. If this is not large in amount, it is of little importance. The protruding vitreous should be excised, the wound rendered as free as possible from the protruding tissues, and the eye bandaged."[21] These statements were corroborated in the 1910 book by John Elmer Weeks, MD, professor of ophthalmology at New York University, entitled '*A Treatise*

of Diseases of the Eye' as follows: "If vitreous is encountered during a cataract operation, it may be excised if only to allow the lips of the wound to be approximated."[22]

Despite the view of possible excision of the vitreous held by the few, above-mentioned physicians and later ophthalmologists like Schafer, Schepens, and Kasner, most ophthalmologists at that time looked on the vitreous as a substance not to be tampered with since they understood from bitter experience that surgical manipulation of the vitreous usually resulted in serious complications such as chronic inflammation, macular degeneration, retinal detachment, glaucoma, and infectious endophthalmitis.[23] Indeed, one prominent ophthalmologist who learnt about Shafer's procedure of aspirating abnormal vitreous asserted that it was a malpractice to touch the vitreous.[23]

It was not until Kasner proposed an 'open sky' vitrectomy technique in 1968, and Machemer's subsequent development of a Vitreous Infusion suction cutter (VISC) for vitreous surgery in 1971, did the attitude of ophthalmologists change towards the vitreous.[24, 25] These initial pursuits sparked the interest of more scientists to delve into vitreous research, and the evidence generated thereof has contributed immensely to this area of research. These initial pursuits also fuelled the rapid advancement of vitreoretinal surgical techniques and instrumentation that have ensured the safe removal of the vitreous without significant detriment to ocular health. These surgical

advancements also succeeded in consolidating the original viewpoint about the vitreous as a contributor to only intraocular clarity and intraocular pressure and hence, no physiological importance was ascribed to it.[26, 27]

The rapid evolvement of technology in the last 25 years has allowed for the development of sophisticated imaging devices and biochemical analytical methods for vitreous research. These technological breakthroughs have provided scientists with invaluable insights into the molecular constitution of this seemingly invisible ocular structure, and its contribution to ocular health and disease.[1, 28] Thus, our present understanding of the vitreous transcends its historically-assigned role as a space filler; it is an integral tissue that actively participates in vision. Today, we know that the vitreous is essential for maintaining molecular and mechanical homeostasis of the eye.[29] This understanding guides our present research into healthy and diseased states of the vitreous, and dictates what future research should entail.

1.3 MAJOR STRUCTURAL COMPONENTS OF THE VITREOUS

1.3.1 Vitreous Embryology

The formation of the human vitreous is a complex process which remains unclear to date.[30] This complex process begins with the primary vitreous stage, characterised by the formation of a primary vitreous body and vascularisation of the primary vitreous, and the secondary vitreous stage, typified by production of acellular secondary vitreous, hyalocyte migration, and hyaloid artery regression.[30, 31]

1.3.1.1 Primary Vitreous stage

During the third to fourth week of gestation at the 4 to 5mm stage, the lens surface ectoderm separates from the neural ectoderm to allow for the formation of the primary vitreous.[30, 32] The developing space is then bridged with a fibrillar meshwork of periodic acid-Schiff (PAS)-positive and Alcian blue.[33] Concurrently, mesodermal cells migrate from the superior border of the anterior optic vesicle through the area of the embryonic choroidal fissure into the area of primary vitreous.[34] This contribution by the lens ectoderm into the primary vitreous content is terminated with the formation of the hyaline lens capsule at the sixth week of gestation.[30]

At the 10mm stage, vascularising mesodermal cells enter the optic cup via the foetal fissure into the vitreous space.[30] These mesodermal cells are derived from the dorsal ophthalmic artery which branches off the internal carotid system.[30] The mesodermal cells develop into the hyaloid artery which diverges into *vasa hyaloidea propria* towards the anterior portion of the optic cup and anastomoses with the vasoproliferative tissue of the early tunica vasculosa lentis (Figure 1.3).[31, 34] By the 30mm stage, vascularisation of the primary vitreous is fully developed. More importantly, the anterior portion of the optic fissure closes the optic cup by 10 to 12mm stage, making the eye a closed system from this point forward.[31]

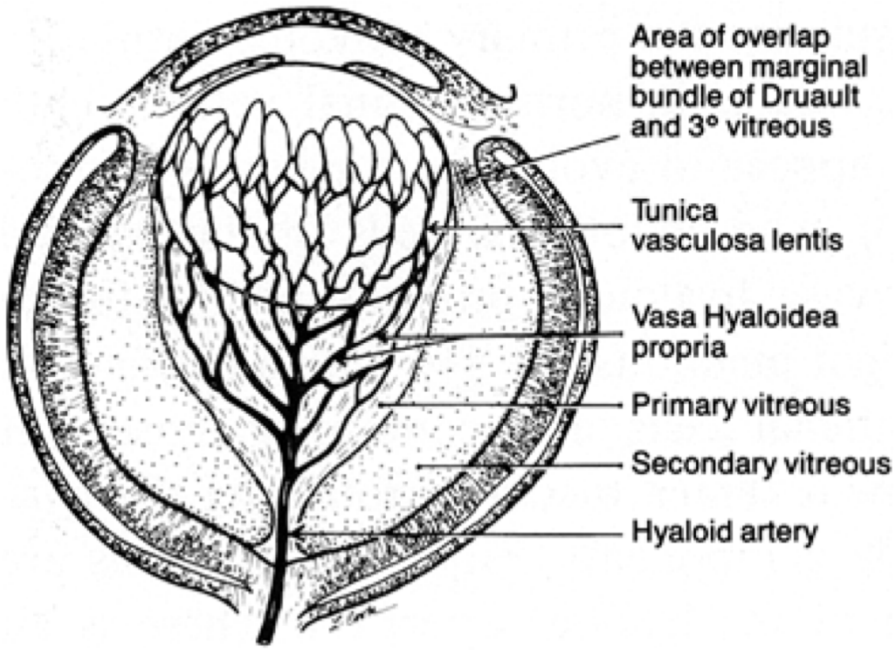


Figure 1.3: *Structures of the embryonic vitreous. Image courtesy Sang.*[30]

1.3.1.2 Secondary vitreous stage

The secondary vitreous stage is characterised by the production of the acellular secondary vitreous, migration of hyalocytes, and regression of the hyaloid system.[30] The acellular secondary vitreous, derived from the neural ectoderm, develops during the 13 to 70mm embryonic stage and fills the space between the internal layers of the retina and the peripheral border of the primary vitreous.[31] This acellular vitreous is an extracellular matrix consisting largely of collagen II fibres and a small amount of hyaluronan (HA).[30] The demarcation line or 'intravitreous membrane' between the primary and secondary vitreous is what later becomes the walls of Cloquet's canal (Figure 1.4).[32, 33]

Monocytic phagocytic cells, hyalocytes, which are believed to originate as reticular cells of the bone marrow, traverse the hyaloid artery system and embed in the cortical secondary vitreous gel.[30] Hyalocytes synthesise HA during their non-phagocytic phase. The HA is then superimposed on the collagen fibres produced by the fibrocytes, which are fibroblast-like cells associated with the hyaloid vasculature.[30, 33]

The hyaloid system begins to atrophy posteriorly in the 40mm embryonic stage during the ninth week of gestation and by the 65mm embryonic stage, the Cloquet's canal can be identified as the junction of the primary and secondary vitreous, extending from the optic disc posteriorly to the degenerating tunica vasculosa lentis anteriorly.[30] The capsula perilenticularis refers to the junction between the primary and secondary vitreous behind the lens, and it houses the Berger's space or the retrolental space of Erggelet (Figure 1.4).[35, 36] The secondary vitreous attaches to the posterior lens surface at the Egger's line, which condenses later to form Wieger's hyaloideocapsular ligament.[30]

It is not clear what triggers the regression of the hyaloid system. However, experimental evidence has shown that a protein native to the vitreous, which impedes angiogenesis, may be involved in this process. The cellular mechanisms that result in this regression include accumulation of increased glycogen and lipid deposits, and subsequent endothelial cell loss and pericyte degeneration. Hyalocytes migrate into the adventitia of the hyaloid artery and phagocytise the degenerated cell components

of the hyaloid artery. Following complete regression of the hyaloid artery, only residual, atrophic strands of ghost cells remain in the Cloquet's canal. By the 240mm stage during the seventh month of gestation, the antenatal vitreous has reached its final stages of development and possesses the characteristics of a fully developed vitreous. At this stage, the hyaloid system and the tunica vasculosa lentis have almost completely regressed and the primary vitreous has atrophied.[30]

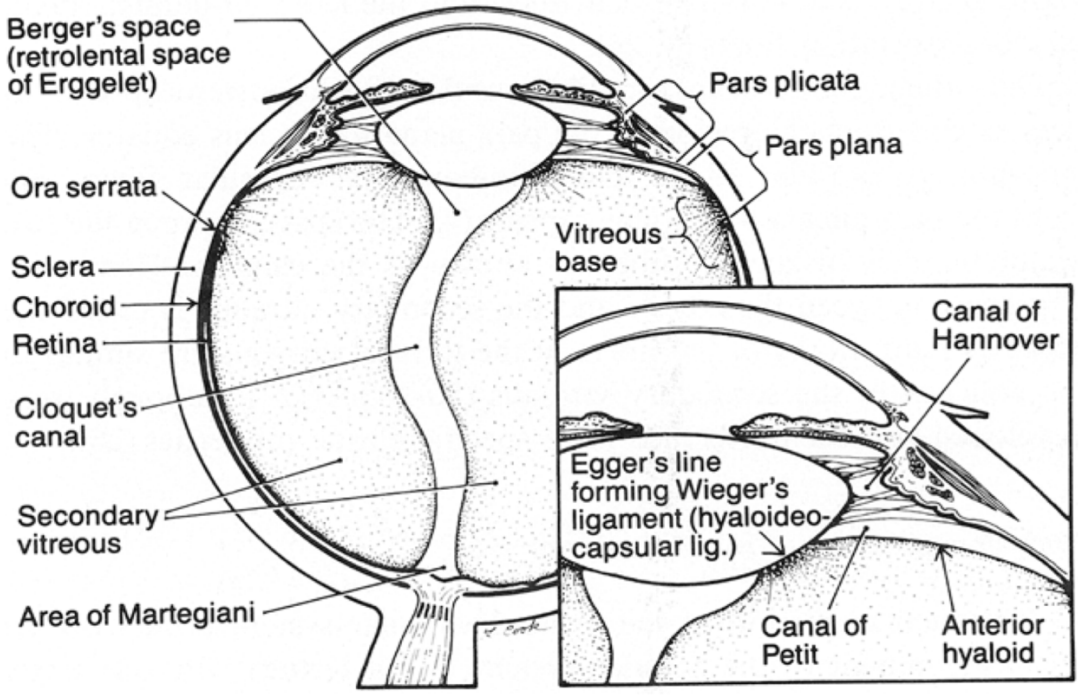


Figure 1.4: *Structures of the developed vitreous. Image courtesy Sang.*[30]

1.3.2 The Adult Vitreous

A look at the adult vitreous body reveals that it is the largest structure within the eye.[14] With a total volume of approximately 4mL, the vitreous body is composed of mainly water (about 98-99%), collagen fibres, glycosaminoglycans [GAGs; predominantly HA]; non-collagenous proteins (including opticin and versican), and small amounts of trace metals and elements.[37, 38] The gel nature of vitreous is credited to the interaction between its two principal components, collagen and HA (Figure 1.3).

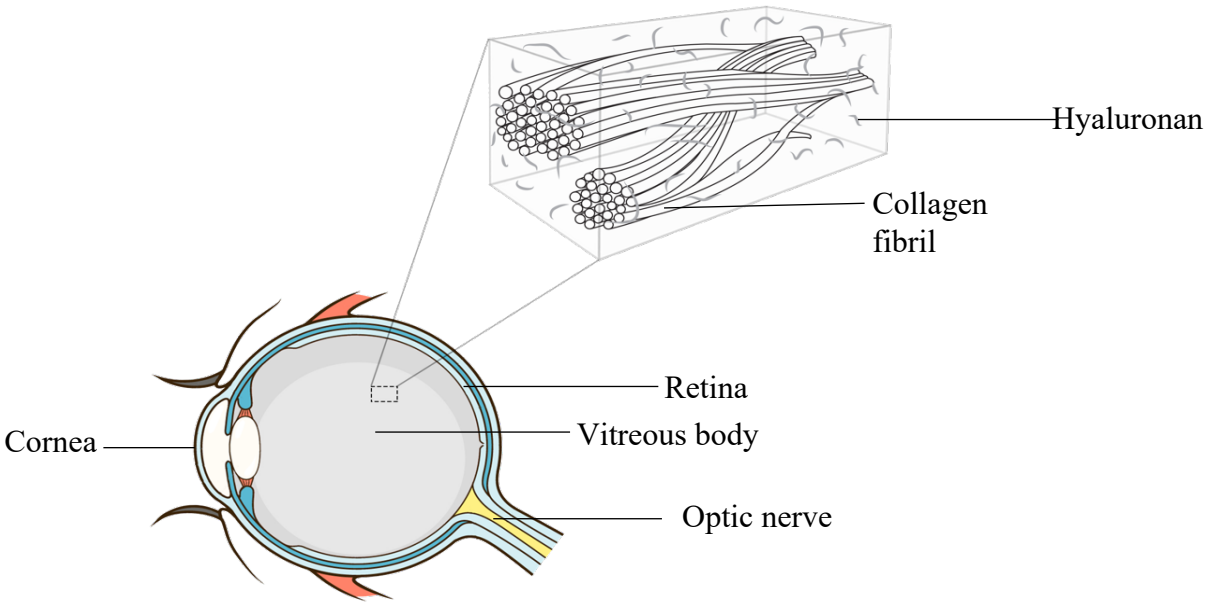


Figure 1.5: *Cross-sectional diagram of the human eye showing the vitreous and the interaction between its two principal components, collagen and hyaluronan. Image courtesy Ankamah et al.[14]*

1.3.2.1 Vitreous Collagen

Collagen concentration within the human vitreous body approximates to 300µg/ml, accounting for 0.5% of the total vitreous protein.[39] The vitreous assembles collagen fibres in a heterotypic fashion that consist of collagen types II, V/XI, VI and IX, with collagen II being the most abundant. Vitreous collagen fibrils are thin and unbranched with uniform diameter ranging between 10 to 20nm (depending on the species).[40] Collagen is not uniformly distributed in the vitreous, the highest concentration is present at the vitreous base, reflecting the main site of synthesis.[41] Collagen constitutes the essential structural component of the vitreous and its removal *in vitro* results in vitreous liquefaction.[26, 42]

1.3.2.1.1 Collagen II

Collagen II is the predominant collagen within the vitreous, accounting for 60-75% of the vitreous collagen.[43] It is described as a fibrillar collagen along with collagens I, III, V, XI, V/XI, XXIV and XXVII.[26] Collagen II comprises three identical α-chains, with a composition of $\alpha1(II)_3$. When collagen II molecules are secreted into the extracellular environment, they appear in a soluble precursor form, procollagen, with terminal extensions called amino-propeptide (N-propeptide) and carboxy-propeptide (C-propeptide).[44] Once in the extracellular environment, these extensions are removed or "processed" by specific enzymes leaving short non-collagenous telopeptides at each end of the main triple-helical region.[41] This process reduces the solubility of the collagen molecules and allows them to participate in fibril formation. Collagen II,

together with type V/XI, self-assemble into staggered arrays and cross-link to form the rope-like core of the collagen fibrils of the vitreous.[45]

1.3.2.1.2 Collagen V/XI

Collagen V/XI is a hybrid collagen molecule which contains chains from both types V and XI, and forms approximately 10% of the vitreous collagen.[44] Similar to collagen II, collagen V/XI is secreted as a procollagen with N- and C-propeptides, just that with collagen V/XI, the N-propeptide is only partially processed whilst the C-propeptide is removed by processing. The type V/XI collagen assembles into heterotypic (mixed composition) fibrils in the vitreous along with type II and type IX collagen.[41]

1.3.2.1.3 Collagen VI

Type VI collagen exists in small quantities in human and bovine vitreous as separate microfibrils from the heterotypic collagen fibril assembly.[44] Collagen VI has been shown to anchor heterotypic collagen fibrils and HA to each other, and are thus perceived to be involved in the (posterior) vitreoretinal attachment and detachment processes.[46]

1.3.2.1.4 Collagen IX

Collagen IX is a fibril-associated collagen with interrupted triple helices (FACIT; other FACIT collagens include types XII, XIV, XVI and XIX) found on the surface

of collagen fibrils.[41] Type IX collagen is composed of three different α-chains, i.e. α1(IX), α2(IX) and α3(IX).[46] Type IX collagen is composed of three collagenous domains (COL 1 to 3) interspersed between four non-collagenous domains (NC1 to 4), and it is covalently bound to type II collagen through the COL2 domain.[45] In the vitreous, collagen IX is a proteoglycan with a single 15-60 kDa chondroitin sulphate GAG side-chain covalently linked to the α2(IX) chain of the NC3 domain.[45] The proteoglycan content of collagen IX approximates to 25 μg protein/ ml vitreous gel. [47]

Collagen IX, with its chondroitin sulphate side-chains, shields collagen II from exposure on the fibril surface.[41] Reduction or loss of this shielding effect by aging or disease causes exposure of the "sticky" surfaces collagen II and subsequent lateral fusion of fibrillar collagen II (a mechanism associated with vitreous degeneration).[45]

1.3.2.2 Hyaluronan

Hyaluronan (HA), a polydisperse, uronic acid-containing, anionic polysaccharide, is the predominant GAG within the vitreous.[48] The concentration of HA within the vitreous ranges between 0. 02 – 1 mg/cm^3.[49] Others have estimated intravitreal concentrations of HA to be approximately 240 μg/ml vitreous gel, which equates to 90% of the total uronic acid-containing macromolecules within the vitreous.[47] HA, unlike other GAGs, is synthesised on the cytoplasmic surface of the plasma membrane (not in the Golgi apparatus) via the action of three HA synthesizing enzymes: HAS1, 2, and 3.[47] As the primary mediator of the internal adhesivity of the vitreous, HA

forms a highly entangled mesh that plays a synergistic role with collagen and proteoglycans in regulating the stiffness of the vitreous.[50] In that, the charges on HA provide an internal osmotic pressure to swell the vitreous tissue and suspend collagen fibres.[47]

1.4 ANATOMICAL REGIONS OF VITREOUS

The vitreous body is subdivided into 3 broad anatomical regions: vitreous cortex (anterior and posterior), central vitreous, and vitreous base.

1.4.1 Vitreous Cortex

The vitreous cortex is a lamellar structure attached to the ILM of the retina posterior to the peripheral vitreous by an extracellular matrix "adhesive" consisting of fibronectin, opticin, laminin, heparan sulfate, and chondroitin sulphate. Anteriorly, the vitreous cortex is attached to the lens.[51] The vitreous is relatively acellular with only a monolayer of mononuclear phagocytes, hyalocytes, located within the posterior vitreous cortex, about 50μm from the ILM.[52]

1.4.2 Central Vitreous

The central vitreous is the largest anatomical zone of the vitreous and is enclosed by the vitreous cortex. Compared with other zones, the central vitreous is the zone with the lowest concentration of collagen fibres.[53] Collagen fibres within the central vitreous are secreted by the ciliary body into the vitreous body.[41] In children, the central vitreous is typified by a homogenous distribution of collagen fibrils separated

by HA molecules, and hence causes only a little light scattering. [54] In older adults, the central vitreous is usually subjected to liquefaction (see section 1.3.1), resulting in significant light scattering. [54] Within the central vitreous is the Cloquet's canal, a clear central space that was once occupied by the hyaloid artery *in utero,* and courses through the central vitreous to the posterior vitreous cortex. [55]

1.4.3 Vitreous Base

The vitreous base is the location of the highest collagen concentration as well as the strongest point of insertion for collagen fibrils within the vitreous.[53, 56] The anterior border of the vitreous base is located approximately 2 mm anterior to the ora serrata, and the posterior border, about 1 to 3 mm posterior to the ora serrata[57]. In addition, it is the only zone where collagen fibres course perpendicular to the retina. The vitreous base is not a flat structure; collagen fibres in the vitreous base extend into the anterior vitreous body and mechanically inserts into the nonpigmented epithelium of the ciliary body as well as the neuroglia of the peripheral retina.[53] This strong mechanical adhesion possibly explains the strong, continuous attachment of the vitreous at the vitreous base even when there is PVD elsewhere and the propensity for retinal tears to occur at the posterior border of the vitreous base.[53, 58]

1.5 FUNCTIONS OF THE VITREOUS

The vitreous contributes to intraocular media clarity, the regulation of intraocular oxygen tension, and the maintenance of IOP.[59] It also confers protection by acting as

a shock absorber, done by the collagen fibres which reduce the compressive forces of HA when the globe is exposed to external pressure.[60, 61] The vitreous acts as a reservoir for nutrients and metabolites that it receives by synthesis within the non-pigmented ciliary epithelium and retinal pigment epithelium.[26, 62-64] Hyalocytes play a vital role in modulating intraocular inflammation in non-inflamed eyes, thereby contributing to intraocular transparency.[52]

1.6 SIMILARITIES BETWEEN VITREOUS AND SYNOVIAL TISSUE

As a connective tissue matrix, the vitreous shares similar biochemical properties with the synovial tissue around joint spaces. Both the vitreous and synovial fluid are viscoelastic tissues consisting mainly of collagen and HA. Vitreous collagen type II, however, differs slightly in chemical composition due to the presence of terminal peptide constituents in its collagen.[65] Particular to the vitreous and cartilage is an acidic glycoprotein, with a five-armed configuration, *cartilage oligomeric matrix protein.*[66] Its function in the vitreous is, however, yet to be identified. The vitreous and synovial fluid separate tissues and protect against friction and high-frequency stresses.[67] The similarities in macromolecular structure between the vitreous and joints is the underlying explanation as to why both tissues show characteristic clinical manifestations in inherited collagen disorders such as Marfan and Ehlers-Danlos syndrome.

1.7 VITREOUS ANTIOXIDANTS PROFILE IN HEALTH AND DISEASE

As previously defined, an antioxidant is a substance that, when present in low concentration compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of the substrate.[6] The vitreous accumulates a high concentration of hydrosoluble antioxidants which contribute to protection of the globe from oxidative stress and radiation damage (Figure 1.4).[68] The vitreous antioxidants can be broadly classified into enzymatic and non-enzymatic antioxidants (Figure 1.5).

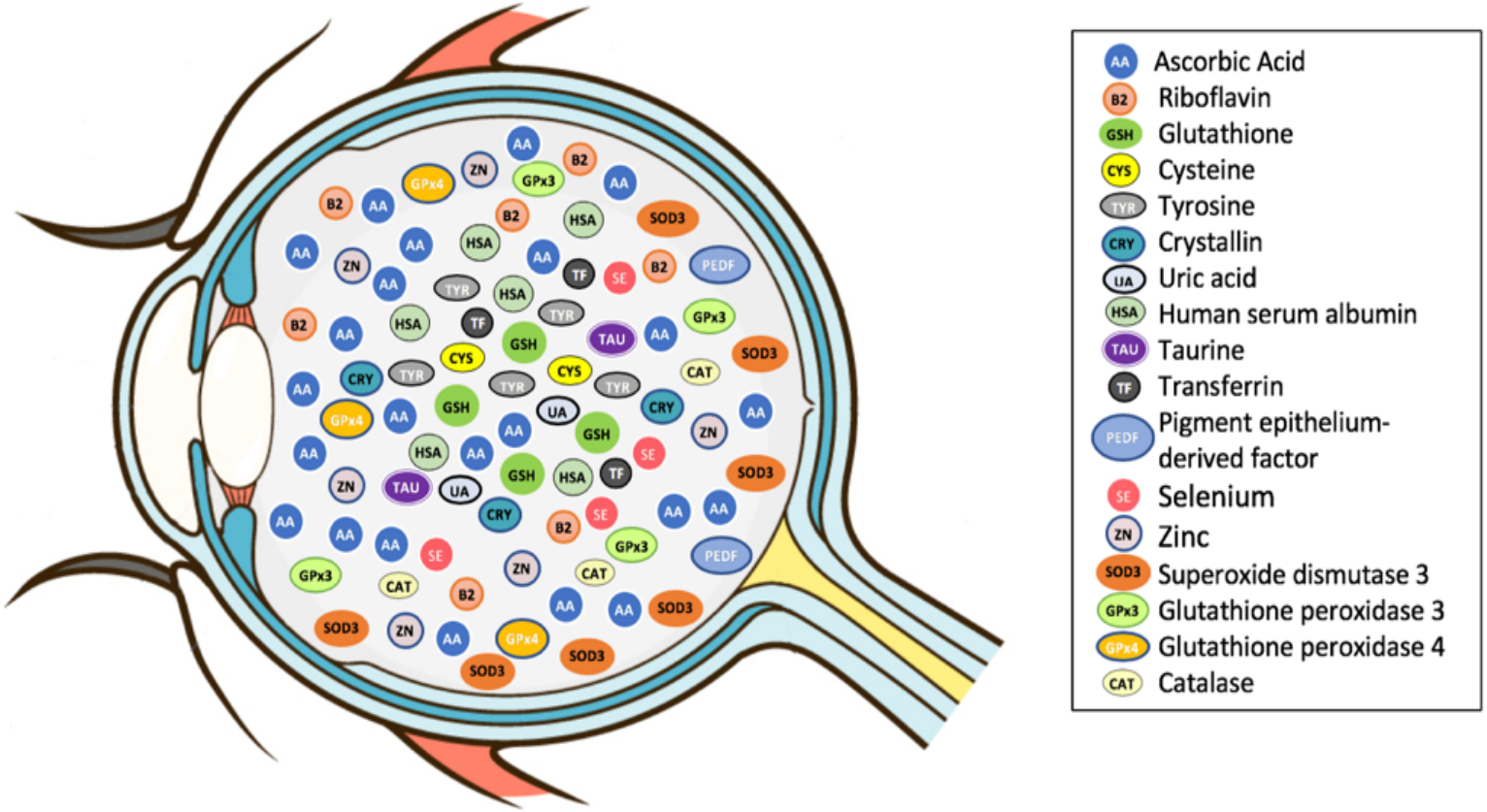


Figure 1.6: *Diagram showing the antioxidants within the vitreous. Image courtesy of Emmanuel Ankamah, Waterford, Ireland.*

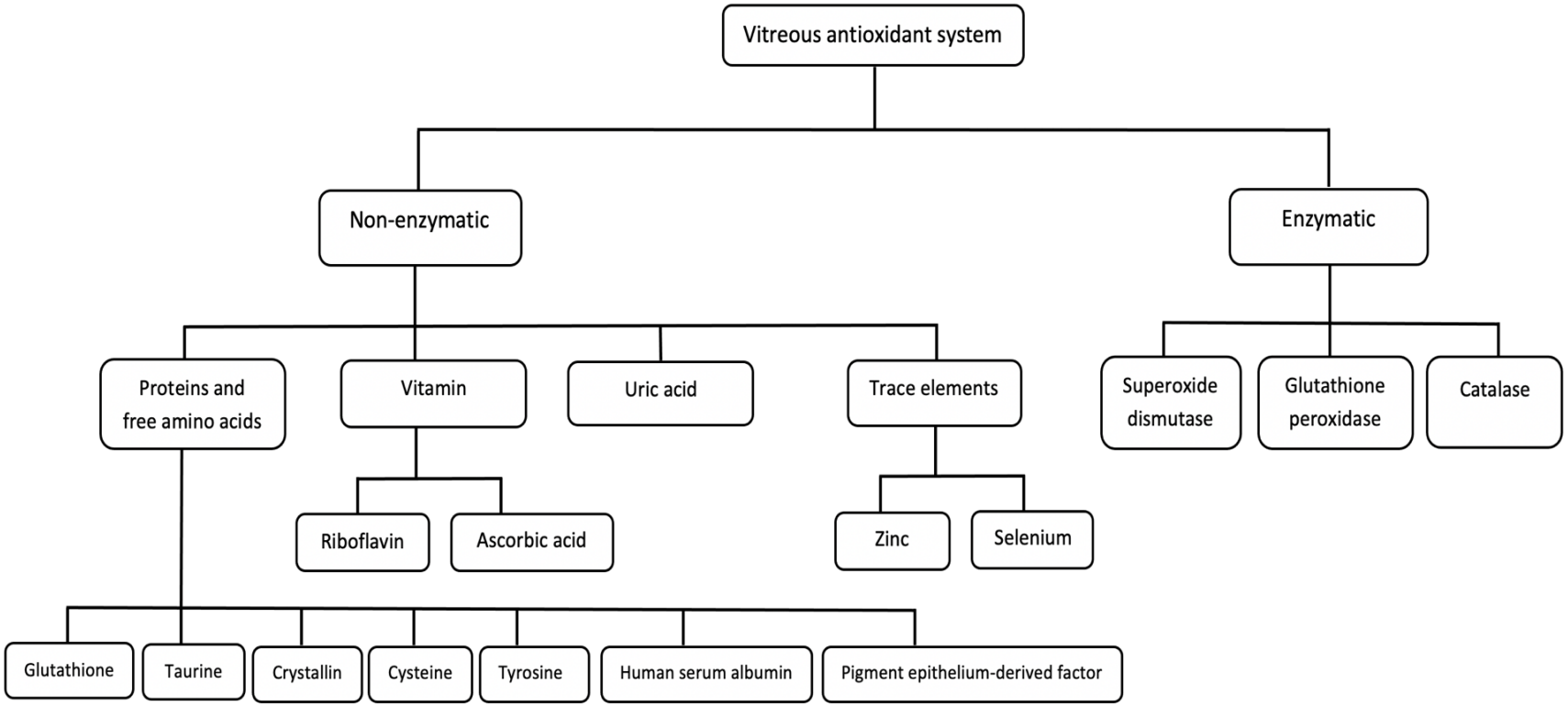


Figure 1.7: *Classification of the vitreous antioxidants. Image courtesy of Emmanuel Ankamah, Waterford, Ireland.*

1.7.1 Non - enzymatic vitreous antioxidants

Antioxidants within this class comprise non-enzymatic molecules that are capable of rapidly inactivating radicals and oxidants.[69] Based on the source of non-enzymatic vitreous antioxidants, they can be classified into metabolic and nutrient non-enzymatic antioxidants. Metabolic antioxidants are endogenous antioxidants produced by the body and include glutathione, metal-chelating proteins, uric acid, and transferrin. Nutrient antioxidants include the class of non-enzymatic antioxidants that are exogenously sourced through foods and supplements, for example, vitamin C, vitamin B_2, and trace metals (zinc and selenium).[70]

1.7.1.1 Vitamins

1.7.1.1.1 Vitamin A

Vitamin A is a general term that describes a group of compounds including retinol, retinaldehyde, and retinoic acid.[71] Humans source this lipophilic micronutrient from dietary sources including fish oils, red palm oil, dark leafy vegetables, carrots, milk and milk products.[72] Vitamin A has been described as an "indirect antioxidant" whose function is to transcriptionally regulate a number of genes involved in mediating the body's antioxidant responses.[73] Vitamin A is also crucial for retinal pigment epithelium (RPE) cell proliferation and extracellular matrix modification.[74] Vitamin A has been shown to be essential for the development of the foetal vitreous body.[75] Indeed, the evidence points towards vitreous developmental defect (an absence of vitreous body) in late vitamin A deficient foetuses and newborns.[75, 76] Vitamin A has

not been detected in the adult human vitreous and the reason for this is not clear. Since the adult vitreous is largely an aqueous medium, it is reasonable to think that a lipid-soluble vitamin may not readily dissolve in it.

1.7.1.1.2 Vitamin C

Also referred to as ascorbic acid (AA), Vitamin C is a water-soluble molecule present in most tissues in its anionic state.[13] Humans cannot synthesise AA *de novo* and source this molecule exogenously.[77] The vitreous gel receives its supply of AA from the plasma by an active transport from the ciliary process of the ciliary body.[78] AA concentration within the vitreous body approximates to 2 mmol/L, about 33 times higher than plasma concentration.[79] Also, AA within an intact gel vitreous is higher than in a liquefied vitreous and in the vitreous of proliferative diabetic retinopathy (PDR) patients.[59, 80] It also appears that there is an association between vitreous vitamin C and serum vitamin C as evidenced by the reduction in both serum and vitreal levels of vitamin C in PDR patients.[80]

As an antioxidant, AA is oxidised in order to convert superoxide anions and lipid hydroperoxidases into stable forms, thereby preventing lipid peroxidation, the oxidative damage of lipids. AA consumes oxygen released at the vitreo-retinal interface, in an ascorbate-dependent fashion, and guards against intraocular oxidative stress and nuclear cataract development.[59] In fact, the evidence indicates that the depletion of intravitreal vitamin C may cause macular ischemia in PDR patients owing to an increase in intravitreal, hypoxia-induced oxidative stress.[80] AA also functions as

an intrinsic modulator of hyalocyte proliferation and of extracellular matrix production by hyalocytes.[81, 82] AA serves as an enzyme co-factor to a number of enzymes, especially hydroxylases, which are involved in collagen synthesis.[83]

1.7.1.1.3 Vitamin B2

Riboflavin has been detected in both human and animal vitreous, with 0.8 µg/ 100 ml and 8.0 µg/L average concentrations detected in the ox and bovine vitreous, respectively.[84, 85] Riboflavin plays an essential role in the glutathione redox cycle and guards against lipid peroxidation.[86] Riboflavin acts as the precursor for two coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are involved in energy metabolism. FAD is essential for the activity of glutathione reductase, which converts oxidised glutathione (GSSH) into reduced glutathione (GSH) (see discussion of GSH below).[87] Also, riboflavin functions as an antioxidant through the oxidation of dihydroriboflavin, the reduced form of riboflavin, to produce reducing equivalents for the deactivation of hydroperoxides.[88] Dihydroriboflavin also protects against reperfusion oxidative damage by reducing oxidised iron in hemeproteins.[89, 90]

Riboflavin directly scavenges for free radicals produced by mutagens, thereby inhibiting their mutagenicity.[91] For a review of the antioxidant ability of riboflavin, see [86, 87]. On the other hand, riboflavin is a photosensitiser which can mediate a riboflavin-sensitised photochemical reaction and result in age-related liquefaction of

the vitreous.[92] Thus, therapeutic use of riboflavin for eye diseases may be a two-edged sword that needs to be wielded carefully to achieve salubrious outcome.

1.7.1.2 Proteins and free amino acids

Proteomic analysis of the human vitreous has revealed proteins and several amino acid constituents that play important roles in ocular development as well as function as antioxidants.[93, 94] The majority of the vitreous antioxidant proteins are located within the central vitreous. They include glutathione, taurine, crystallin, cysteine, uric acid, tyrosine, human serum albumin, transferrin, and pigment epithelium-derived factor.[95]

1.7.1.2.1 Glutathione (GSH)

Glutathione is a cysteine-containing peptide and a thiol antioxidant with an average concentration of 0.26 mmol/l.[96-98] The concentration of glutathione within the vitreous is relatively lower compared to AA.[97] As an antioxidant, glutathione can directly remove selected oxygen radicals and indirectly assist in the recycling of vitamins C and E.[99] Also, GSH inhibits the degradation of HA by acting as a scavenger for hydroxyl radicals.[100, 101]. GSH is a cofactor for glutathione peroxidase activity of reducing lipid hydroperoxides, producing alcohol and GSSH in the process.[87]

Reduced intravitreal GSH level has been linked with the pathological complications of inflammation and neovascularisation in PDR and Eales' disease.[98, 102] Other reports, on the contrary, indicate an increase in intravitreal GSH in PDR eyes.[103] This increase may describe a protective response to detoxify the diabetes-associated redox

alteration of the vitreous. Indeed, profound structural abnormalities have been identified in the human vitreous that are independent of diabetes effects on the retina.[54, 104, 105]

1.7.1.2.2 Taurine

Taurine is a free amino acid that abounds in tissues during development.[106] Taurine has been detected in the rat vitreous at a concentration of 1.72μmol/ml.[107] Although the exact role of taurine within the vitreous is yet to be elucidated, taurine, as an organic osmolyte, has been proposed to be involved in the vitreous-mediated ionic exchanges that occur between the retina and the anterior segment.[108] In addition, it has been proposed that the retina possibly receives its supply of taurine from the vitreous.[109, 110] Taurine provides antioxidative and neuroprotective functions to ocular tissues, although this mechanism has not been fully understood in the human eye.[111] Depletion or deficiency of taurine leads to loss of photoreceptors and can impede visual function in man and in animal models.[109, 112]

1.7.1.2.3 Crystallin

Crystallin is a chaperone or stress protein which accumulates within the lens more than all other ocular tissues.[113] Both α- and β- crystallins have been isolated in the rat vitreous.[114] β-crystallin B2 (molecular weight ~ 23kDa) has been recently identified by matrix -assisted laser desorption ionisation time of flight (MALDI-TOF) in the normal human vitreous.[115] β-crystallin S, β-crystallin A4, β-crystallin A3, α-crystallin

B chain, and γ-crystallin C have also been found in the vitreous body of both PDR patients and controls.[116] Crystallin levels were significantly lower in the vitreous from PDR patients compared with controls. Crystallins perform an anti-apoptotic role by inhibiting the formation of ROS, thereby reducing oxidative stress.[117]

1.7.1.2.4 Cysteine

Cysteine, a non-essential amino acid with a highly reactive thiol group, is found in most peptides and proteins. Cysteine acts as the rate limiting precursor for the synthesis of GSH.[118] As an antioxidant, its reactive thiol group is oxidised to cystine disulphide and aids in maintaining a redox equilibrium within a cell, tissue or biofluid.[119]

1.7.1.2.5 Tyrosine

L-tyrosine is a monophenolic amino acid and a byproduct of the pentose phosphate pathway.[120] The concentration of tyrosine within the adult vitreous is 91 μmol/l.[121] Antioxidant activities of tyrosine, as observed *in vitro,* include anti-lipid peroxidation, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities.[120]

1.7.1.2.6 Human serum albumin (HSA)

HSA is an anionic globular protein with a molecular weight of approximately 69 kDa.[115, 122] HSA is sourced by filtration from blood and constitutes about 80% of the

average protein concentration within the healthy vitreous body.[115, 123] The molecular structure of HSA confers multiple antioxidant properties on it including an ability to bind potential ROS-generating ligands (for example, the transition metals copper and iron), scavenge hydroxyl radicals through its reduced cysteine residue (Cys34), and scavenge peroxynitrite through its thiol (-SH) group (for a review of the antioxidant properties of HSA, see [124]).

1.7.1.2.7 Transferrin

Transferrin (molecular weight ~ 80kDa) is a glycoprotein with two specific high-affinity binding sites for iron.[125, 126] The vitreous contains a mean transferrin concentration of 0.0878 g/L.[28] As an antioxidant, transferrin is an iron chelator which keeps ionic iron sequestered at physiological PH and minimises the involvement of iron in iron-dependent radical reactions.[127] This property helps to reduce intravitreal iron toxicity during vitreous haemorrhage.[128]

1.7.1.2.8 Pigment Epithelium-Derived Factor (PEDF)

PEDF is a 50 kDa glycoprotein, a member of the serine protease inhibitors, which is produced by the retinal pigment epithelium.[3] The mean concentration of PEDF has been shown to be higher in the vitreous from diabetic macular oedema patients (2.03 µg/ml) compared to the normal vitreous (0.83 µg/ml).[129] Also, PEDF has been detected by proteomic and western blot analyses in the vitreous of normal and PDR patients, with a downward regulation of PEDF in the vitreous of PDR patients.[116] A

more recent study, however, has indicated that PEDF is absent in the normal vitreous but present in the vitreous during ischemic retinopathies.[130] PEDF exerts anti-angiogenic activity within the eye, and its loss has been implicated in the pathogenesis of AMD.[131]

1.7.1.3 Trace elements

Two trace elements detected in human vitreous are selenium and zinc.

1.7.1.3.1 Selenium

Selenium is an essential trace element found within both the adult and infant vitreous, with an average concentration of 0.1035μmol/L.[28] A trend of higher concentrations of selenium has been reported in the adult male vitreous compared to the female.[132] High selenium rich sources include sea foods, meat products, and cereals. Low levels sources include milk, fruits, and vegetables.[106, 133] Selenium can exist in biological systems as a selenoprotein (an enzymatic antioxidant; eg., selenoprotein P and glutathione peroxidase), an organic selenium compound (eg., selenomethionine and dimethyselenide) or inorganic forms (as selenites and selenates).[134] Selenium functions indirectly as an antioxidant through its incorporation in antioxidant enzymes, selenoenzymes.[132]

1.7.1.3.2 Zinc

Zinc is the second most essential trace metal in the body and the most abundant within the eye.[135, 136] Zinc has been detected in both the adult and infant vitreous.[28, 137] Zinc concentration within the adult human vitreous approximates to 1.95 μmol/L.[28] While its specific roles within the vitreous are yet to be elucidated, zinc is known to exert its antioxidative properties by protecting sulfhydryl groups from oxidation. Also, zinc acts as a stimulus for the synthesis of the cysteine-rich, metal-binding protein, metallothionein.[136] Metallothionein functions as a scavenger for damaging oxygen free radicals (e.g., hydroxyl radicals) and protects tissues from various forms of oxidative injury including lipid peroxidation and glycoxidation (a phenomenon which can result in vitreous degeneration).[138] In fact, in Eales' disease, in which oxidative stress has been implicated as a potential causative mechanism, studies have reported reduced levels of zinc and increased levels of oxidation and peroxidation products within the vitreous.[102, 136]

1.7.1.4 Uric acid (UA)

Uric acid, a degradation product of the metabolic breakdown of purine nucleotides, functions as an antioxidant at normal concentrations. In the presence of oxidative stress, however, there is upregulation of UA concentrations and a concurrent shift in redox balance, causing UA to become oxidant.[139, 140] As a water-soluble physiological antioxidant, UA reacts highly with peroxyl or hydroxyl radicals to yield urate, its intermediate radical, which is subsequently reduced by ascorbate as part of an overall

antioxidant effect.[141, 142] In bovine vitreous, the concentration of UA is 170μM.[143] Intravitreal UA levels of 156 - 170 μmol/l have been reported for subjects with diabetic macular oedema, 3-fold higher than non-diabetic controls (52 – 70 μmol/l).[139, 144]

***Table 1.1**: Table showing the concentrations of antioxidant molecules in the human, animal, and diseased vitreous from previous studies*

Antioxidant	Author	Human vitreous*	Animal Vitreous	Vitreous from diseased eye
Ascorbic Acid	Duarte & Lunec [79]	2 mmol/l		
	McGahan [145]		0.43 mmol/kg - rabbit	
	Park[80]	172.7 µg/mL		19.1 µg/mL - PDR
Riboflavin	Philpot & Pirie [84]		0.8 µg/ 100 ml - ox	
	Long [85]		8.0 µg/L - bovine	
Glutathione	Sulochana et al.[102]			2.8 µg/mg protein – ED 17.7 µg/mg protein – DVH
	Cicik et al.[98]	0.26 mmol/l		0.58 µmol/l – PDR 15.7 µmol/l – PVR
	Géhl et al.[103]	2.35 µmol/µg protein		4.54 µmol/µg protein - PDR
Taurine	Diederen et al.[146]	22.6 µM		26.0µM - RRD 28.1µM – PDR
	Heinämäki et al.[107]		1.72µmol/ml	
Uric acid	Sebag [143]		170µM - bovine	
	Krizova et al.[139, 144]	156 - 170 µmol/l		52 – 70 µmol/l - DME
Tyrosine	Shih [121]	91 µmol/l		
Transferrin	Kokavec [28]	0.0878 g/L		
Selenium	Kokavec [28]	0.1035µmol/L		
Zinc	Kokavec [28]	1.95µmol/L		
Superoxide dismutase	Sulochana et al. [102]			0.9 IU / mg protein – ED 22.1 IU / mg protein - DVH
Glutathione peroxidase	Sulochana et al. [102]			0.61[#] - ED 0.49[#] - DVH
Catalase	Mayer [147]	58 µl O_2/mg protein		
PEDF	Ouchi et al.[129]	0.83 µg/ml		2.03 µg/ml - DME

*, Samples used were from cadaver or eyes undergoing vitrectomy for idiopathic macular holes or epiretinal membranes; ED, Eales' disease; DVH, Diabetic vitreous haemorrhage; PDR, Proliferative diabetic retinopathy; PVR, Proliferative vitreoretinopathy; RRD, Rhegmatogenous retinal detachment; DME, Diabetic macular oedema; #, µmol of GSH utilised/mg protein/ min; PEDF, Pigment epithelium-derived factor

1.7.2 Enzymatic vitreous antioxidants

The antioxidant enzymes detected in the vitreous are superoxide dismutase, glutathione peroxidase and catalase.

1.7.2.1 Superoxide dismutase (SOD)

SOD is a metalloprotein enzyme that catalyses superoxide radicals to hydrogen peroxide and molecular oxygen.[148] SOD is comprised of 3 isoforms: cytosolic SOD (SOD1), the mitochondrial SOD (SOD2), and the extracellular SOD (SOD3).[149-151] SOD1 and SOD3 are copper-and-zinc-containing SOD (Cu/Zn-SOD) whereas SOD2 is a manganese-containing SOD (Mn-SOD).[13, 148] SOD3 isoenzyme, an interstitially-located, homotetrameric, Cu/Zn-containing SOD, is distinctively concentrated at the vitreous base and cortex.[148, 152] SOD3 interacts with specific proteoglycans at the vitreous base and cortex, and functions to regulate oxidative stress response in the vitreous and to prevent oxidative damage to the adjacent neural retina.[152] Thus, dysregulation of SOD3 activity at the vitreous base may belong to the pathophysiological mechanism for oxidative-stress-related vitreo-retinal pathologies such as diabetic vitreo-retinopathy.[152] Average intravitreal concentrations of SOD reported in Eales' disease and diabetic vitreous haemorrhage are 0.9 IU/ mg protein and 22.1 IU/mg protein.[102]

1.7.2.2 Glutathione peroxidase (GPX)

Of the five isoenzymes belonging to this family of selenoenzymes, the extracellular GPx3 and phospholipid GPx4 are found within the vitreous body.[153] As a homotetrameric protein, GPx3 catalyses the reduction of organic hydroperoxides and hydrogen peroxides (H_2O_2) to alcohol and water by employing GSH as an electron donor. GPx4, a monomeric protein, is capable of directly reducing phospholipid and cholesterol hydroperoxides.[154] However, evidence from bioanalytical studies of this enzyme indicates that less than 50% of GPx is active within the vitreous. Also, the antioxidant enzyme activity of GPX has been attributed to the tetrameric form and not the monomeric.[153] This antioxidant activity depends on the availability of reduced GSH.

1.7.2.3 Catalase

Catalase is a tetrahedral hemoprotein that protects tissues from the toxic effects of peroxide by converting peroxides into water and oxygen.[155] The human vitreous body has an average concentration of 58 µl O_2 per mg soluble protein of catalase.[147] Catalase has been detected in the vitreous of PDR patients leading to the suggestion that catalase may be a potential candidate for the treatment of acute ischemic diseases of the retina, although this association requires significant further investigation.[94]

1.8 CHAPTER SUMMARY

The literature summarised in this chapter suggests that the vitreous is principally composed of water, collagen and hyaluronan. In addition, the vitreous is a repository for a number of enzymatic and non-enzymatic antioxidant molecules that contribute to the integrity of the vitreous. Some aspects of this chapter have been published, as the world's first comprehensive review of vitreous antioxidants, in *Antioxidants* (Impact factor: 4.520) under the title, '***Vitreous antioxidants, degeneration and vitreoretinopathy: exploring the link'*** (see Appendix F1 below).

Chapter 2

VITREOUS DEGENERATION

Degeneration of the human vitreous is ubiquitous during life, mainly resulting from aging and disease. Two principal and inter-related processes account for vitreous degeneration: liquefaction (synchysis senilis) and vitreo-retinal dehiscence, which in combination result in posterior vitreous detachment (PVD).[53]

2.1 LIQUEFACTION

Liquefaction is a physico-chemical degenerative change that disrupts the homogeneity of the gel vitreous. It is characterised by dissociation of HA from collagen, aggregation of collagen fibrils (syneresis), and formation of lacunae (collagen-free liquid spaces) within the vitreous body.[58, 156] There is also evidence suggesting collagen degradation as a mechanism for liquefaction.[157] Liquefaction commences quite early in life, with 12.5% of the vitreous gel being liquified by age 18. After increasing during growth and development, the volume of the gel remains stable until about the fifth decade when it begins to decrease in parallel with an increase in liquid vitreous.[158, 159] While the causative mechanisms for vitreous liquefaction have not been fully unravelled, our up-to-date understanding of this process could be summed up into oxidative stress-induced liquefaction and enzymatic-induced liquefaction.[160, 161]

2.1.1 Oxidative stress-induced liquefaction

ROS have been proposed to be the main cause of vitreous structure alteration in aging.[92] Liquefaction has been reported with *in vivo* and *in vitro* animal model experiments investigating the effect of free radicals, generated from photosensitiser and white-light irradiation, on the vitreous.[92, 162-164] Light-induced free radicals have also been shown to decrease the molecular weight of HA, induce HA depolymerisation and, consequently, liquefaction.[92, 118, 165, 166] Liquefaction caused by light-induced ROS has been described to be age-related.[163] This could be because riboflavin, the naturally present photosensitiser molecule within the vitreous, is irradiated by white light on a daily basis during the course of a lifetime. This results in an age-dependent build-up of free radicals that contribute to the molecular alteration of vitreous collagen and HA.[92]

2.1.2 Enzymatic liquefaction

A number of proteolytic enzymes have been implicated in vitreous gel liquefaction. The mechanisms of action of these enzymes in liquefaction can be understood by observing their effects on collagen and HA. Although contrasting views abound in literature regarding the fate of collagen and HA with enzymatic activity, the evidence points to proteolytic enzymatic activity as a mechanism for vitreous liquefaction.

2.1.2.1 Enzyme Effects on Vitreous Collagen

There is evidence to suggest that increased enzymatic activity causes liquefaction either by collagen cleavage or collagen degradation. Vaughan and associates have reported an increase in the level of the enzyme plasmin(ogen) in the vitreous with age. Plasminogen activates a matrix metalloproteinase (MMP), progelatinase or proMMP-2, which results in the cleavage of collagen and subsequently, vitreous liquefaction.[161] While it is true that the mere observation of an increase with age is only an association and not proof of causation, this is an avenue of research to pursue.

Góes et al. reported a degradation of the chondroitin sulphate chains of collagen IX after rabbit vitreous was treated with chondroitin ABC lyase.[167] A morphological change of collagen IX diminishes the shielding effect it renders to the surface of collagen II. This makes collagen II fibres susceptible to lateral fusion, fibrillar aggregation, and liquefaction.[45] It is not clear, however, that chondroitin ABC lyase is active in the human vitreous body.

Bioanalytical studies found that collagenase and trypsin degrade collagen type II *in vitro*, altering the mechanical behaviour of the vitreous and inducing liquefaction.[168-170] This has been confirmed by experimental models of the aging eye, which have shown increased liquefaction, reduced vitreous viscosity, and decreased elasticity, after intravitreal treatment with the active enzyme collagenase.[168, 169] Also, Los and colleagues observed collagen fragmentation in

the lacunae of the vitreous undergoing liquefaction and attributed the cause of this phenomenon to active enzyme activity within the aging vitreous.[157]

2.1.2.2 Enzyme Effects on Vitreous Hyaluronan

The reported effect of enzymatic activity on HA are equivocal. A study by Bishop and colleagues reported only a reduction in vitreous gel wet weight, but not a destruction of its gel state, following complete depolymerisation of HA by Streptomyces HA lyase, chondroitin ABC lyase, and testicular hyaluronidase. Other studies, on the contrary, have reported vitreous liquefaction following extensive HA depolymerisation by hyaluronidase.[163, 171, 172]

2.2 POSTERIOR VITREOUS DETACHMENT (PVD)

2.2.1 Definition

Significant vitreous liquefaction (synchysis) with simultaneous vitreo-retinal interface dehiscence, results in innocuous PVD, characterised by the complete separation of the vitreous cortex from the ILM of the retina in all areas posterior to the vitreous base.[53] Although innocuous PVD is usually not sight-threatening, there are often visual complaints associated with this phenomenon, notably floaters (see section 1.4 below) and photopsia or Moore's light flashes, which are the perception of sudden flashes of light.[53]

2.2.2 Prevalence of PVD

Evidence from autopsy studies has indicated a 51% prevalence of PVD in the seventh decade, which increases further to 63% in the eighth decade.[58] Hikichi

and colleagues found that the prevalence of PVD increased with age in the fifth through the ninth decades when healthy white and Japanese eyes were compared (i.e., from 4%, 24%, 37%, 59%, to 87% in the whites, and from 5%, 21%, 43%, 72%, and 82% in the Japanese).[173] Other clinical studies have shown a prevalence of PVD of 65 % after the age of 65.[53]

2.2.3 Grading and/or stages of PVD

Our understanding of the grading of the stages of PVD have come from slit lamp biomicroscopy, B-scan ultrasound imaging, and OCT (both spectral domain OCT (SD-OCT) and swept source OCT (SS-OCT)] techniques. In 2010, Johnson summarised what was then the up-to-date understanding of the stages of PVD. He explained that age-related PVD followed these stages: stage 0, absence of PVD; stage 1, perifoveal PVD with vitreofoveal adhesion; stage 2, macular PVD (no vitreofoveal adhesion); stage 3, near-complete PVD with vitreopapillary adhesion only; and stage 4, complete PVD (Figure 2.1).[174] The major contribution of Johnson to this subject matter was that "age-related PVD appears to be an insidious, chronic event that begins in the perifoveal macula and evolves over a prolonged period of time prior to vitreopapillary separation."[175]

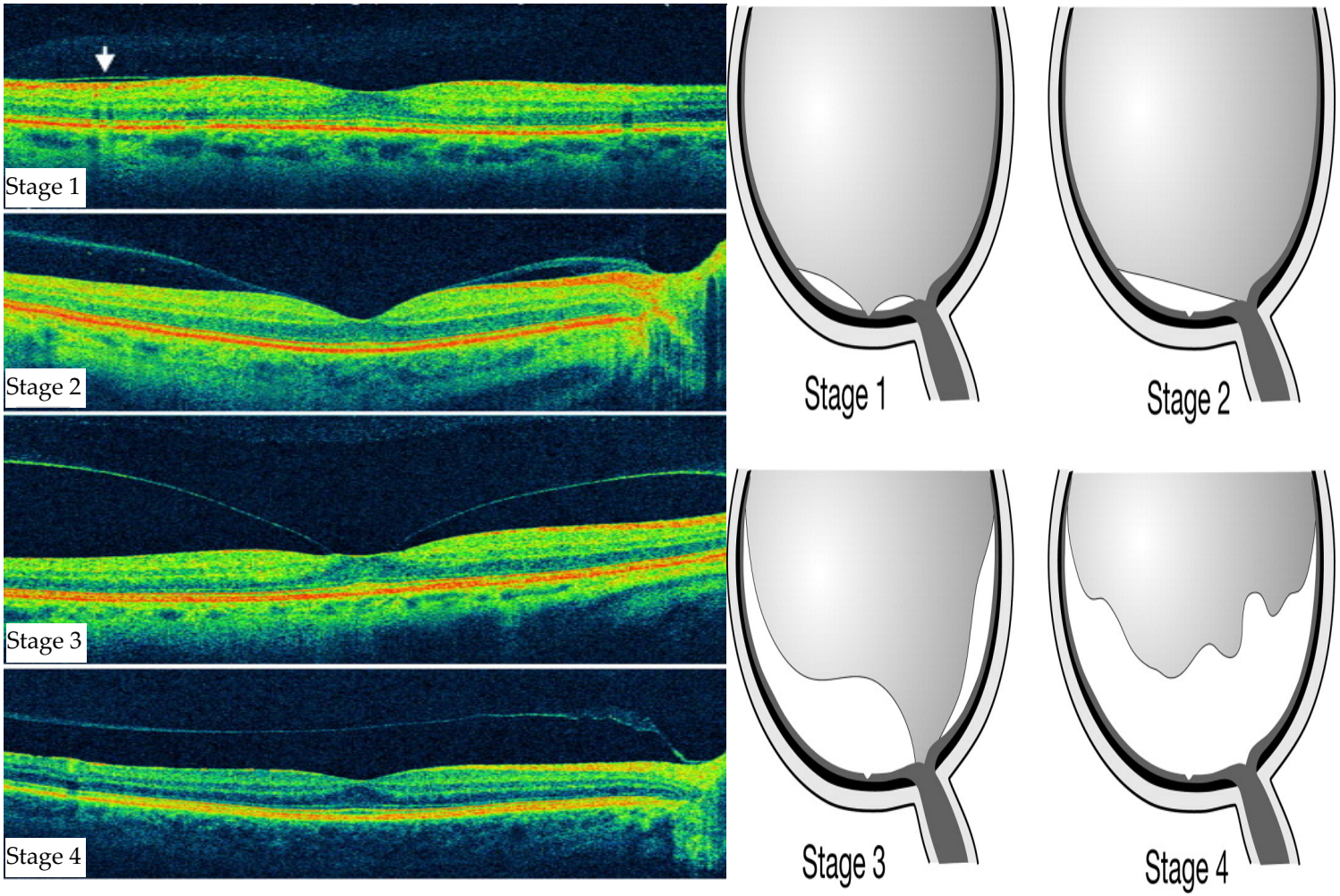


Figure 2.1: *Early stages of PVD according to Johnson A) Spectral-domain optical coherence tomography B) schematic illustration; stage 1, perifoveal PVD with vitreofoveal adhesion; stage 2, macular PVD (no vitreofoveal adhesion); stage 3, near-complete PVD with vitreopapillary adhesion only; and stage 4, complete PVD. Courtesy of Johnson*[174]

Later on, Tsukahara and colleagues employed wide-angle OCT to image the vitreoretinal interface from the macula to the periphery and classified PVD into 5 stages: stage 0, no PVD; stage 1, peripheral PVD limited to paramacular to peripheral zones; stage 2, perifoveal PVD extending to the periphery; stage 3, peripapillary PVD with persistent vitreopapillary adhesion alone; and stage 4, complete PVD (Figure 2.2).[176] Contrary to Johnson's earlier work which suggested that PVD originates in the perifoveal region and after the sixth decade, Tsukahara showed that PVD first appears as early as in the third decade of life and in the paramacular-peripheral region where the vitreous gel adheres to the retina.

Recently, Moon and colleagues, employing both ultrasonography and OCT, graded PVD into No PVD, Partial PVD, and complete PVD (Figure 2.3).[177] Using a single enhanced vitreous imaging (EVI) image that traversed both the optic disc and fovea, no PVD was defined as complete attachment of the posterior vitreous cortex (PVC) to the perifoveal and parafoveal areas, fovea, and optic disc. In their study, PVD outside the parafoveal area (2500 μm in diameter) were also considered as no PVD. Partial PVD was defined when detached PVC was observed within the parafoveal area (2500 μm in diameter). Complete PVD was defined as no observable attached PVC in the macular or optic disc area.[177]

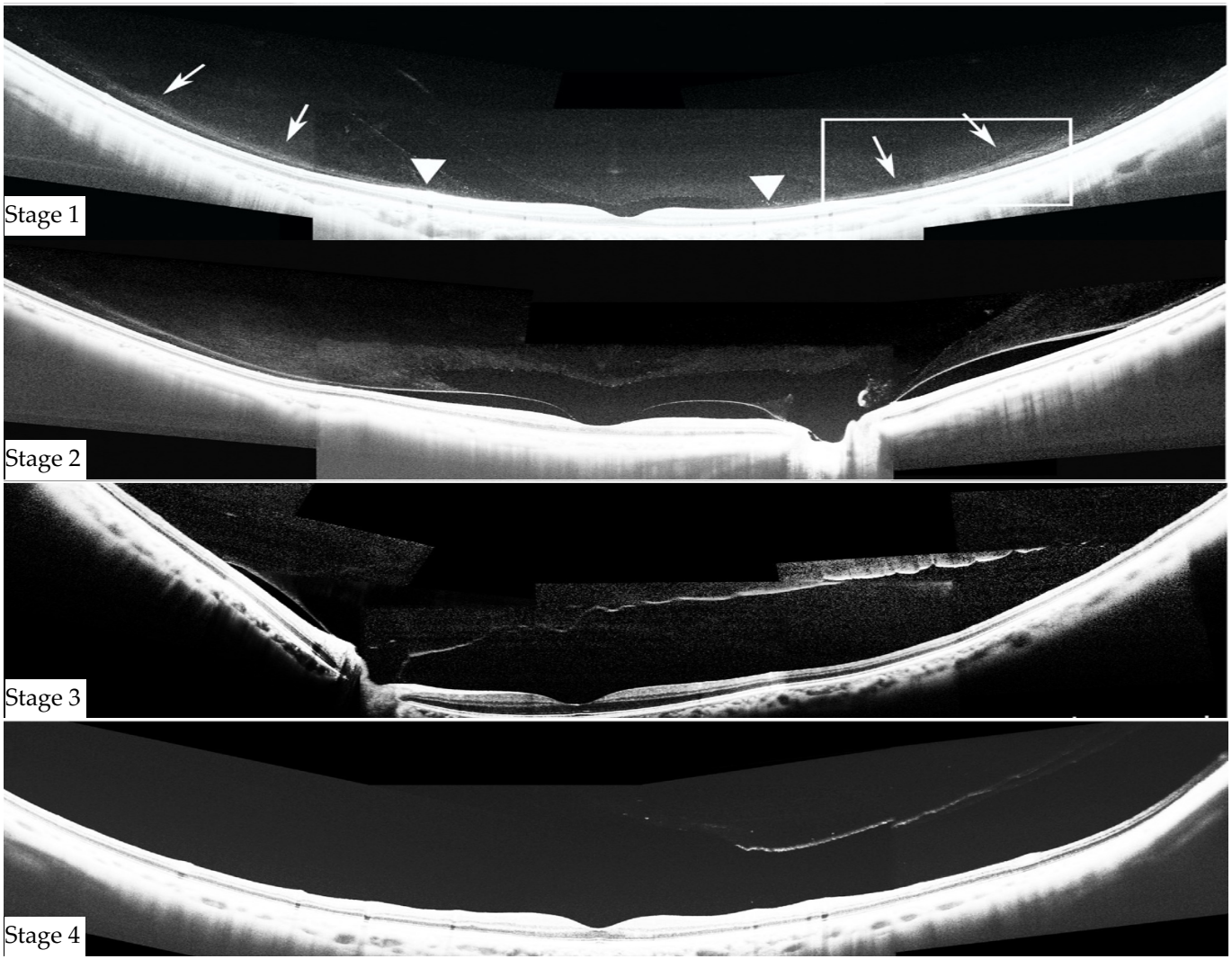


Figure 2.2: *Montaged images of vitreoretinal OCT cross-sections in an eye with PVD. Stage 1, peripheral PVD limited to paramacular to peripheral zones; Stage 2, perifoveal PVD; stage 3, peripapillary PVD; stage 4, complete PVD. Courtesy of Tsukahara et al.*[176]

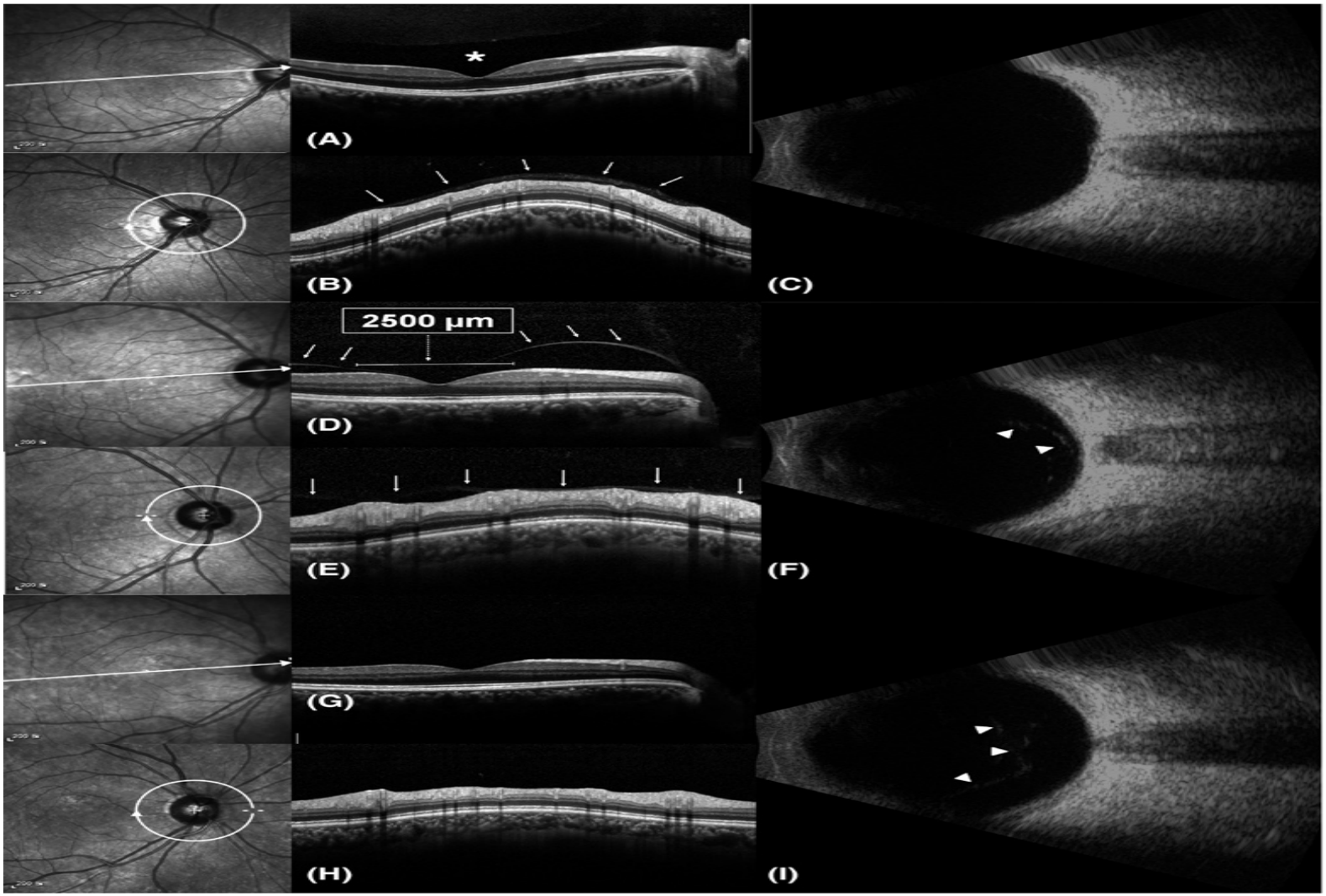


Figure 2.3: *Posterior vitreous detachment (PVD) grading based on optical coherence tomography (OCT) and ultrasonography (US). (A–C) No PVD. (D–F) Partial PVD. (E–I) Complete PVD. Courtesy of Moon et al.*[177]

Putting these studies together, it becomes evident that PVD occurs as either a partial or complete detachment. Further, PVD commences within the paramacular and peripheral vitreoretinal interfaces, and progresses in an age-dependent fashion towards the perifoveal zones.

2.2.4 Risk factors for PVD

Aging aside, high myopia, menopause, and hereditary extracellular matrix syndromes such as Stickler syndrome, Ehlers-Danlos syndrome, and *LAMA5* multisystem syndrome, are other known risk factors for PVD.[178-181] PVD occurs earlier in higher myopes compared to emmetropes, owing in part to precocious liquefaction of the myopic vitreous.[180, 181] The higher incidence of PVD in postmenopausal women has been attributed to hormonal changes associated with menopause (i.e., lowered levels of oestrogen in postmenopausal compared to premenopausal women).[179]

2.2.5 Anomalous PVD

In the case where firm vitreo-retinal adhesions persist in the face of significant vitreous synchysis, anomalous PVD (APVD) can occur.[182, 183] This unifying concept in vitreo-retinopathies is based upon the premise that same initiating abnormality (i.e., excess liquefaction without concurrent vitreo-retinal dehiscence) can explain several seemingly disparate vitreo-retinal disorders ranging from retinal tears/detachments to axial vitreo-macular traction syndrome and tangential vitreo-maculopathies such as macular pucker and macular holes. APVD has also been observed in eyes with PDR, Eale's disease, high myopia, and congenital collagen disorders such as Ehlers-Danlos syndrome.[54, 105, 184, 185]

APVD can be classified as full-thickness, when the entire PVC remains attached to the retina at specific locations, or partial-thickness where the PVC splits (referred to as "vitreoschisis"), with its outer layer adhering to the retina.[186] Clinical manifestations include rhegmatogenous events (when traction is exerted on the peripheral retina), vitreo-papillopathies (when traction is exerted at the optic disc), neovascularisation and vitreous haemorrhage in ischemic retinopathies (when traction is on abnormal blood vessels arising from the retina and/or optic disc), and the two aforementioned forms of vitreo-macular traction.[186-188] At the level of the macula, vitreoschisis results in macular pucker (when the split occurs anterior to the level of the hyalocytes and vitreous is separated from the optic disc) or macular hole (when the split occurs posterior to the hyalocytes and the vitreous remains attached to the optic disc).[189] There are also cases of macular hole that do not involve vitreoschisis, although better imaging technologies are needed to fully characterise these vitreo-maculopathies.

2.2.6 Diagnosis of PVD

PVD can be detected and imaged by OCT (Figure 2.4; see 2.2.3 for more details on PVD assessment with OCT) and B-scan ultrasound (Figure 2.5).

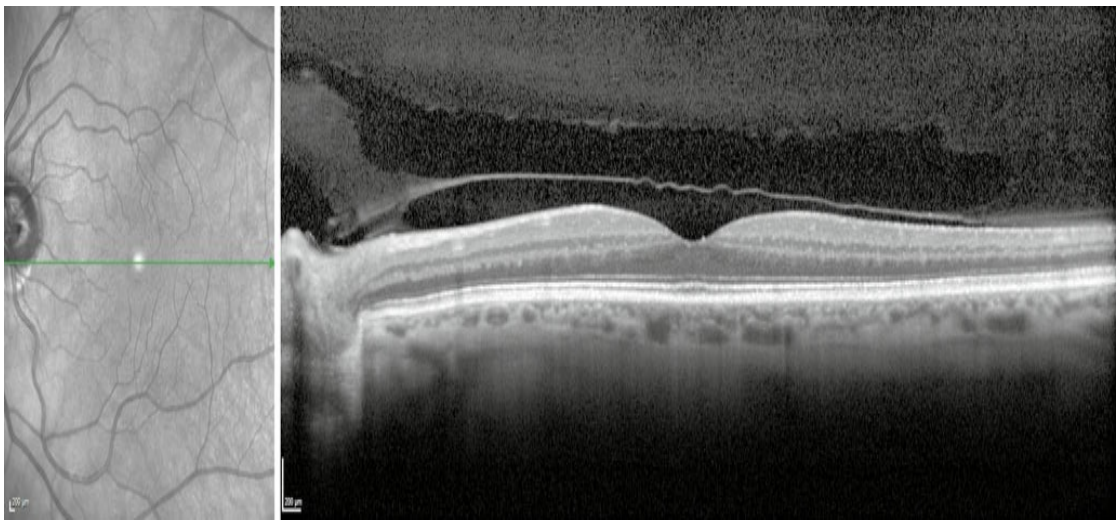


Figure 2.4: *SD-OCT Imaging of posterior vitreous detachment in vivo. SD-OCT of left eye of asymptomatic 64-year-old male subject demonstrating PVD extending from the edge of the optic nerve head and fully detached at the fovea (From Sebag J, Silverman RH, Coleman DJ: To see the invisible - the quest of imaging the vitreous. In Vitreous – in Health and Disease [J. Sebag, ed][1]).*

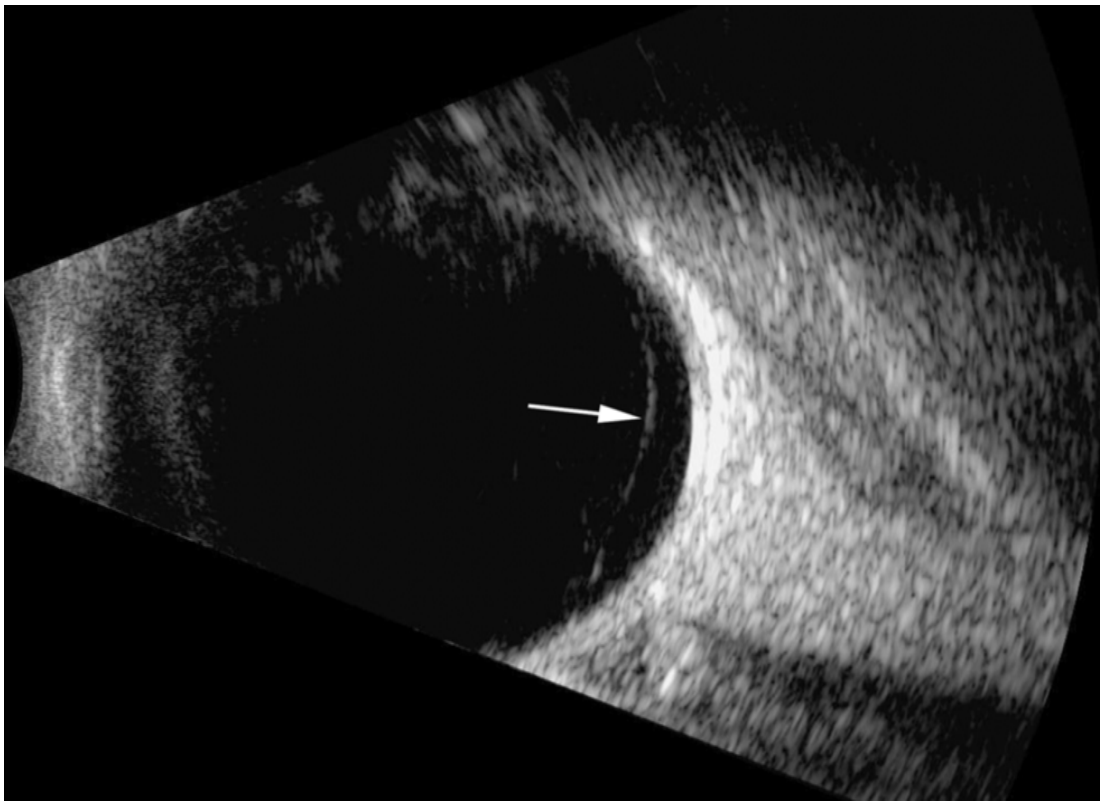


Figure 2.5: *10MHz ultrasound B-scan of vitreous demonstrating PVD; The arrow indicates the detached PVC (From Sebag J, Silverman RH, Coleman DJ: To see the invisible - the quest of imaging the vitreous. In Vitreous – in Health and Disease [J. Sebag, ed][1]).*

2.3 VITREOUS FLOATERS

2.3.1 Definition

Significant vitreous degeneration translates into an entoptic phenomenon, vitreous floaters or myodesopsia, which describes the perception of flies, cobwebs, tracts, lamellae, membranes, and fine bundles within the visual field (Figure 2.6). Recently, Prof. Sebag has advocated for the term, *vision degrading myodesopsia,* to be used to characterise vitreous floaters that significantly degrade visual function (specifically contrast sensitivity) and quality of life.[190]

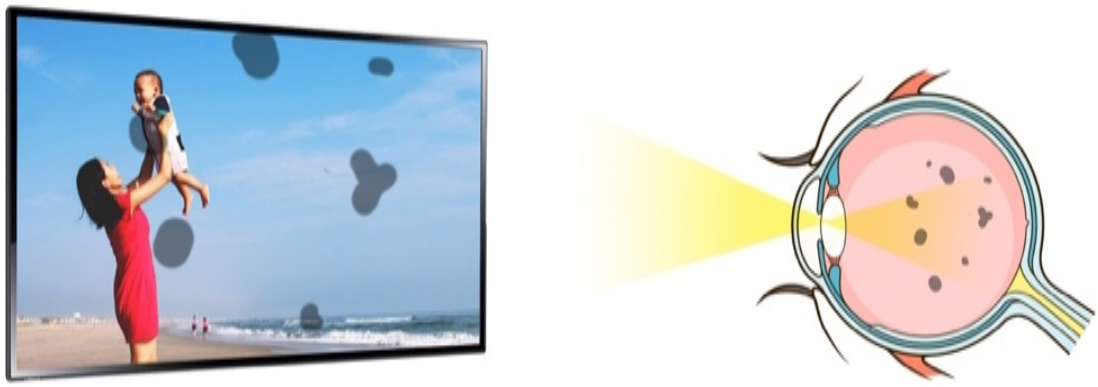


Figure 2.6: *Visual perception of eyes with vitreous opacities (floaters). Image courtesy of Emmanuel Ankamah*

2.3.2 Historical background

Vitreous floaters, also called muscae volitantes (Latin for flying flies), has been a commonly known vitreous disorder in ancient historical medicine. Galen, the second century scientist, described vitreous floaters as "circumscript condensations of the aqueous."[191] This opinion was upheld by Arabian medical scholars and even in the science of the Renaissance. It was not until Galen's theory

of the crystalline lens being the principal organ of visual perception was debunked that scholars began to look for different explanations for vitreous floaters.[191]

Evidence from 13th century literature suggests that blocked optic nerve was considered the cause of muscae volitantes. As indicated by Benvenutus Grassus, a 13th century ophthalmologist, “muscae volitantes are treated with an electuary, a sugar-based medicine to be swallowed, to open the optic nerve presumed to be blocked.”[192]

In the early 19th century, the quest to understand and explain muscae volitantes grew rapidly. The physician, J. Ware, published the outcomes of his study, 'Muscae volitantes of nervous persons' in which he described floaters as an outcome of unusual nervous agitation.[193] It was not until 1823 when Purkinje correctly identified muscae volitantes as opacities floating in the vitreous.[191] Purkinje employed an approach which was different from previous scholars. Rather than treating muscae volitantes as pathological states, he characterised them as 'visual truths', whose 'objective grounds' had to be established. To do so, Purkinje surveyed the various forms and movements of floaters as well as the circumstances under which they appear. He described the cause of floaters as follows: “intense movements such as lifting 'something heavy with one's head bent' or 'vigorous leaps' followed by a 'fixed stare' on a bright surface.”[193] Such an activity would cause an individual to experience fly-like objects as well as moving black spots and lines in one's visual field. Because the spots and lines could be seen only with open eyes and only in external light, and because they had shadows, Purkinje

inferred that they must be bodies. He added that, “their form and the fact that they appeared more frequently when the observer in question was excited or agitated indicated that they were caused by blood globules, as excitement was generally accompanied by higher activity of these globules.”[193]

2.3.3 Pathophysiology of vitreous floaters

To date, we do not fully understand the pathophysiology of vitreous floaters. Principally, liquefaction and posterior vitreous detachment underlie vitreous floaters. Oxidative stress, increased intravitreal proteolytic enzymes, and reduction/depletion in vitreous antioxidant capacity have been proposed as the underlying mechanisms for these aforesaid degenerative processes.[14, 160, 161] Significant degeneration due to aging or disease causes exposure of the “sticky” surfaces of collagen II, dissociation of collagen fibres from HA, and subsequent lateral fusion of fibrillar collagen II.[45] These collagen fibres clump together irregularly as vitreous opacities, which translate into vitreous floaters (Figure 2.7). Other sources of floaters include amyloid, asteroid bodies, macrophages of Whipple disease, blood (called synchysis scintillans), opercula, and endophytic retinoblastoma.[194]

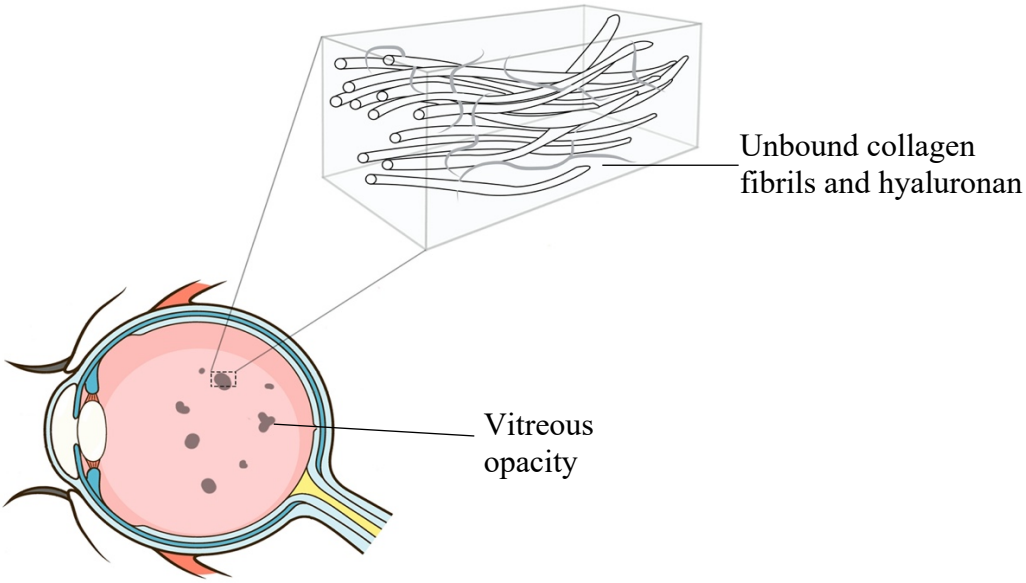


Figure 2.7: *Cross-sectional diagram of the human eye showing aggregation of collagen fibrils as vitreous opacities. Courtesy of Emmanuel Ankamah.*[14]

2.3.4 Categories of vitreous floaters

Vitreous floaters can be categorised into primary and secondary floaters.

2.3.4.1 Primary floaters

Primary floaters are caused by vitreous opacities that arise from structures endogenous to the vitreous body. With advancing age, collagen fibrils dissociate from HA and aggregate into visible opacities that first appear in the central vitreous. Primary vitreous floaters cause disruption and scattering of light, and are appreciated as mobile dark lines and spots or nodules within the visual field.

2.3.4.2 Secondary floaters

Secondary floaters are opacities in the vitreous body whose origin is exogenous to the vitreous body including opacities resulting from posterior uveitis, vitreous haemorrhage, proteins, amyloid, or RPE cells.[194, 195] The most common cause of

secondary vitreous floaters is preretinal or vitreous haemorrhage, which induces the sudden onset of floaters and hazy vision.[194]

2.3.5 Risk factors of floaters

Aside liquefaction and PVD, other risk factors for vitreous floaters include high myopia, trauma, menopause, diabetes, and hereditary extracellular matrix syndromes such as Stickler, *LAMA5* multisystem, Marfan's, and Ehlers Danlos syndromes.[160, 161, 178-181]

2.3.6 Quantification of vitreous floaters

2.3.6.1 Quantitative Ultrasonography

Wa et al. and Mamou et al. have developed quantitative ultrasonography methods to quantify inhomogeneities within the degenerated vitreous.[159, 196] The rationale for this method hinged on the ability of B-mode ultrasound scans to image the entire vitreous. Using a customised 15-mHz probe, both longitudinal and transverse b-mode ultrasound scans were taken in primary gaze, and a horizontal longitudinal scan through the premacular vitreous in temporal gaze (Figure 2.8). Each scan set had 100 frames of log-compressed envelope data. Within each frame, two regions of interest (ROIs) were analysed (whole-central and posterior vitreous) to yield three parameters (energy, E; mean amplitude, M; and percentage of the vitreous filled by echodensities, P50) averaged over the entire 100-frame dataset.[159] B-scan ultrasound imaging for the vitreous are limited by lower resolution, the skill of the operator, experience of the investigator in interpreting images (which can be rather subjective), and patient cooperation.[197]

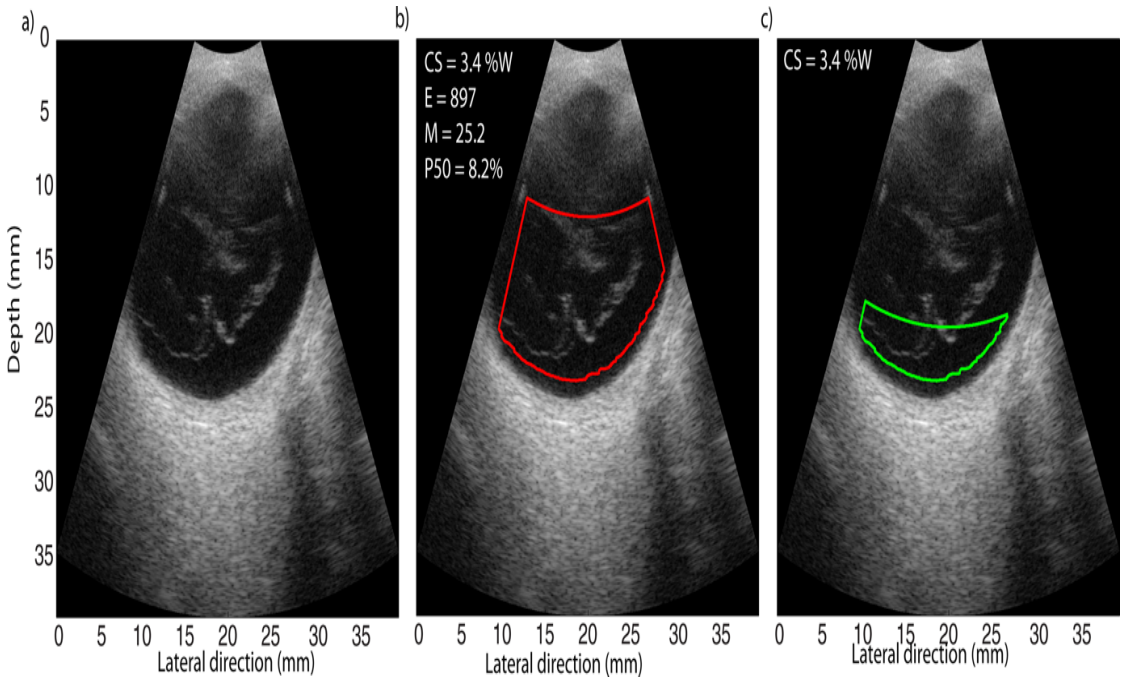


Figure 2.8: *(a) Illustrative ultrasound image of an eye with visible echodensities. (b) Whole-central vitreous ROI outlined in red. (c) Posterior vitreous ROI outlined in green. Courtesy of Mamou et al.*[159]

2.3.6.2 Optical coherence tomography

Both swept-source OCT (SS-OCT) and spectral-domain OCT (SD-OCT) have been used to image the vitreoretinal interface for PVD.[176, 177, 197] Recently, Ruminiski and colleagues have employed a 3D SS-OCT technique to image the anterior human vitreous for age-related changes. They have shown that SS-OCT can be used to successfully image opacities within the anterior vitreous (Figure 2.9). OCT-derived indices of vitreous optical density (VOD), vitreous opacification ratio (VOR), and lens optical density (LOD) were correlated with AL and double-pass assessment of retinal point spread function (Objective Scatter Index [OSI]).[198] Assessment of vitreous floaters with OCT has been limited to date since OCT does not permit imaging of the entire vitreous.[159]

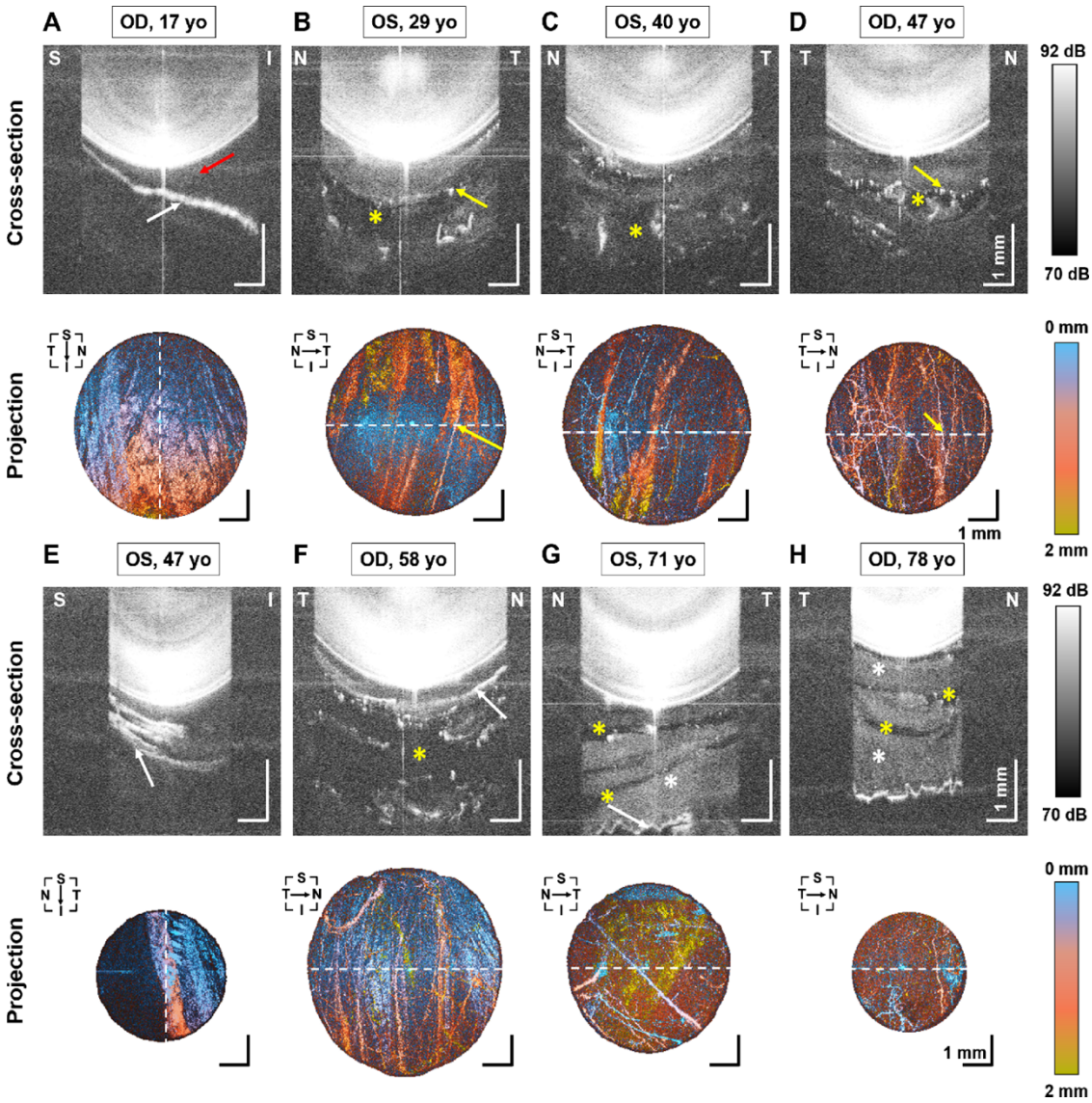


***Figure 2.9**: Characteristic features of retrolental vitreous in 3-D SS-OCT images. Cross-sectional images and projection images of the anterior vitreous. (**A**) From a 17 year old subject, OD. (**B**) From a 29 year old subject, OS. (**C**) From a 40 year old subject, OS. (**D**) From a 47 year old subject, OD. (**E**) From a 47 year old subject, OS. (**F**) From a 58 year old subject, OD. (**G**) From a 71 year old subject, OS. (**H**) From a 78 year old subject, OD. Dashed lines represent section direction. White arrows indicate laminar structures. The red arrow indicates the Berger's space. The yellow arrows denote fiber-like opacities. The yellow asterisks indicate lacunae with liquid vitreous. The white asterisks correspond to the gel vitreous. T = temporal, N = nasal, S = superior, I = inferior. Scale bar = 1 mm. Courtesy of Ruminiski et al.*[198]

2.3.6.3 Infrared Fundus photography

This imaging method was described by Sun et al. as part of a laser vitreolysis study to quantify vitreous floaters. In this study, 30° or 55° field, infrared images were captured with the Heidelberg Retina Angiograph 2 (Heidelberg Engineering,

Germany; Figure 2.10). Using an open source software, image J (version 1.43u, National Institute of Health, USA) the target areas with floater shadows were circled on each image and measured automatically for each patient.

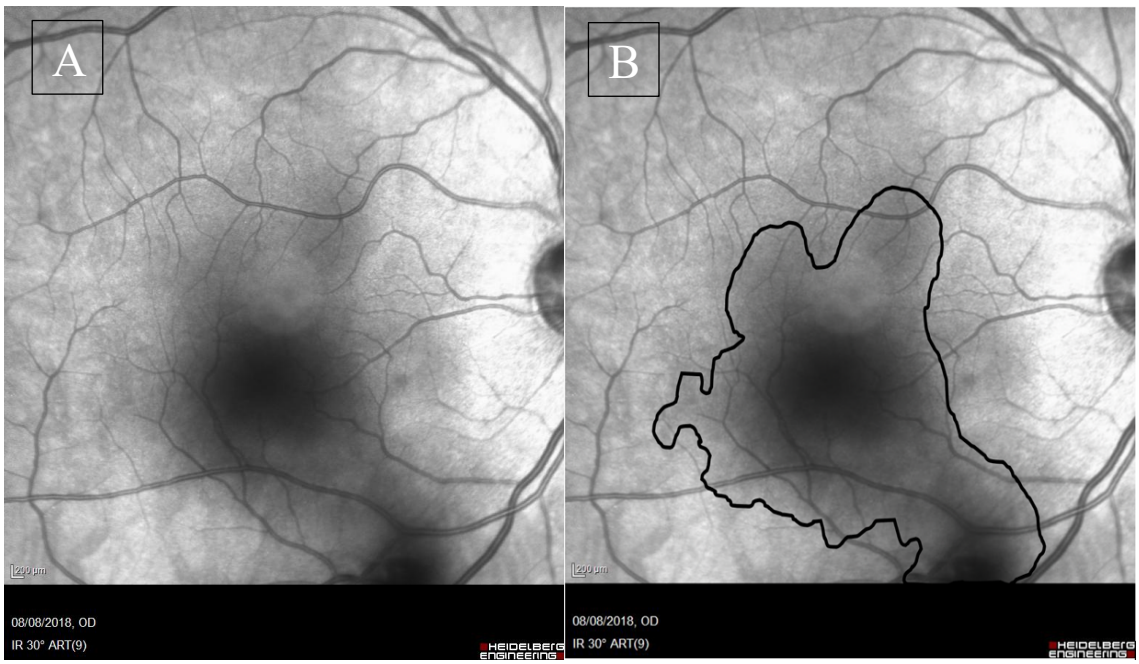


Figure 2.10: *A 30° field, 768 x 868 pixel infrared image showing vitreous opacity shadows (A); highlighted vitreous opacity areas (B). Courtesy of Emmanuel Ankamah, Waterford, Ireland.*

2.3.6.4 Ultra-widefield infrared vitreous imaging

This imaging technique was developed as part of this PhD program for quantifying floaters. Using the ultra-widefield angiography module (UWF-Module) of the Spectralis HRA + OCT Multicolour, a 30-second, 102° field confocal scanning ophthalmoscopy video was recorded of the vitreous. Images were then obtained using the Heyex software and analysed using ImageJ. This imaging has been described in detail in chapters 4 and 5 below.

2.3.7 Management of floaters

Floaters have been described by patients as a visual nuisance that impinge on their quality of life.[199] As a result, some patients are willing to trade 1.1 years of every 10 years of their remaining life to get rid of their floaters.[200-202] The available management options for vitreous floaters are:

2.3.7.1 Observation or Watchful waiting

Watchful waiting or observation remains the conventional treatment mostly offered to floater sufferers. In that, patients are only monitored after they have been either reassured that their floaters will resolve with time, encouraged to ignore their floaters or counselled to adapt to their new visual experience.[203] This is usually proffered after clinicians have successfully ruled out the possibilities of retinal pathologies following the onset of floaters. The assertion that floaters resolve on their own is unfounded in literature. Wagle and associates have demonstrated that utility values, an objective quantification of the functional quality of life associated with a specific health state, are similar for both chronic and acute sufferers of vitreous floaters.[202] This possibly explains why patients continue to seek medical intervention to their floaters from one facility to another, at the expense of doctors' advice of resolution of or adaptation to their condition.[38]

2.3.7.2 Pars plana vitrectomy

Pars plana vitrectomy remains the definitive treatment for vitreous floaters, substantiated by beneficial outcomes on objective and subjective measurements.[204-206] However, the risks associated with this procedure, which

include retinal detachments, nuclear cataracts, iatrogenic retinal tears, transient high postoperative intraocular pressure, transient postoperative hypotony, cystoid macular oedema and vitreo-retinal haemorrhage, cannot be overlooked.[206, 207]

2.3.7.3 Neodymium-doped:yttrium-aluminium garnet (Nd:YAG) laser vitreolysis

Aside vitrectomy, Nd:YAG laser vitreolysis is the other management option for floaters.[60, 203] It is often described as an 'off-label' treatment since prospective studies assessing the safety and efficacy of this procedure are lacking to date.[203] Complications associated with laser vitreolysis include posterior lens capsule rupture, prolonged elevation of IOP, retinal detachments, retinal tears, laser injury-related transient posterior pole retinal haemorrhage, worsening floaters, refractory open-angle glaucoma, and rapid progression of pre-existing cataracts.[208-211]

2.4 RATIONALE AND RELEVANCE OF THE THESIS

2.4.1 Towards personalised medicine: The role of nutritional supplementation in future eyecare

Conventional medicine, as has been practiced over the years, is a disease-oriented and reactive approach of treating patients' complaints as well as ensuring that clinically measured disease-related indices are normalised. This approach of 'disease care' appropriates the majority of health resources to the management of clinical manifestation of severe pathologies, and fails to address the entirety of health, which is a state of complete physical, mental and social wellbeing, and not just the absence of disease.[212]

Personalised medicine, a predictive, preventive, and individual-specific approach to healthcare, on the other hand, focusses on identifying distinct profiles of a person's health: genetic, biological, and environmental, with the ultimate goal of either avoiding the manifestation of diseases in individuals or providing treatments customised to the person in question.[213] Facilitated by the constant innovations in biochemical, genomic and diagnostic apparatus, the trajectory towards personalised medicine will involve everything ranging from lifestyle modifications (physical exercise and dietary or nutritional prescriptions), health promotion campaigns, screening exercises, predictive algorithms to isolate individuals with high risk of disease, telemedicine monitoring and assessment, early and appropriate diagnosis, to genetic-tailored therapies for diseases. Not only will this preventive and specific healthcare delivery improve patient well-being but also reduce the financial burden on patients and healthcare systems.[214]

As oxidative-stress and depleted intraocular antioxidant levels account for numerous eye disorders such as Fuch's dystrophy, AMD, and cataracts, dietary supplementation with antioxidants, aimed at mitigating oxidative stress and injury, may subserve the preventive aspects of personalised medicine in eye care. This idea, previously employed in the management of ocular surface and retinal diseases, is yet to be applied to the vitreous.

The vitreous body is laden with antioxidant molecules that could protect against oxidative stress and diseases of the vitreous as well as surrounding tissues. Concerning the former, vitreous gel liquefaction and degeneration may be due at least in part, to depletion of vitreous antioxidants initiating gel liquefaction. Thus,

a plausible mechanism for retarding vitreous degeneration lies in supplementing with exogenous nutrients that have direct antioxidant effects or elevate endogenous antioxidant levels within the vitreous body. Concerning the latter, deficient vitreous antioxidant capacity might contribute to chronic diseases such as cataracts, glaucoma, diabetic vitreo-retinopathy, and age-related macular degeneration. Thus, future strategies might include administration of exogenous nutrients such as ascorbic acid, hesperidin, zinc, leucocyanidin, l-lysine, and verbascosides which have been shown to have inhibitory effects on the proposed mechanisms of vitreous degeneration, albeit only indirectly and not *in vivo*, certainly not in humans.[215-219]

In terms of the role of exogenous nutrients to augment the vitreous, an important aspect to consider relates to the currently unproven efficacy of increasing intravitreal levels of exogenous micronutrients and the mode of delivery of these nutrients into the vitreous. To date, there is limited data available to help explain this process, so we can only conjecture based on evidence from toxicology studies. Fluorometry studies and post-mortem toxicological analysis have shown that transfer of molecules from systemic circulation into the vitreous are mediated by diffusion, hydrostatic and osmotic pressure gradients, convection, and active transport, through the blood-aqueous and blood-retina barriers.[62, 78, 220-222] Given that some of the aforesaid exogenous nutrients have been previously detected in the human vitreous, one can theorise that these nutrients utilise the above-mentioned pathways to accumulate in the vitreous, in spite of the fact that specific delivery channels have not been isolated for most of these nutrients.[28, 79, 223, 224]

The potencies of these putative channels are unknown, and thus, achieving sufficient therapeutic doses of exogenous nutrients within the vitreous may require repeated long-term administration of these agents.[225] However, to guard against systemic adverse effects due to chronic use and to ensure safety, concentrations of exogenous nutrients administered should be the daily *Dietary Reference Intake* values for these nutrients.[226] In the case where no recommended values are available for a micronutrient, concentrations to be consumed should be guided by data on the adverse effects observed with different concentrations of the same micronutrient.

2.4.2 Justification

The above rationale informed this PhD project, which was designed to explore vitreous degeneration as a disease as well as to investigate the potency of targeted nutritional supplementation as a management alternative for symptomatic vitreous degeneration. A significant research gap that needed to be filled was the investigation into the impact of vitreous degeneration on photopic and mesopic luminance contrast at high spatial frequencies. This was warranted because the visual disturbance associated with vitreous degeneration are most noticeable against bright environments. In addition, given that the vitreous contributes to the optical factors for the decline of retinal image contrast, the impact of vitreous degeneration on contrast sensitivity may be more striking on high spatial frequencies compared to low frequencies (more on this in chapter 3).

Another part of this thesis concerns the use of a novel imaging methodology, ultra-widefield infrared vitreous imaging, developed as part of this PhD, to image vitreous opacities. This is reported first in a study that assessed the long-term effectiveness of laser vitreolysis for managing vitreous floaters (Chapter 4) and subsequently, in a nutritional interventional study for managing symptomatic vitreous degeneration (Chapter 5).

In investigating a management alternative for symptomatic vitreous degeneration, a randomised, double-blind, placebo-controlled clinical trial was conducted to investigate the impact of 6-month supplementation with a formulation of selected antioxidative and antiglycation micronutrients on the symptomatology associated with vitreous degeneration [Floater Intervention Study (FLIES), trial registration number: ISRCTN15605916]. This study was warranted since a low-risk yet effective management alternative is needed to boost the vision-related quality of life of patients suffering from symptomatic vitreous degeneration.

2.4.3 Research Questions

This PhD thesis presents findings from experiments that answer the following questions:

1. What is the impact of vitreous degeneration on photopic and mesopic contrast sensitivities at high spatial frequencies?

2. Can ultra-widefield infrared vitreous imaging be used to assess treatment effectiveness in patients who have undergone laser vitreolysis for symptomatic vitreous degeneration?

3. What is the impact of 6-month supplementation with a targeted antioxidative and antiglycative micronutrient formulation on subjective visual symptomatology, visual function, and objective vitreous imaging parameters, in patients with symptomatic vitreous degeneration?

2.4.4 Objectives

The following objectives and the respective chapters in which they are addressed have been presented below:

Objective 1: To determine the impact of vitreous degeneration on photopic and mesopic contrast sensitivity (Chapter 3).

Objective 2: To evaluate ultra-widefield infrared vitreous imaging as a determinant of treatment effectiveness of YAG laser vitreolysis for managing vitreous floaters (Chapter 4).

Objective 3: To determine the impact of targeted nutritional supplementation with antioxidative and antiglycation micronutrient formulation on the subjective disturbance, objective vitreous imaging parameters, and visual function, in patients with symptomatic vitreous degeneration (Chapter 5).

2.5 CHAPTER SUMMARY

This chapter presents a literature review of the specific area of study, vitreous degeneration, and highlights the research questions that are answered in this PhD thesis. In brief, this chapter has shown that vitreous degeneration is explained by two broad processes, liquefaction and posterior vitreous detachment. Further, reduction, or possibly, depletion of vitreous antioxidants is the precursor for

vitreous degeneration and subsequent potential sight-threatening, vitreo-retinal complications. Some aspects of this chapter have been published in *Antioxidants* (Impact factor: 4.520) under the title, ***Vitreous antioxidants, degeneration and vitreoretinopathy: exploring the link*** (see Appendix F1 below)

Chapter 3

VITREOUS DEGENERATION COMPROMISES PHOTOPIC AND MESOPIC CONTRAST SENSITIVITY

3.1 INTRODUCTION

Visual acuity (VA) and contrast sensitivity (CS) represent the two main tests that are typically used to assess spatial vision. Visual acuity, the conventional method of assessing spatial vision in clinical practice, requires patients to correctly name small letters that are close to 100% contrast and have spatial features that approach the resolving power of the eye.[227] The difficulty of the task varies across letters and subjects often achieve VA values within 'normal limits', even when the contrast of the retinal image is lowered as a result of aberrations and scattered light.

Contrast sensitivity, on the other hand, is a measure of the contrast threshold for seeing a target.[228] Contrast thresholds for either spatially periodic patterns or single optotypes such as Landolt rings can provide a sensitive measure of spatial vision that can be used to detect changes in retinal image contrast even when individual observers manage to achieve VA values within the normal range.[229, 230] The size of the optotype employed is usually fixed at three times the mean acuity limit of 5 min arc (6/6) and the reciprocal of the contrast threshold needed to resolve the gap

is usually described as functional contrast sensitivity (FCS).[231] Although VA and CS are associated with each other, they assess different aspects of spatial vision.[228] Thus, when a CS test is conducted in conjunction with a conventional VA test, the combined results provide a more informative assessment of spatial vision.[232]

Aging and ocular disease have been shown to degrade contrast sensitivity (CS). Factors responsible for CS degradation are broadly categorised as either optical or neural. Optical aberrations, increased lenticular optical density (leading to reduced retinal illuminance), and increased intraocular light scatter (resulting from either increased lenticular opacity or increased vitreous opacification) constitute optical factors whereas parafoveal loss of rods and ganglion cell complex thinning, secondary to retinal ganglion cell loss, comprise the neural factors for CS decline.[233-235] It is worth mentioning that the influence of neural factors in CS decline are more pronounced within the low to mid spatial frequencies, whereas optical factors are more striking in high spatial frequencies.[236]

Vitreous degeneration results in the entoptic phenomenon, vitreous floaters or myodesopsia. Further degeneration of the vitreous gel results in the weakening of the vitreoretinal adhesions and the separation of the posterior vitreous cortex from the inner limiting membrane of the retina, at the vitreoretinal interface, a phenomenon referred to as posterior vitreous detachment (PVD).[175] PVD has been described as the principal underlying phenomenon for the sudden onset of primary floaters (that is, vitreous opacities that arise from structures endogenous to the vitreous body).[194] That notwithstanding, primary floaters can occur

asynchronously from PVD, especially when sufferers are myopic. Both vitreous opacities and PVD have been shown to compromise CS under mesopic luminance.[159, 204, 206, 237, 238] It follows from the foregoing that when vitreous opacities occur in tandem with PVD, there should be a further decline in CS. Also, the impact of vitreous opacities and PVD on CS under photopic luminance have not yet been studied and reported. This is particularly necessary given the fact that the visual disturbances associated with vitreous floaters mostly occur against uniform and bright backgrounds.[239, 240] Moreover, no study has been conducted yet to compare the effect of vitreous degeneration on both photopic and mesopic CS.

In view of the aforesaid, it seems plausible that a CS test designed to assess predominantly high spatial frequencies (and under photopic luminance) may be more suitable for assessing the impact of vitreous degeneration on contrast threshold. In this study, we sought to ascertain the impact of vitreous opacities and PVD, if any, on contrast thresholds while controlling for other potential confounding factors to contrast threshold decline - mainly age, lenticular opacity and retinal dysfunction. An age-matched sample was used for this study to control for the effect of age on contrast thresholds. In order to control for contrast threshold loss due to lenticular opacity, participants included in this study had clear lenses or were pseudophakes fitted with monofocal intraocular lenses and had no posterior capsular opacification.[241, 242] We assessed retinal function by employing a 15 Hz flickering stimulus test, which remains largely uninfluenced by small changes in the optics of the aging eye. Age-related loss of flicker threshold is attributed to changes in or loss of retinal ganglion cells, which in turn cause a

degradation of spatial contrast.[243] An age-matched sample implied no interaction of age with retinal function measured by flicker threshold and a significant difference between samples would indicate the presence of a neural confounding factor to contrast threshold decline.

3.2 METHOD

3.2.1 Study design and sample

An age-matched sample of 115 subjects, comprising 30 subjects with primary floaters (cases) and 85 subjects devoid of vitreoretinal disease (controls) were included, in a 1:3 case-control fashion, into this cross-sectional study. This study design was ideal because the evidence has shown that, in order to have enough power to detect a difference between the two groups, the ratio of cases to controls should not be greater than 1:3.[244] All volunteers who were aged 35 years and above were included in this study, as floaters are usually developed at the fourth decade of life, with myopic vitreopathy occurring 5 to 10 years before the fourth decade.[194] Cases were recruited from a vitreoretinal sub-specialty clinic (Institute Of Eye Surgery, Waterford) and as such the diagnosis, while current, was not prospective. For the cases, the eye in subjects with unilateral floaters was selected as the study eye. In cases with bilateral floaters, the eye described by the subject as more bothersome was selected as the study eye. For the controls, the dominant eye (as determined by the convergence near-point method) or the eye with the better best corrected visual acuity (BCVA) was selected as the study eye.[245]

Exclusion criteria included prior treatment for floaters (laser vitreolysis or vitrectomy), secondary floaters (as described by Milston et. al,[194]); lens opacification including posterior subcapsular cataracts, nuclear and cortical opacification; pseudophakes fitted with multifocal intraocular lenses; neural, developmental and retinal diseases (for example, glaucoma, age-related macular degeneration, diabetic retinopathy, branch retinal vein occlusion, vitreomacular traction, congenital hypertrophy RPE, toxoplasmosis, myelinated nerve fibre, macular oedema, and amblyopia); and BCVA worse than 6/12 (0.3logMAR).

3.2.2 Ethics

The study was conducted following ethical approval from the Research Ethics Committee, Waterford Institute of Technology, Waterford, Ireland, and from the Research Ethics Committee, Health Service Executive, South Eastern Area, Ireland (WIT2019REC0007). The study adhered to the tenets of the declaration of Helsinki. Informed consent forms were completed by all participants prior to enrolment into the study.

3.2.3 Assessments

3.2.3.1 Visual Acuity and Contrast Threshold

BCVA and functional contrast threshold were assessed using the *Acuity-Plus* test from the Advanced Vision and Optometric Test (AVOT; https://www.city.ac.uk/avot).[235, 246] Stimuli were presented on a high resolution EIZO Color liquid-crystal display (LCD) Monitor (ColorEdge CS240, 21.5 inches; EIZO Corporation, Japan) with 1900 x 1200 pixels and 10 bits per primary colour.

The visual display was calibrated spectrally for each primary colour for both spectral radiance and luminance using bespoke software (LUMCAL; City Occupational Ltd., London, UK). The monitor incorporates EIZO's digital uniformity equaliser (DUE) technology to avoid fluctuations in brightness and chromaticity on different parts of the screen. The display was located 3 metres from the observer.

The *Acuity-Plus* test involved a range of positive and negative polarity Landolt ring optotypes with varying gap sizes which were presented randomly during the test. A staircase procedure with 12 reversals was used to vary the gap size of the stimulus using a two-down, one-up procedure. All participants were tested first using the photopic protocol (background luminance of 34 cd/m^2). This was then followed by similar measurements using the high mesopic protocol (background luminance of 1 cd/m^2) which involved short dark adaptation. Spectrally-calibrated 'neutral density' filters were employed for the mesopic testing. Participants wore their distance spectacle prescription, if any, for VA testing.

The *Acuity-Plus* test employed diagonal guides which pointed towards the centre of the screen. A small outline square and cross, flashed briefly in the centre of the screen to attract the participant's point of regard. A short time afterwards, a Landolt ring, whose gap orientated towards one of four oblique guides, was presented to the eye for 160ms. This stimulus arrangement facilitated fixation at the centre of the screen and accommodation in the plane of the screen. In addition, the stimulus employed also eliminated eye movements and multiple fixations during the task.[231]

The participant's task was to register the direction of the gap in the Landolt ring optotype, or to guess its location, by pressing one of four buttons arranged to simulate the geometry of the screen. VA results generated by the program were displayed as the minimum angle of resolution (MAR). These were converted to logMAR values for reporting. In this study, photopic and mesopic VA as assessed with Landolt ring stimuli of 100% negative luminance contrast are presented.

Functional contrast threshold was assessed as described previously.[247] To summarise, functional contrast threshold was measured by randomly displaying either a positive or negative polarity Landolt ring optotype with a fixed gap size of 3 min arc. The latter contains a range of spatial frequencies centred around 10 cycles per degree. The reasons for selecting this contrast test protocol is because the use of a Landolt ring optotype with a 3 min arc gap size is rich in high spatial frequencies, which are most affected by increased scatter, residual refractive errors and higher order aberrations.[231, 248] Any smaller gap size, say 1 or 2 min arc, may become undetectable in some subjects, even at maximum contrast. In addition, this protocol is easy to carry out and the test has been shown to be sensitive to changes in the retina as well as reduction in image contrast caused by increased light scatter in the eye.[248]

A staircase procedure with 10 reversals was used to vary the luminance contrast of the stimulus using a two-down, one-up procedure, reducing the chance response probability to 1/16. Participants were tested first under photopic luminance, followed by testing under mesopic luminance. The same, spectrally-calibrated

'neutral density' filters were also employed for assessing mesopic functional contrast threshold. All tests were conducted while subjects wore spectacles that ensured optimum refraction. Functional contrast threshold results represent the percentage contrast thresholds needed to achieve ~ 73% correct response. Photopic and mesopic functional contrast threshold with negative polarity stimuli results have been reported in the present study. The relative percentage differences in contrast threshold between the study groups were calculated as:

$$\%\, difference = 100 * \left(\frac{CTcases}{CTcontrols} - 1\right)$$

where CT_{cases} = mean contrast threshold of cases [that is, either the entire cases (n = 30), cases with PVD (n=12) or cases without PVD (n=18)]; $CT_{controls}$ = mean contrast threshold of controls.

3.2.3.2 Flicker threshold

Rapid flicker thresholds under high mesopic conditions was measured using the *Flicker-Plus* test from the AVOT suite. The test measures flicker thresholds at five discrete locations in the visual field using an efficient experimental technique based on a five-alternative forced-choice (AFC) procedure with five randomly interleaved staircases.[249] Temporal contrast modulation thresholds were measured using a 1-up, 2-down procedure and the thresholds were estimated by averaging the last 6 staircase reversals.[250, 251] The disc stimuli were modulated sinusoidally at a frequency of 15 Hz and subtended 45 min arc at the fovea and 60 min arc, 5° away from fixation in each of the four quadrants. The stimulus presentation time was 600 ms and the time-averaged luminance remained equal to that of the uniform

background (i.e., 0.5 cd/m^2). In addition to the low luminance, the spectral composition, size and temporal modulation frequency of the test stimuli were selected appropriately to favour rods.

Prior to the stimulus presentation, a small outline square and cross flashed briefly in the centre of the screen to attract the participant's point of regard. Participants viewed the display from 1 metre through spectrally-calibrated 'neutral density' filters. Participants had to indicate the location of stimulus presentation by pressing one of five buttons arranged to simulate the stimulus positions on the screen. A separate button indicated that the subject was totally unaware of any stimulus. When this button was pressed, the program allocated the subject's response randomly to one of the five buttons.

The staircase algorithm requires two consecutive correct responses at a given stimulus location during the random sequence presentation before a reversal occurs and the stimulus contrast is reduced for the following presentation. The five, randomly-interleaved staircases makes the test procedure statistically efficient since in the absence of any signal, the chance probability of a correct response is only 1/25. Flicker thresholds under high mesopic adaptation were computed in central vision and at each of the 4 parafoveal locations. For this report, the thresholds for the parafoveal locations were averaged to produce a single threshold, as a measure of parafoveal flicker threshold. Results were reported as flicker thresholds (%).

3.2.3.3 PVD Assessment

PVD was diagnosed based on assessment of optical coherence tomography (OCT) scans, measured using the Spectralis HRA + OCT Multicolour (Heidelberg Engineering GmbH, Heidelberg, Germany) and funduscopic examination findings. Macula and peripapillary disc OCT scans were obtained for all participants who had floaters. We employed the high resolution 19-line raster scan protocol and a 20º x 20º scan angle of the macula to obtain IR+OCT horizontal line scans of the macula area of each participant. The scan was only initiated when sufficient vitreous was identified on the B-scan to enable PVD assessment.

The horizontal line through the foveal area was used to assess PVD within the parafoveal area. The optic disc protocol was used to obtain a circumferential papillary scan and was assessed for detachment. PVD was diagnosed based on the observation of a Weiss ring upon indirect ophthalmoscopic examination and/or a complete separation of the PVC from the ILM along the horizontal macula area and from the optic disc on the macula and peripapillary disc scans, respectively.

3.2.4 Outcome measures

Outcome measures for this study were photopic and mesopic functional contrast thresholds.

3.2.5 Statistical analysis

The statistical package IBM SPSS® Statistics version 25 was used for all analyses, and the 5% level of significance was applied. The differences in study outcomes

between cases and controls were assessed using independent samples *t*-test for BCVA, functional contrast thresholds, and flicker threshold variables, and chi-squared test for sex. All quantitative variables were expressed as means ± standard deviations (Mean ± SD). In our sub-analysis to assess the effect of PVD on functional contrast thresholds, subjects were split into cases with PVD (n = 12), cases without PVD (n = 18) and controls (n = 85). One-way analysis of co-variance (ANCOVA) was used to compare the mean functional contrast thresholds between the 3 groups. The Bonferroni's correction procedure was used for subsequent pairwise post-hoc comparisons.

3.3 RESULTS

3.3.1 Effect of vitreous floaters on contrast thresholds

Table 3.1 presents the demographic and visual function measurements of cases and controls. BCVA (photopic and mesopic) and flicker thresholds (foveal and parafoveal) were not significantly different between the two groups ($p>0.05$ for all; Figures 3.1 and 3.2). Compared with controls, cases recorded significantly worse contrast thresholds at photopic and mesopic luminance ($p = 0.028$ and $p < 0.001$ for photopic and mesopic contrast thresholds, respectively; Figure 3.3). Photopic and mesopic contrast thresholds were lower by 37.4% and 27.5%, respectively, when the cases were compared with the controls.

Table 3.1: Demographic and visual function variables for study participants.

Variables	**Cases (n = 30)**	**Controls (n = 85)**	**Sig.**
Age (years)	50.87 ± 7.82	48.02 ± 6.65	0.057
Females, No. (%)	16 (53.3)	49 (57.6)	0.831
BCVA, LogMAR			
Photopic	0.06 ± 0.13	0.02 ± 0.11	0.158
Mesopic	0.30 ± 0.12	0.29 ± 0.10	0.480
Flicker threshold (%)			
Foveal	6.74 ± 2.71	6.32 ± 2.06	0.389
Parafoveal	4.36 ± 1.38	4.22 ± 1.26	0.618
Functional contrast threshold, (%)			
Photopic	15.46 ± 9.66	11.25 ± 4.59	0.028*
Mesopic	61.48 ± 19.65	48.20 ± 15.10	<0.001*

Data displayed are mean ± SD for numerical data and percentages, n (%) for categorical data; Sig., the statistical difference between the two groups; * statistically significant difference between the two groups at the 0.05 level; Best Corrected Visual Acuity (BCVA) recorded as logarithm of minimum angle of resolution (LogMAR), a score of 0.00 corresponds with 6/6; Flicker threshold recorded as %; Functional contrast threshold measurements recorded as contrast threshold (%), lower % value implies better contrast.

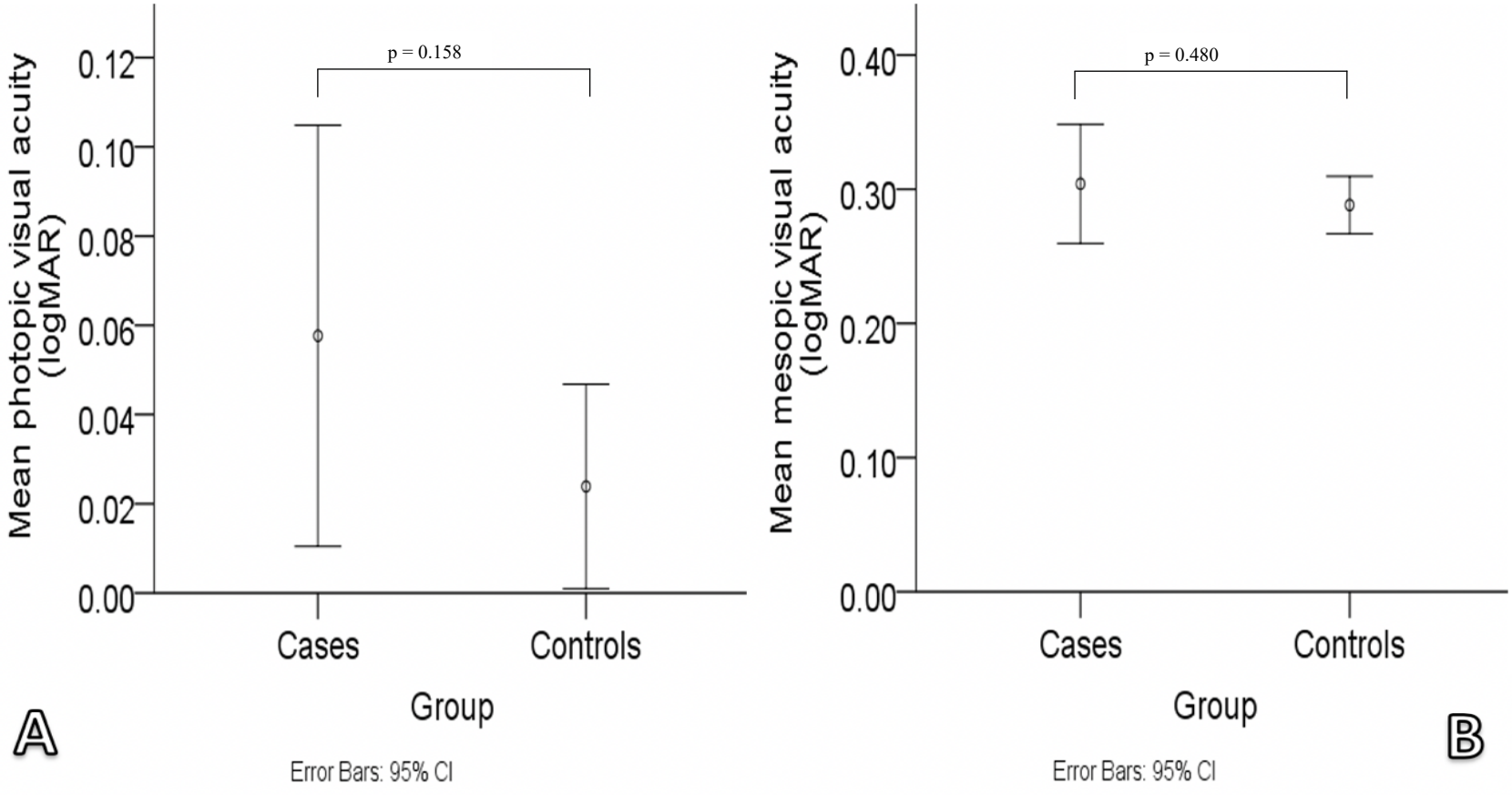


Figure 3.1: *Error plots showing the mean photopic (A) and mean mesopic (B) visual acuity of cases and controls. (Cases = subjects with symptomatic floaters; controls = subjects with healthy eyes)*

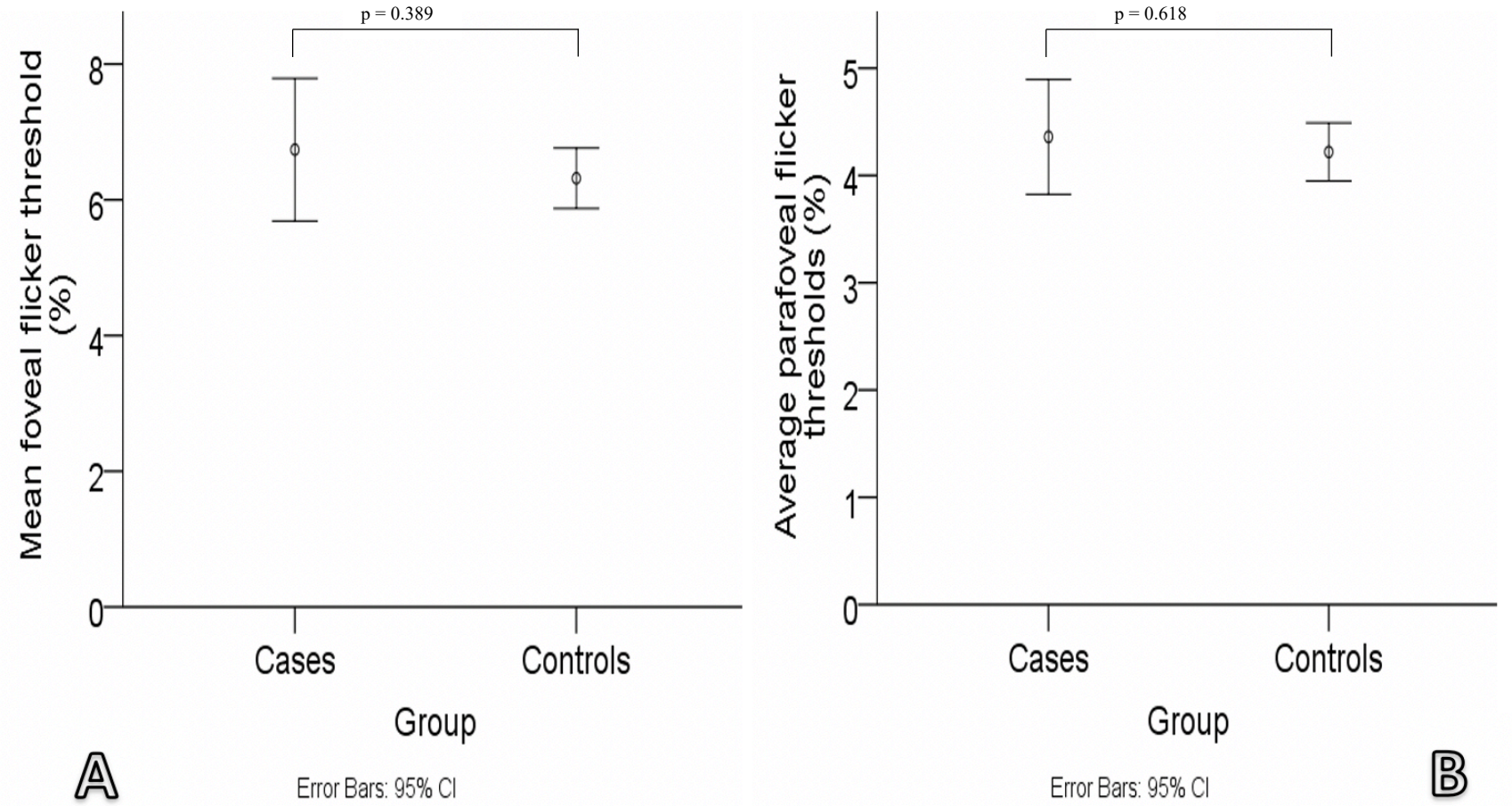


Figure 3.2: *Error plots showing the mean foveal (A) and average parafoveal (B) flicker thresholds of cases and controls. (Cases = subjects with symptomatic floaters; controls = subjects with healthy eyes).*

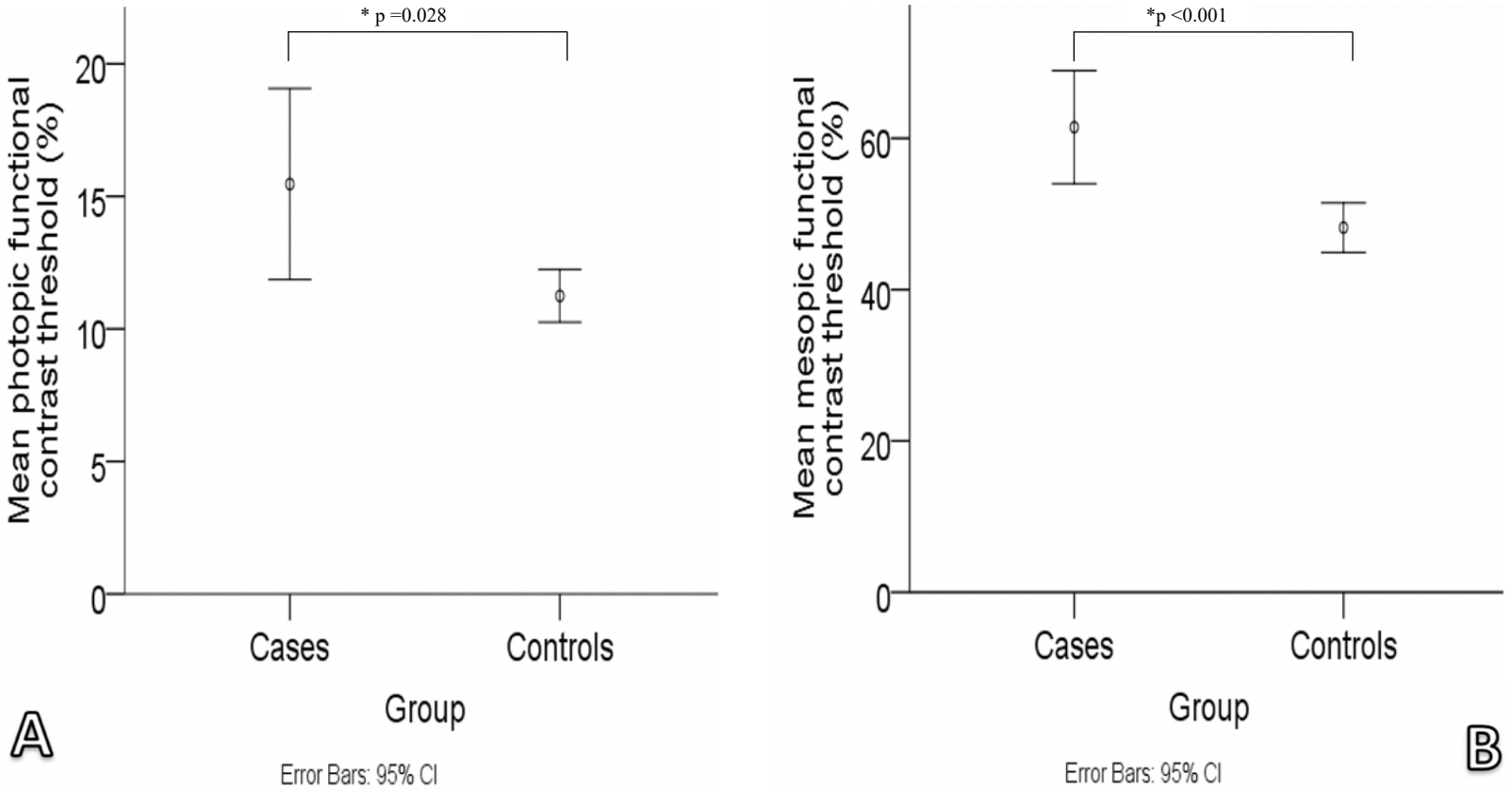


Figure 3.3: *Error plots showing the photopic (A) and mesopic (B) functional contrast thresholds of cases and controls. (Cases = subjects with symptomatic floaters; controls = subjects with healthy eyes;* ******* *statistically significant difference between the two groups at the 0.05 level).*

3.3.2 Effect of PVD on contrast thresholds

To assess the effect of PVD on functional contrast threshold, subjects were split into cases with PVD (n = 12), cases without PVD (n = 18), and controls (n=85). Cases with PVD were then compared with cases without PVD and controls to determine the influence of PVD on contrast threshold (Table 3.2). Foveal and parafoveal flicker thresholds were not significantly different between the three groups [F (2,112) = 0.433; p = 0.650 and F (2,112) = 2.024; p = 0.137 for foveal and parafoveal flicker thresholds, respectively]. In addition, photopic and mesopic VA were not statistically significantly different between the three groups [F (2,112) = 1.088; p = 0.340 and F (2, 112) = 0.338; p = 0.714 for photopic and mesopic VA, respectively]. Cases with PVD were significantly older than cases without PVD and controls [F (2, 112) = 3.974; p = 0.022). Therefore, subsequent analyses to determine the effect of PVD on contrast thresholds were controlled for age by employing a one-way ANCOVA test.

One-way ANCOVA was conducted to compare mean differences in functional contrast thresholds between the three groups. Photopic functional contrast was shown to be significantly different for the three groups [F (2, 112) = 5.875; p = 0.004]. Post hoc pairwise comparisons indicated a significantly lower photopic functional contrast thresholds in the cases with PVD compared with controls (a relative percentage difference in functional contrast threshold of 64.0%; p = 0.001). However, photopic contrast threshold in cases without PVD did not significantly differ from cases with PVD (p = 0.100) or controls (p = 0.504).

Mesopic functional contrast thresholds was significantly different in the three groups [F (2, 112) = 5.605; p = 0.005]. Post hoc analysis indicated significantly lower mesopic functional contrast thresholds in cases with PVD (a relative percentage difference of 30.3%; p = 0.014) and in cases without PVD (a relative percentage difference of 25.6%; p = 0.017) when compared with controls. However, mesopic functional contrast thresholds in cases without PVD did not significantly differ from cases with PVD (p > 0.999).

Table 3.2: *Visual function outcomes of the 3 groups.*

Variable	**Cases with PVD (n=12)**	**Cases without PVD (n=18)**	**Control (n= 85)**	**Sig.**
BCVA, LogMAR				
Photopic	0.07 ± 0.12	0.05 ± 0.14	0.02 ± 0.11	0.340
Mesopic	0.31 ± 0.13	0.30 ± 0.11	0.29 ± 0.10	0.714
Flicker threshold (%)				
Foveal	6.92 ± 2.45	6.62 ± 2.93	6.32 ± 2.06	0.650
Parafoveal	4.94 ± 1.72	3.98 ± 1.00	4.22 ± 1.26	0.137
Functional contrast threshold (%)				
Photopic	18.45 ± 12.45	13.48 ± 6.95	11.25 ± 4.59	0.004*
Mesopic	62.80 ± 16.82	60.54 ± 21.89	48.20 ± 15.10	0.005*

All data are expressed as mean ± SD; Sig., the statistical significance between the three groups (One-way ANCOVA for functional contrast threshold and ANOVA for age, flicker threshold, and BCVA); * statistically significant difference between the three groups; Cases, subjects with vitreous floaters; PVD, posterior vitreous detachment; Best Corrected Visual Acuity (BCVA) recorded as logarithm of minimum angle of resolution (LogMAR), a score of 0.00 corresponds with 6/6; Flicker threshold recorded as %; Functional contrast threshold measurements recorded as contrast threshold (%), lower % value implies better contrast.

3.4 DISCUSSION

The findings of this study indicate that contrast sensitivity is compromised in subjects with vitreous degeneration under both photopic and mesopic luminance. The aim of the study was to investigate whether the degeneration of the vitreous and the corresponding increase in scattered light can be detected as a change in functional contrast threshold by employing a stimulus rich in high spatial frequencies, which is known to be significantly influenced by optical factors (including ocular forward light scatter) while controlling for cofounders including age, lens opacity and neural factors.

Contrary to earlier reports of the vitreous not contributing to intraocular light scatter, recent studies have shown increased intraocular light scattering and hence, increased straylight, with vitreous degeneration.[252-254] The crystalline lens has been proposed to be the most likely cause of and major contributor to increased forward scatter and as a result, optical degradation, in the ageing eye, particularly at photopic luminance when the contrast sensitivity of retina is high.[255-257] Also, a rapid increase in forward scatter, with an associated reduction in photopic CS, has been reported for subjects over 45 years, in a study which excluded subjects with significant cataract.[248, 256] This suggests that there is a contribution to forward scatter and consequent reduction in photopic CS beyond the fourth decade that may be attributable to other intraocular light scattering sources, including vitreous degeneration.

With cataract and neural factors controlled for in the present study, we have attributed the difference in contrast thresholds observed in this case-control

comparison to vitreous degeneration due to the following reasons. Firstly, while residual, higher-order optical aberrations such as spherical aberration and coma have been implicated in the decrease in CS in older eyes, we assumed that the effect of these aberrations on CS, if any, would be similar for our age-matched sample such that the decrease in contrast herein observed could be attributed to vitreous degeneration.[258] Secondly, senile miosis, observed in the ageing eye, has been shown to improve depth of focus and to limit spherical aberration and coma. With the exception of reduced retinal illuminance, smaller pupils may contribute to enhanced spatial vision in our study population.[259] In addition, the directional sensitivity of cones also limits the detrimental effects of spherical aberration, when large pupils are involved.[260]

3.4.1 *Impact of vitreous degeneration on photopic contrast threshold*

Photopic functional contrast threshold was lower by 37.4% when cases were compared with controls, and by 64.0% when the PVD subgroup was compared with controls. To the best of our knowledge, this is the first study to assess the impact of vitreous degeneration on photopic contrast threshold by employing a contrast test that maximises the contribution of optical factors to the loss of retinal image contrast. It can be inferred from the present study that vitreous degeneration can account for at least 35% of photopic contrast threshold loss when compared with healthy eyes and worsens further with increasing vitreous degeneration. Intraocular light scattering, arising from the degenerated vitreous, casts a veil of undesired light upon the retinal image, resulting in decreased contrast sensitivity.[261, 262] This reduction in image contrast remains regardless of increases

in light level and may even result in a concurrent increase in glare as the illumination increases, causing a further reduction in contrast.

While the major contributor to photopic contrast decline has been attributed to cataractous lens changes, the data here are novel and support the hypothesis that vitreous degeneration contributes to the loss of photopic contrast threshold.[255] This significant decrease in photopic contrast threshold possibly explains the visual discomfort expressed by patients while performing important activities of daily life that involve bright backgrounds such as reading a book, working on a computer, enjoying outdoor sceneries, and driving during the day.[263]

3.4.2 *Effect of vitreous degeneration on mesopic contrast threshold*

In the present study, mesopic contrast was 27.5% less in cases compared with controls. Also, cases with PVD demonstrated a 30.3% lower mesopic contrast when they were compared with controls. Sebag and colleagues performed an age-matched analysis of 16 subjects with floaters, who were considered for minimally invasive vitrectomy, with 16 healthy controls and reported that mesopic CS was worse by 67% in the floaters group.[264] The same group prospectively studied previously normal eyes of subjects who subsequently developed PVD and reported a decline in mesopic CS of 52.5% following PVD development.[237] A retrospective study by the same group, designed to investigate the effect of the aging vitreous on CS, reported that CS was 51.2% worse in eyes with PVD compared with eyes with no PVD.[238] The same group has recently published a retrospective study

describing a 91% reduction in mesopic CS when 195 eyes of 145 subjects with vitreous floaters (of whom 77.9% had PVD) were compared with controls.[206]

The outcome of mesopic visual function assessment is influenced by the level of retinal illuminance, retinal location tested, and the spectral and spatial content of the stimuli. Each of these parameters can affect the interaction between rod and cone signals in the mesopic range and hence, the outcome of the test.[265] As these parameters differ for the commercially available mesopic contrast tests, it is not unusual to observe differences in mesopic CS outcomes when comparing different test devices. The previous studies employed either 3 cpd or a composition of low to medium spatial frequencies to assess mesopic CS while we employed a stimulus optotype rich in high spatial frequencies, which is arguably more relevant when assessing functional spatial vision.

High spatial frequencies contribute less to spatial vision at lower light levels. This is because high spatial frequencies are affected more, compared to lower spatial frequencies, at lower light levels and results in loss of retinal sensitivity to contrast.[262] Subsequently, one would expect that an increase in scattered light, which attenuates preferentially the high frequency end of the stimulus spectrum, should cause the greatest loss of CS at higher light levels, when the retinal sensitivity to contrast is high, and less so, at lower light levels, when high spatial frequencies are no longer resolved by the retina.[255, 266] The difference between this finding and results from other studies, which reported greater loss of CS in the mesopic range, illustrates why the method of assessing CS is important and can affect significantly the outcome of the experiment.

A limitation to this study is that residual, high order aberrations were not measured and corrected, particularly for large pupil sizes in the mesopic range. While we acknowledge the impact of higher-order aberration on CS, the age-matched study design assumes that both groups are affected similarly by the effects of these aberrations, and the difference in contrast thresholds observed can, therefore, be attributed to vitreous degeneration. Higher order aberrations are indeed important, but less so with smaller pupil size (at photopic light levels) and with a Landolt ring stimulus three times above the mean acuity limit. Pupil size was undoubtedly larger under mesopic conditions but the lower, expected, retinal sensitivity to contrast in the mesopic range makes the increase in higher order aberrations less effective.[267]

Another limitation is that, the sample sizes of the cases with PVD and cases without PVD were small and a larger study is needed to confirm the findings when comparing contrast thresholds of the three groups. While smaller samples could have provided results that were not sufficiently powered to detect a difference between the groups and a higher propensity for a type II error to occur, the significant difference in contrast thresholds observed between the groups underscore the major impact of the varying degrees of vitreous degeneration on contrast. In our study, we did not assess the severity of vitreous degeneration with quantitative ultrasonography, which has been shown to correlate with loss of contrast sensitivity.[159] Overall, the findings of this study confirm previous reports and add to our knowledge of the contribution vitreous degeneration makes to the loss of contrast threshold, especially under photopic luminance.

3.5 CONCLUSION

Vitreous opacities and PVD diminish spatial contrast under photopic and mesopic luminances. The findings from this study also highlight the importance of the method employed to assess contrast thresholds at photopic and mesopic light levels. In addition to eliminating the symptomatic effects caused by vitreous opacities, any treatment that can safely reduce or eliminate vitreous degeneration will also improve CS.

3.6 CHAPTER SUMMARY

This study is the first to assess the impact of symptomatic vitreous degeneration on photopic and mesopic contrast thresholds at a high spatial frequency. What this study has shown is that, vitreous degeneration degrades both photopic and mesopic contrast thresholds at high spatial frequencies. The reduction in photopic contrast threshold may explain the visual discomforts, and the subsequent reduction in vision-related quality of life, of patients suffering from symptomatic vitreous degeneration. This study has been published in Clinical and Experimental Optometry (Impact factor = 1.918; Manuscript ID, CEOptom-20-453-OP.R1) under the title, '***The impact of symptomatic vitreous degeneration on contrast thresholds: a case-control study***' (Appendix F3).

Chapter 4

ULTRA-WIDEFIELD INFRARED IMAGING OF VITREOUS OPACITIES

4.1 INTRODUCTION

Intravitreal opacities that arise from the degeneration of the vitreous body itself cause light scattering and cast shadows on the retina, and this results in the entoptic phenomenon described as primary vitreous floaters. Symptomatic floaters cause intermittent blurred vision, sensitivity to glare and visual haze, consequently impacting on visual function and vision-related quality of life of its sufferers.[202, 237]

Until recently, diagnosis of vitreous floaters was largely based on patients' subjective report. Optical coherence tomography (OCT) and b-scan ultrasound are two imaging modalities that have been recently employed to diagnose vitreous floaters. Recently, Sebag and associates have shown that quantitative ultrasound based imaging (QUS) of the vitreous is a plausible method for imaging and quantifying the severity of floaters.[38, 159] Sun and colleagues have also shown the use of an open source software, Image J (National Institute of Health, USA), to quantify floater shadow areas on 30° or 55° field infrared fundus photographs of patients who were treated with laser vitreolysis for floaters.[268] A limitation to this

methodology is that because the imaging is focussed on only a small field of the retina, only the floater shadows that appear within this field can be quantified. An improvement on this methodology will be to employ ultra-widefield confocal scanning ophthalmoscopy imaging focussed on the vitreous to allow for a wider visualisation of the vitreous body.

Current treatment options for floaters include watchful waiting, pars plana vitrectomy and laser vitreolysis.[205, 206] Neodymium-doped yttrium-aluminium-garnet (Nd:YAG) laser vitreolysis, is a well described treatment that involves the application of Q-switched laser energy to photodisrupt vitreous opacities.[269] According to the 2018 American Society of Retina Specialist (ASRS) Preferences And Trends (PAT) Survey, at least 90% of retinal physicians have never performed Nd:YAG laser vitreolysis to treat vitreous floaters.[270] Notwithstanding this, modern laser devices which are optimised to provide better visualisation of the entire vitreous as well as to ensure efficient laser energy delivery to target tissues offer the potential for more effective and safer outcomes of laser vitreolysis compared to older generations of laser devices.[271]

The present study was conducted to assess the effectiveness of Nd:YAG laser vitreolysis for treating vitreous floaters using ultra-widefield vitreous imaging.

4.2 METHOD

4.2.1 Study design

Consecutive patients diagnosed with symptomatic primary vitreous floaters between January 2016 and February 2020 which were treated with laser vitreolysis at the Institute of Eye Surgery were retrospectively enrolled into this study. All patients underwent a pre-treatment scan to document the extent of floaters, plan the treatment and categorise the floaters into anterior, posterior or mid-vitreous floaters. Patients with secondary floaters (from for example, uveitis and trauma), massive discrete floater(s) or non-discrete vitreous haze were deemed unsuitable for laser vitreolysis. Only one eye was treated per session per patient.

4.2.2 Ethics

All participants signed an informed consent for their procedure and for their anonymised data to be used for research. Anonymised data were collated from Medisoft Electronic Medical Records (Medisoft Limited, UK). The study adhered to the tenets of the Declaration of Helsinki. The study protocol and study design were approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Co. Cork, Ireland [ECM 4 (z) 10/03/2020] and the Research Ethics Committee of the Waterford Institute of Technology, Co. Waterford, Ireland (WIT2019REC0026).

4.2.3 Clinical assessments and outcomes

Intra- and post-operative complications were recorded. The need for subsequent vitrectomy and patients' satisfaction at each follow-up visit were recorded.

Patients' satisfaction outcome was divided into 4 discrete categories: (a) complete resolution of symptoms (b) improvement of symptoms (c) no improvement in symptoms and (d) deterioration or worsening of symptoms.

Best corrected visual acuity (BCVA) and intraocular pressure (IOP) were measured, at baseline and subsequent follow-ups, with an electronic Early Treatment Diabetic Retinopathy Study (ETDRS) chart (Thomson Xpert 2000, Thomson Software Solutions, UK) and a rebound tonometer (iCare, Finland), respectively.

4.2.3.1 Laser vitreolysis

Laser vitreolysis was performed as an out-patient procedure with the VISULAS YAG III laser (Carl Zeiss Meditec AG, Germany) under topical anaesthesia (Proxymetacaine 0.5%, Bausch & Lomb, USA) by a single surgeon. The study eye was fully dilated with tropicamide 1% and phenylephrine 10% (Bausch & Lomb, USA). All cases were performed with a Karickhoff 30 mm off-axis vitreous lens (Ocular Instruments, USA). For anterior floaters, the procedure was commenced with single burst 2.0 mJ energy, which was increased till a desired effect was achieved. Vitreous opacities within 2 mm distance to the lens were treated cautiously by initially focusing the aiming beam posterior to the floater, before moving the focus anteriorly towards the floater.

For posterior floaters, a single pulse 9.0 mJ energy was used and the number of pulses was increased until the desired effect was achieved. Maximum pulse energy

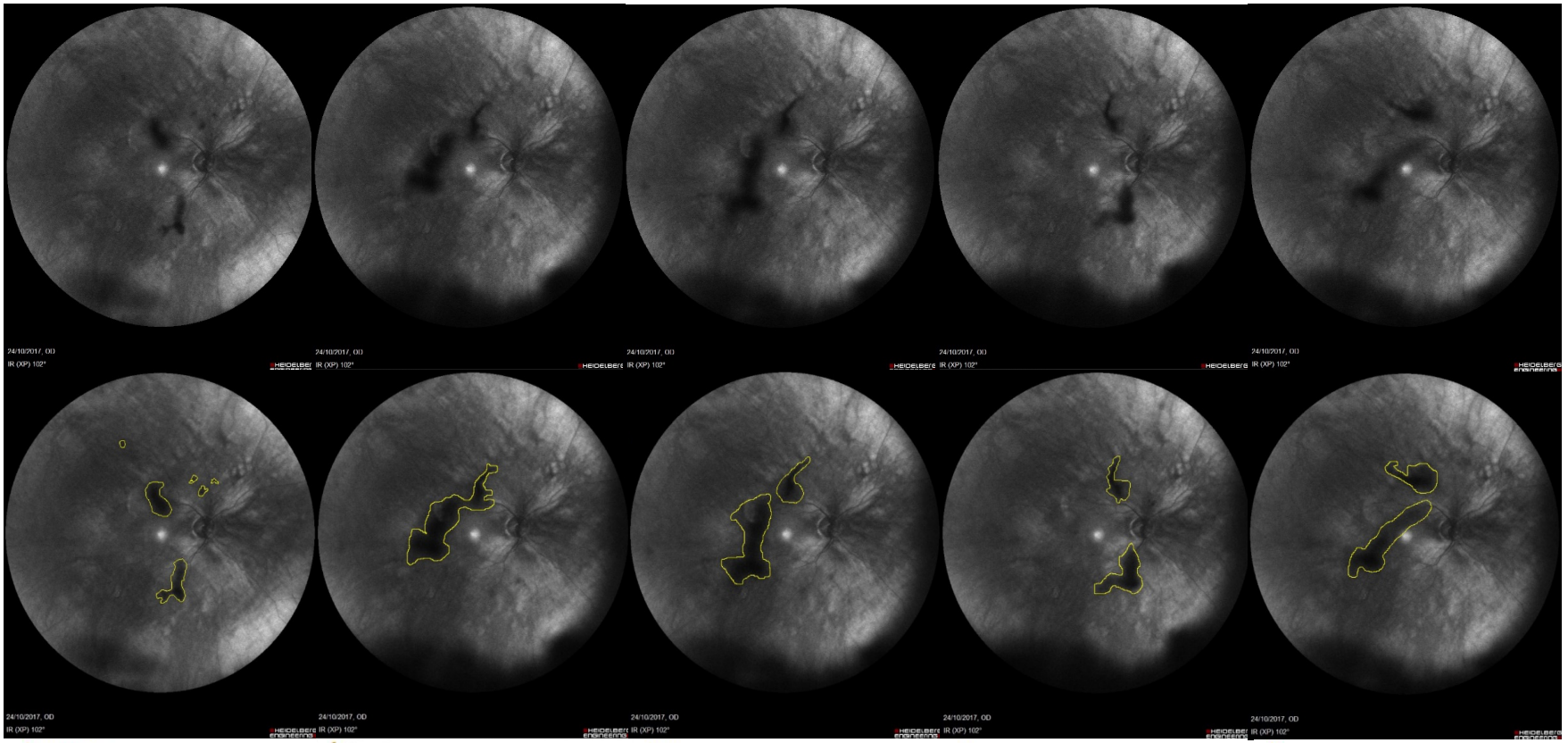
possible on the VISULAS YAG III was in triple burst mode, delivering a maximum energy of 27 mJ. Vitreous opacities adjacent to the retina (where both floaters and retina were similarly focussed) were treated by initially focusing the aiming beam on the retina before moving the focus anteriorly towards the opacity. No medication was prescribed and no patient restriction was advised following the procedure. Patients were offered pars plana vitrectomy if they considered the initial treatment unsuccessful.

4.2.3.2 Vitreous Opacity Area Quantification

A 30-second, 102° field, cross-polarised infrared reflectance [IR(XP)] movie of the vitreous was recorded from the study eye using the ultra-widefield angiography module (UWF-Module) of the Spectralis HRA + OCT Multicolour (Heidelberg Engineering GmbH, Heidelberg, Germany). In recording the video, patients were instructed to gaze in the upward, downward, rightward and leftward directions, and the eye returned back to the internal fixation target of the device after each gaze. This allowed for the vitreous opacities to be sufficiently mobile in order to capture all the potential sizes of the opacities. After the video was acquired, 5 still, 768 x 868-pixel images were obtained from the video using the Heidelberg Eye Explorer software (Heyex; version 1.10.4.0) and the acquisitions were made when the eye was fixated on the internal target after each of the eye movements.

The images were then imported into Image J (version 1.53f, National Institute of Health, USA) and were converted into 8-bit type files, as described by Sun and colleagues.[268] The image scale was set at 1 pixel/mm. Vitreous opacity outlines

were manually traced using the ‘freehand selection’ tool. For precise tracing of opacity outlines, the ‘magnifying glass’ tool was employed to enlarge the entire image before tracing. After the tracing was completed, the ‘measure’ tool was selected from the ‘analyze’ menu and the software automatically generated the vitreous opacity area. The results from the 5 still images were averaged to obtain the vitreous opacity area (in mm^2) and have been reported in cm^2 (Figure 4.1). A single investigator (E.A) conducted all the image analysis for pre-operative and post-operative scans to assess change in floater areas following the treatment.



Results

	Area	Mean	Min	Max
1	7259	40.359	9	93

Results

	Area	Mean	Min	Max
1	15713	31.841	2	95

Results

	Area	Mean	Min	Max
1	18034	33.007	7	85

Results

	Area	Mean	Min	Max
1	9388	43.153	8	127

Results

	Area	Mean	Min	Max
1	16458	37.350	7	116

Figure 4.1: *Vitreous opacity areas quantification using ultra-widefield infrared vitreous imaging and Image J software. Average floater area of 133.70 ± 47.43 cm^2.*

For this study, the change in vitreous opacity areas following laser vitreolysis were grouped into categories as follows: 100% - complete resolution (Figure 4.2); 30 – 99% - improvement (Figure 4.3); 0 – 29% - no / minimal improvement (Figure 4.4); <0% - deterioration (Figure 4.5). Treatment was deemed successful if there was either complete resolution or improvement based on this imaging modality.

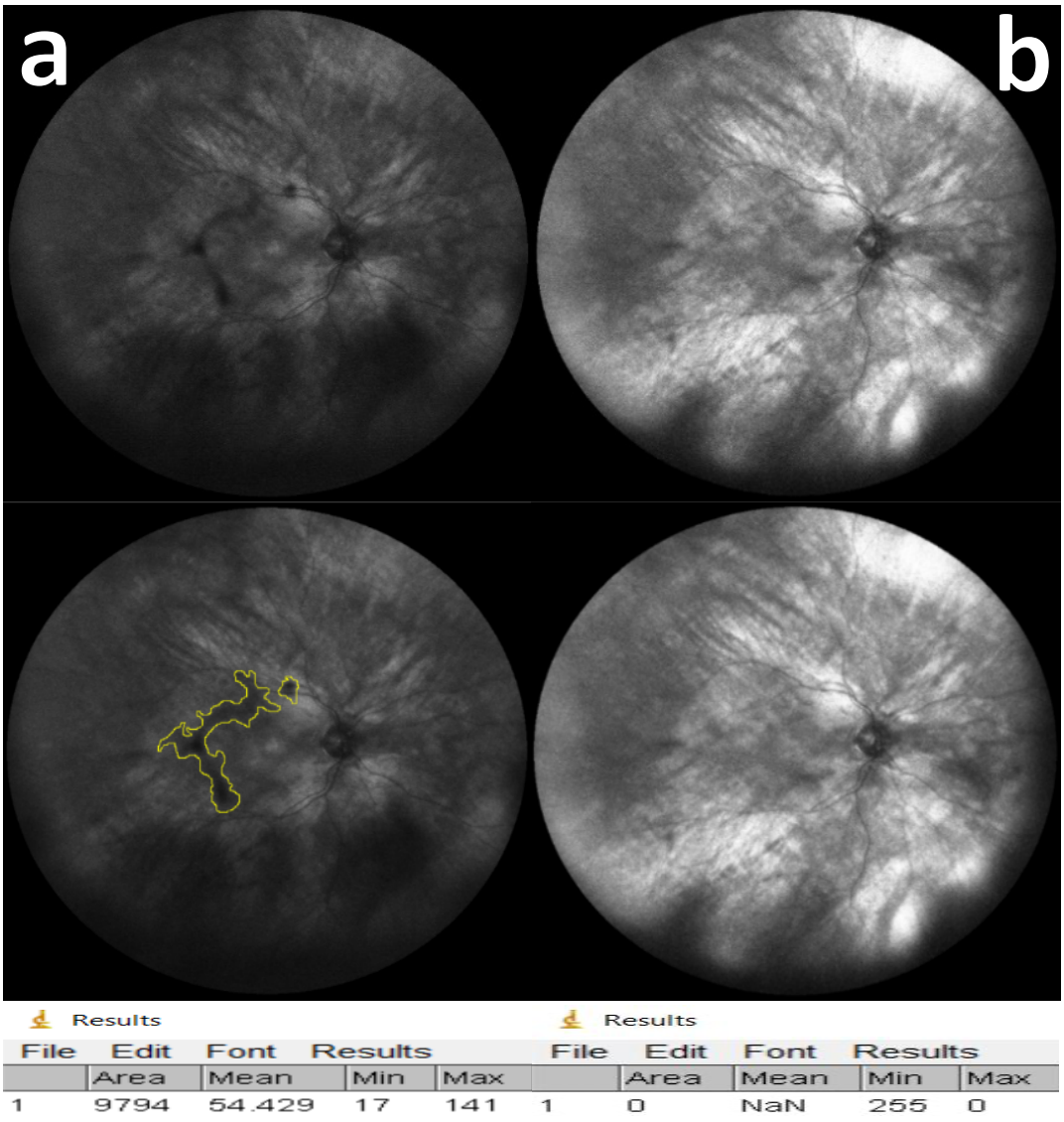


Figure 4.2: *Change in vitreous opacity areas categorised as 'complete resolution' based on pre-operative (a) and final visit (b) scans*

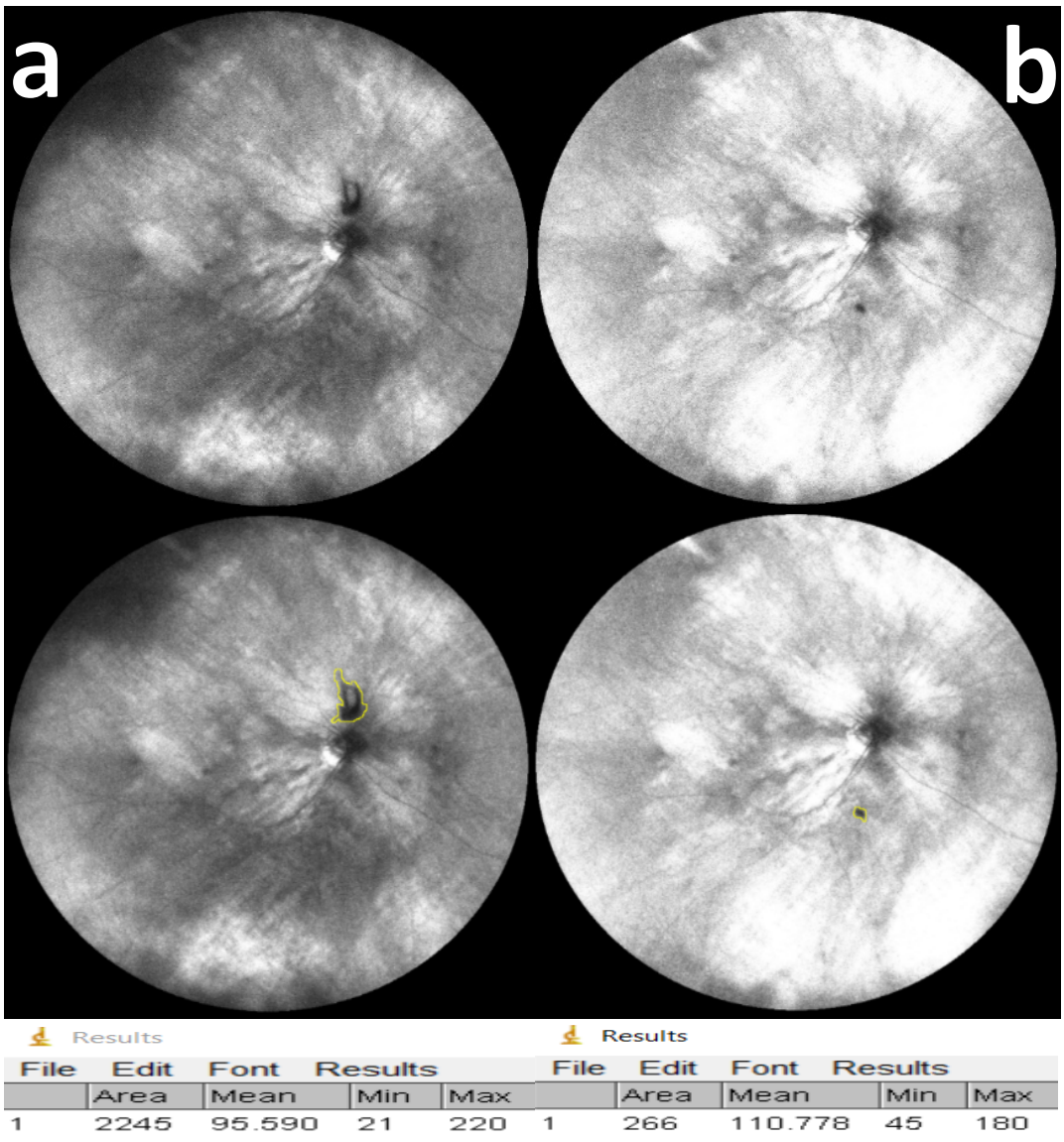


Figure 4.3: *Change in vitreous opacity areas categorised as 'improvement' based on pre-operative (a) and final visit (b) scans.*

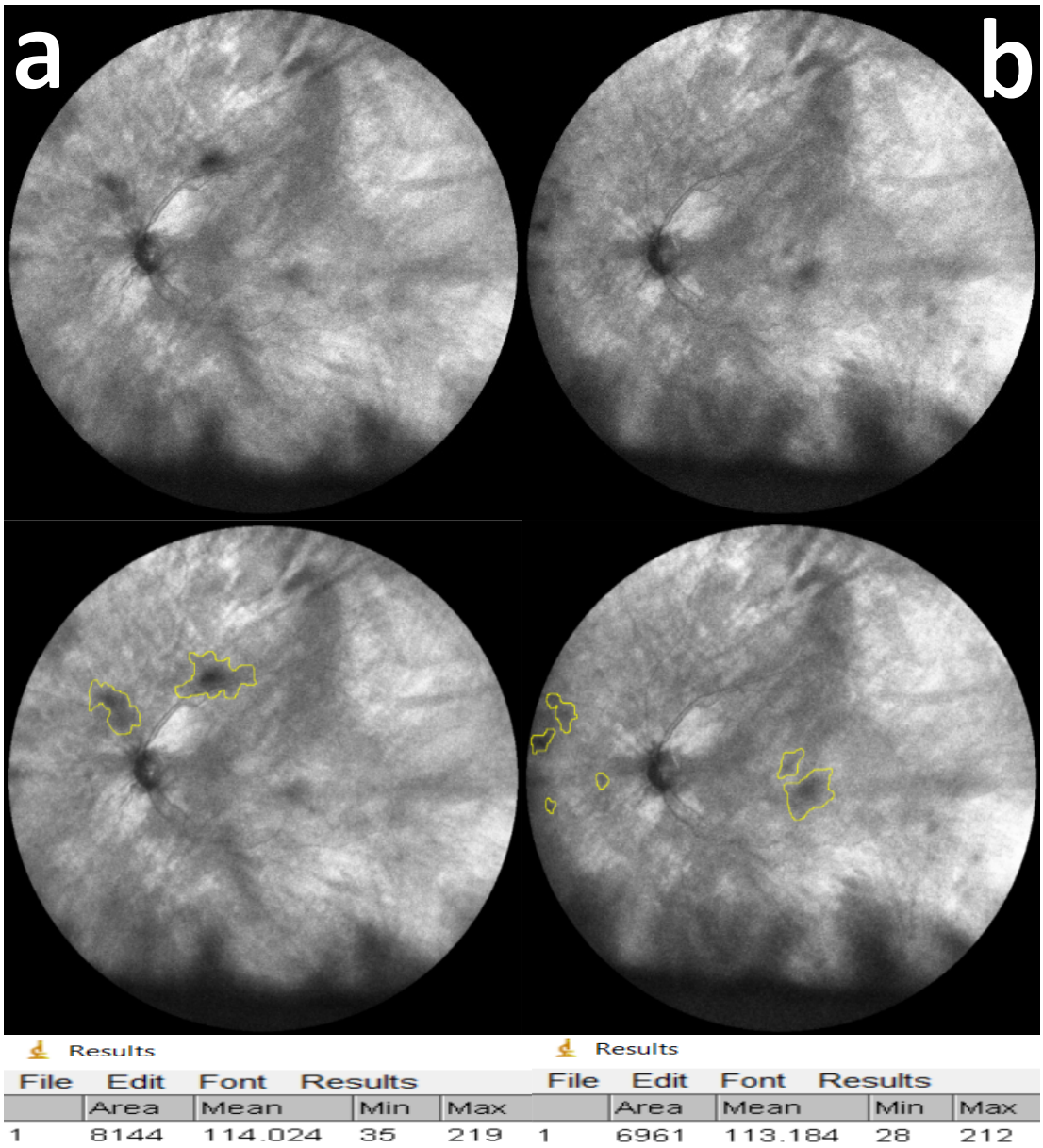


Figure 4.4: *Change in vitreous opacity areas categorised as 'No Improvement' based on pre-operative (a) and final visit (b) scans.*

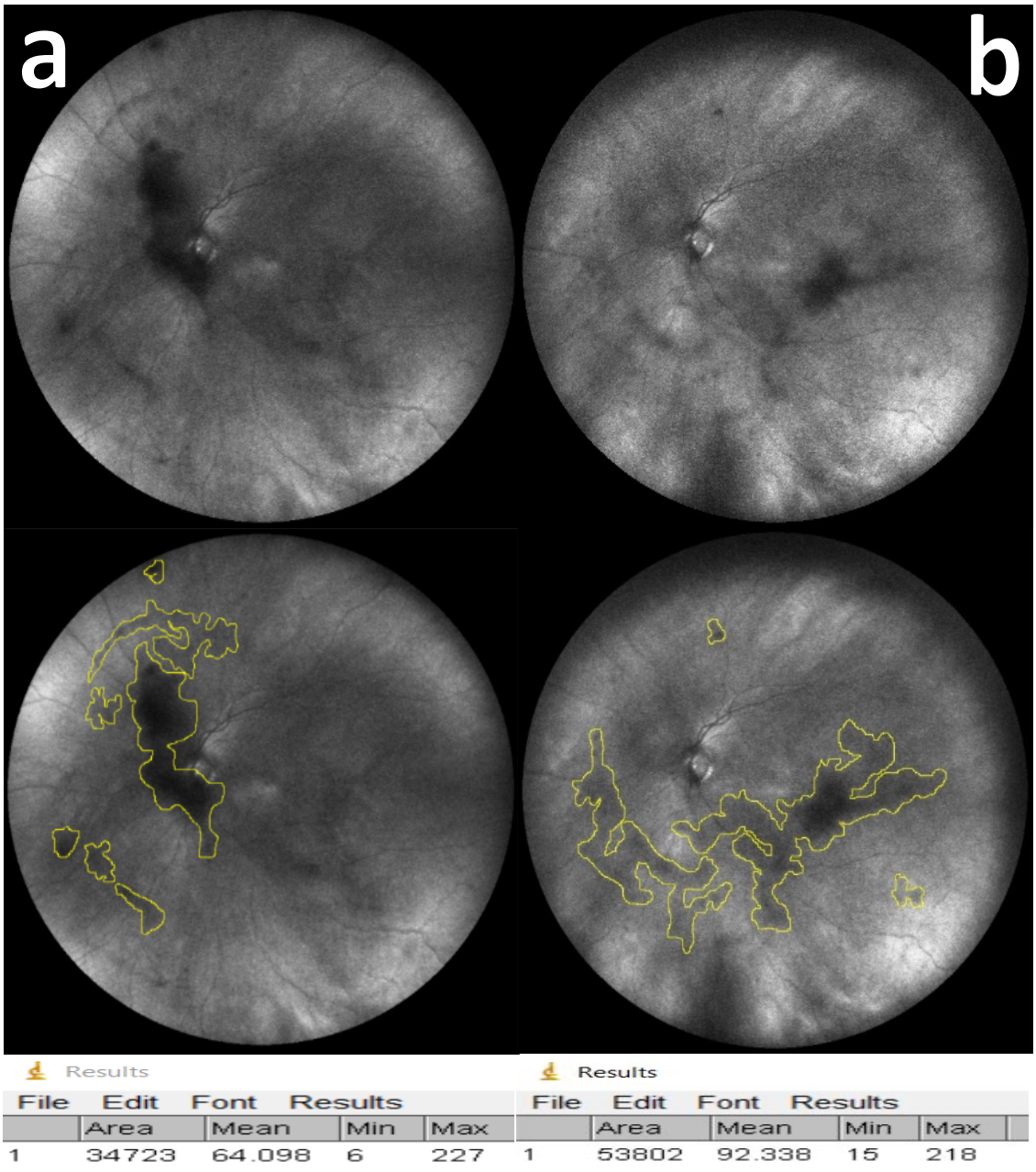


Figure 4.5: *Change in vitreous opacity areas categorised as 'Deterioration' based on pre-operative (a) and final visit (b) scans.*

4.2.4 Statistical analysis

SPSS® Statistics version 25 (IBM, USA) and Excel 2016 (Microsoft, USA) were used for the statistical analyses. Descriptive statistics was used for continuous and categorical outcomes. Paired-sample *t*-tests were used to analyse BCVA, IOP and quantitative vitreous opacity areas for change following laser vitreolysis. A 5% level of significance was applied.

4.3 RESULTS

4.3.1 Baseline characteristics

Table 4.1 summarises the demographic and baseline ocular characteristics of the study population. A total of 77 eyes from 77 subjects were enrolled into the study. The mean age of patients was 61 years old (range: 38 - 82 years). No patient underwent more than 3 sessions of laser vitreolysis (66 eyes were treated with a single procedure, 10 eyes underwent 2 treatment sessions, and only one eye had 3 treatment sessions). Less than half (48%) of the subjects were followed up for less than a year, a third (34%) were followed up for between 1 to 2 years and 18% of subjects were followed up for more than 2 years.

Of the 77 eyes treated with laser vitreolysis, 12 were still symptomatic enough to elect for vitrectomy (mean duration to vitrectomy after laser vitreolysis = 14 months; range: 4 to 47 months). Of these 12 eyes, 4 of the vitrectomies were combined with cataract surgery. Vitrectomy was curative.

Table 4.1: *Demographic, baseline and treatment characteristics of study population (n = 77)*

Variable	**n (%)**
Sex	
Male	31 (40.3)
Female	46 (59.7)
Study Eye	
OD	39 (50.6)
OS	38 (49.4)
PVD status	
PVD	48 (62.3)
No PVD	29 (37.7)
Lens status	
Phakic	50 (64.9)
Pseudophakic	27 (35.1)
Refractive status	
Emmetropic	34 (44.1)
Myopic	33 (42.9)
Hyperopic	10 (13.0)
Location of opacities in vitreous	
Anterior	16 (20.8)
Central	43 (55.8)
Posterior	18 (23.4)

Data displayed are n (%) for categorical data; OD, Right eye; OS, Left eye; PVD, Posterior vitreous detachment

4.3.2 Effectiveness of Nd:YAG laser vitreolysis

4.3.2.1 Objective effectiveness of laser vitreolysis

Figure 4.6 and Table 4.2 describe the objective effectiveness of laser vitreolysis. There was a significant decrease in vitreous opacity areas at the final visit following laser vitreolysis ($p<0.001$). Of the 77 participants, there was complete resolution, improvement, no/minimal improvement and deterioration, of vitreous opacities, in 39.0%, 50.6%, 2.6% and 7.8%, respectively, of participants. Success of laser vitreolysis by this methodology was 89.6%.

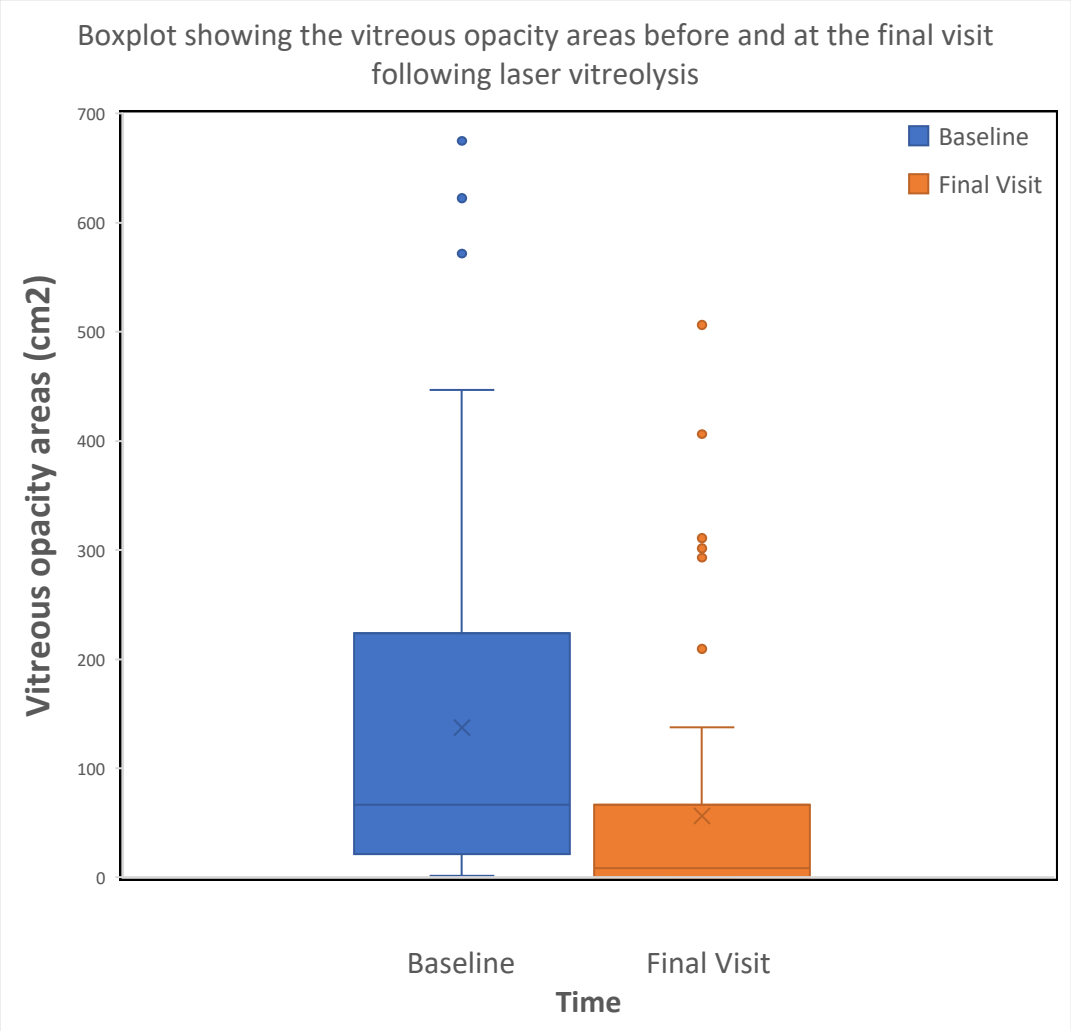


Figure 4.6: *Boxplot showing the vitreous opacity areas at baseline and at the final visit following laser vitreolysis.*

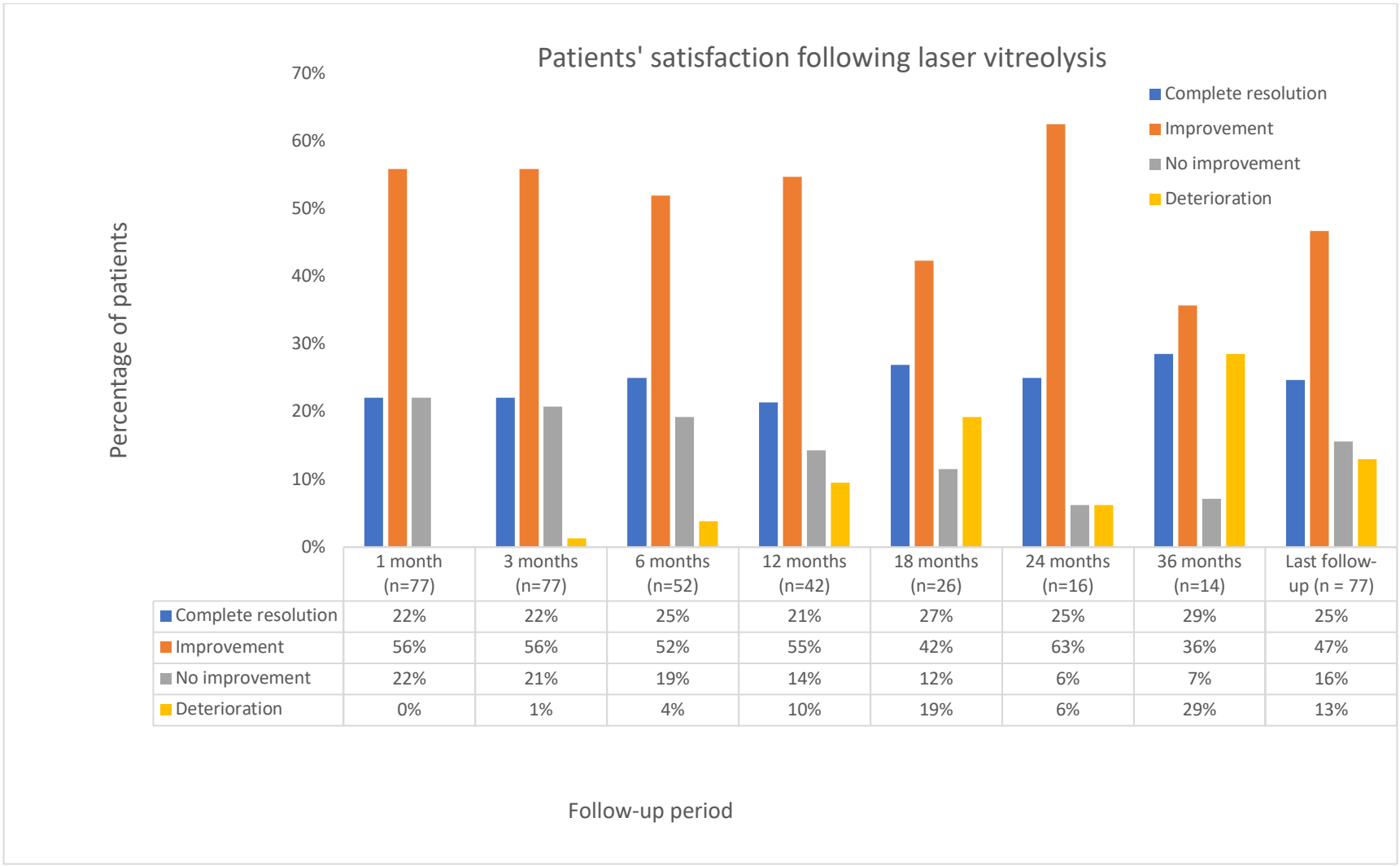
Table 4.2: *Vitreous opacity areas, BCVA and IOP at baseline and post-operative visits (n=77)*

Variables	Pre-operative	Post-operative			
		1 month	p^a	Last follow-up	p^b
Vitreous opacity areas, mean (SD), cm^2	136.04 ±155.80			55.77 ± 108.30	<0.001
BCVA, mean (SD), logMAR	0.15± 0.17	0.14 ± 0.15	0.161	0.11 ± 0.15	0.019
IOP, mean (SD), mmHg)	15.01 ± 2.90	14.47 ± 2.96	0.025	14.06 ± 3.97	0.006

BCVA, Best corrected visual acuity (BCVA) recorded as logarithm of minimum angle of resolution (logMAR), a score of 0.00 corresponds to 20/20 (6/6); IOP, Intraocular pressure (IOP) recorded as mmHg; vitreous opacity areas recorded as cm^2; p^a, paired *t*-test between pre-operative and month 1; p^b, paired *t*-test between pre-operative and last follow-up

4.3.2.2 Subjective effectiveness of laser vitreolysis

Figure 4.7 describes the subjective effectiveness of laser vitreolysis. Sixty eyes (78%) reported either total resolution or improvement, 17 eyes (22%) reported no improvement and no eye reported a deterioration of their visual symptoms at 1-month post-operative visit. At the final follow-up (mean follow-up = 16 months; range: 3 – 53 months), 71% of eyes reported either total resolution or improvement, and 29% reported either no improvement or deterioration, of their visual symptoms (Figure 4.7).



	1 month (n=77)	3 months (n=77)	6 months (n=52)	12 months (n=42)	18 months (n=26)	24 months (n=16)	36 months (n=14)	Last follow-up (n = 77)
Complete resolution	22%	22%	25%	21%	27%	25%	29%	25%
Improvement	56%	56%	52%	55%	42%	63%	36%	47%
No improvement	22%	21%	19%	14%	12%	6%	7%	16%
Deterioration	0%	1%	4%	10%	19%	6%	29%	13%

Figure 4.7: *Patients' satisfaction following Nd: YAG laser vitreolysis at the follow-up visits.*

4.3.2.3 Effect of Nd:YAG laser vitreolysis on BCVA and IOP

Table 4.2 presents the BCVA and IOP pre- and post- Nd:YAG vitreolysis. There was no change in BCVA from baseline at 1-month post-operative visit ($p > 0.05$). There was, however, a significant improvement in BCVA at the final visit ($p = 0.019$). A statistically significant decrease of less than 1mmHg of IOP after YAG vitreolysis at 1 month and last post-operative visit ($p<0.05$ for both) from baseline is not clinically meaningful.

4.3.4 Complications

Two intra-operative complications were recorded. One case (1%) of posterior lens capsule injury occurred due to an inadvertent delivery of laser energy, originally targeted at an anteriorly-located vitreous opacity, to the posterior capsule (Figure 4.8). This adverse event was managed with hydrodelineated phacoemulsification combined with vitrectomy 14 months after YAG laser vitreolysis because initial lens trauma did not cause a visually significant cataract. One case (1%) sustained mild retinal bleed which resolved spontaneously without any treatment (Figure 4.9).

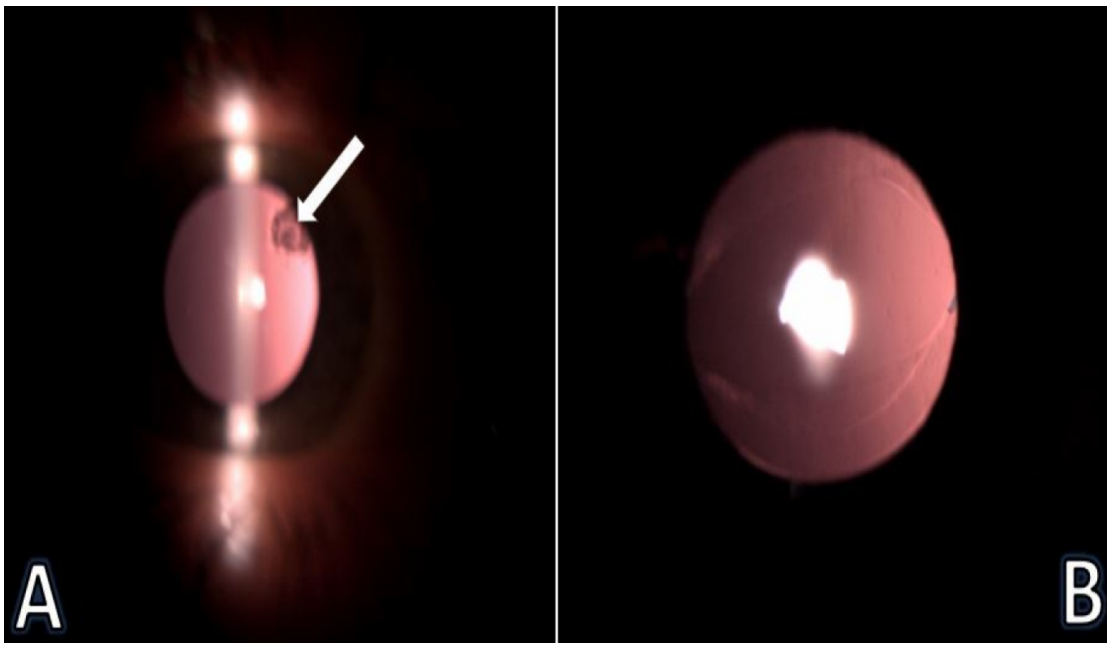


Figure 4.8: *Slit lamp retroillumination image of the crystalline lens showing the posterior capsule injury (A; white arrow) and optic capture of the intraocular lens following cataract surgery (B).*

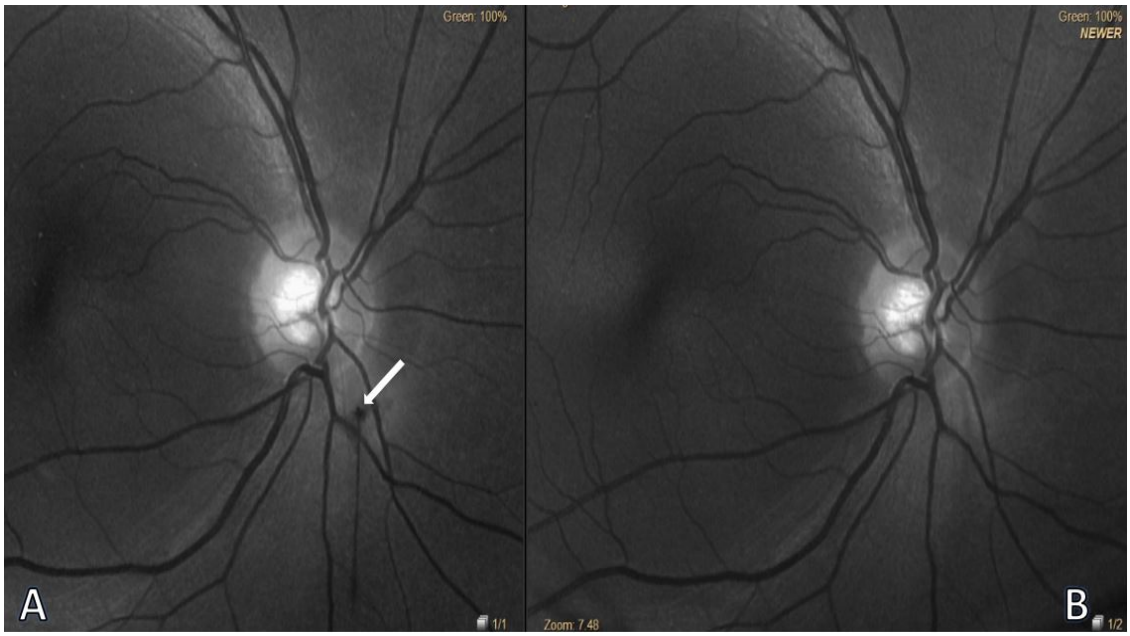


Figure 4.9: *Green channel imaging showing trickle of blood into vitreous from YAG laser retinal injury just infranasal to the optic disc at day 0 (A; white arrow) and spontaneous recovery by week 2 without any intervention (B).*

4.4 DISCUSSION

This study investigated the effectiveness of Nd:YAG laser vitreolysis for treating symptomatic vitreous floaters using the VISULAS YAG III. Our objective success rate and our patient-reported efficacy rate at the last follow-up were 89.6% and 71%, respectively. Also, an intra-operative complication rate of 3% was recorded. Given that only the first eye of all patients was included in this analysis, our success rate was free from the confounding effects of a previously-successful fellow eye treatment. Further, our high efficacy rate may be due to the exclusion of patients with extensive floaters; these patients were offered vitrectomy.

There was a significant reduction in objective vitreous opacity areas following laser vitreolysis in the present study, characterised by a success rate of 90%. In addition, 78% of patients reported complete resolution or improvement of their floater symptoms at 1-month post-operative visit. This response was sustained in 71% of study eyes at the last follow-up. However, 15% of eyes were unsatisfied and subsequently underwent vitrectomy by the last follow-up.

In a randomised clinical trial by Shah and Heier to evaluate YAG laser vitreolysis versus Sham YAG vitreolysis for symptomatic floaters, 34 of 36 patients (94%) in the YAG group had significantly improved or completely resolved floaters compared with 0 in the sham group based on masked grading of fundus photographs.[269] Sun and colleagues retrospectively investigated the treatment efficacy of Nd:YAG laser vitreolysis for symptomatic vitreous floaters using infrared fundus photography and reported objective and subjective improvements

of 94.5% and 92.7%, respectively (improvement here was defined as complete success, significant success or partial success).[268]

It appears from our findings, and that of previous studies, that the objective treatment success of laser vitreolysis observed may not necessarily translate into subjective success. According to Shah and Heier, some patients hold huge expectations prior to laser vitreolysis such that in the event where they do not achieve complete resolution or significant improvement of their symptomatology following the procedure, they tend not to notice the difference between their discomfort prior to the procedure and the discomfort from the residual floaters post the procedure, in spite of the reduction in the vitreous opacities observed on objective analysis. Thus, patient expectations should be well managed prior to the procedure, and the objective diagnostic technique employed should be employed in counselling the patient before and after the procedure.

Shah and Heier recently conducted an observational extension study on the long-term efficacy of Nd:YAG laser vitreolysis on 35 patients, who were enrolled in their previous randomised trial and returned for follow-up.[272] At 2.3 years of follow-up, 50% of patients felt their symptoms were significantly or completely better. Our study, with a mean follow-up of 16 months, had a higher treatment success compared to that of Shah and Heier (71% versus 50%) because our study allowed for re-treatments. Our treatment success with a single treatment was 64%. Other factors that could also influence the efficacies recorded in the different

studies include the type of the laser device and the differences in the length of follow-up.

There was a significant improvement in BCVA from baseline following laser vitreolysis, consistent with previous reports.[273] However, other studies indicated no change in visual acuity following laser vitreolysis.[268, 274] Previous case reports have documented spikes in IOP subsequent to laser vitreolysis, consequently resulting in glaucoma that warranted treatment.[210] However, mean IOP recorded in our study population was similar at baseline and at all follow-ups.

Symptomatic vitreous floaters are commonly a progressive condition and laser vitreolysis does not involve the removal of the source of the floaters (i.e., the vitreous gel). As a result, efficacy of laser vitreolysis may deteriorate with time, as new floaters may develop with time due to progressive degeneration of the vitreous. In our study, there was an increase in the percentage of patients who noted a deterioration in their symptoms at 36 months. This is likely a result of one or a combination of the following: 1) follow-up bias where patients without symptoms are more likely to be lost to follow up 2) small sample size at 36 months and 3) accumulation of vitreous floaters occur beyond 3 years. Also, some patients required vitrectomy many years after initial successful laser vitreolysis. This underscores the importance of long-term follow-up of laser vitreolysis patients.

Complications that occurred in this study were unsurprisingly vitreoretinal in nature. Posterior lens capsule defect has been previously documented as one of the

most significant potential adverse events following YAG laser vitreolysis by the ASRS Research and Safety in Therapeutics (ReST) Committee.[208] In addition, in a randomised clinical trial by Shah and Heier, one posterior chamber intraocular lens was pitted peripherally intra-operatively.[269] Thus, care must be taken when treating anterior floaters.

Sun et al. reported a single intra-operative case of mild retinal haemorrhage, which resolved following glucocorticoid and vitamin C treatment for two weeks.[268] The ASRS ReST Committee has also documented retinal haemorrhage as a potential complication following laser vitreolysis.[208] The case of retinal bleed recorded in our study was mild and resolved spontaneously without any intervention. No post-operative adverse event was noted at any follow-up time point.

A limitation associated with this study is the retrospective nature of the study design. A prospective pivotal clinical trial, the type of study used for regulatory approval of medicines, vaccines and medical devices, would certainly further improve our understanding of a procedure that could be, for many patients, an office procedure to treat a condition that is currently treated with an intraocular procedure (vitrectomy).

4.5 CONCLUSION

The findings of this study indicate that Nd:YAG laser vitreolysis results in both objective and subjective improvements for at least two-thirds of patients who undergo the procedure, and should be considered as a treatment option for patients

suffering from floaters. Caution is, however, required when treating anterior floaters as well as floaters adjacent to the retina. Floater symptoms may recur with time following Nd:YAG laser vitreolysis and may necessitate vitrectomy to alleviate the symptoms following laser vitreolysis.

4.6 CHAPTER SUMMARY

The study shows that at least two-thirds of patients who undergo laser vitreolysis have at least significant subjective improvements (if not resolution) of their floater symptoms, and this improvement was corroborated by improvements in objective assessment of vitreous opacities. The dataset reported in this study has been included in a larger dataset, which has been submitted to Graefe's Archive for Clinical and Experimental Ophthalmology (Impact Factor = 2.10) for review and publication under the title, '***Nd:YAG laser vitreolysis for the treatment of symptomatic vitreous floaters: safety and effectiveness assessment***.'

Chapter 5

DIETARY INTERVENTION WITH A TARGETED MICRONUTRIENT FORMULATION OPTIMISES VITREOUS HEALTH IN PATIENTS WITH SYMPTOMATIC VITREOUS DEGENERATION

5.1 INTRODUCTION

Degeneration of the exquisite vitreous gel is ubiquitous during life, mainly resulting from aging or disease. Two principal processes, liquefaction (synchisis senilis) and posterior vitreous detachment (PVD), account for vitreous degeneration.[38] Oxidative stress, increased intravitreal proteolytic enzymes and decrease in vitreous antioxidant capacity have been proposed as the underlying mechanisms for these degenerative processes.[14, 160, 161] Vitreous degeneration culminates in vitreous floaters, the perception of linear strands and dark grey spots primarily within the central visual field.[263]

Floaters have significant negative impact on visual function and vision-related quality of life of its sufferers.[199, 237] Unfortunately, the conventional treatment mostly offered to these sufferers, after clinicians have successfully ruled out the possibilities of retinal pathologies following the onset of floaters, is watchful

waiting. Aside watchful waiting, pars plana vitrectomy and Nd:YAG laser vitreolysis are the other treatment options available. Importantly, the potential sight-threatening risks associated with these treatment options, including retinal detachments, cataract development/progression, iatrogenic tears, vitreo-retinal haemorrhage, worsening floaters, prolonged elevation of intraocular pressure, and refractory open-angle glaucoma, deter clinicians from recommending these treatments, especially when the desired benefits do not significantly outweigh the potential risks.[206-211] It follows from the above that a low-risk yet effective therapy is warranted to boost the vision-related quality of life of patients with floaters.

In terms of a low-risk yet effective therapy for floaters, a plausible idea to pursue relates to management with micronutrients that can retard the afore-mentioned mechanisms underpinning vitreous degeneration. This rationale derives from *in vitro* experimental evidence indicating the potency of exogenous micronutrients such as hesperidin, verbacosides, leucocyanidins, and l-lysine against vitreous degeneration mechanisms.[216, 217, 219, 275] Given that some of these micronutrients accumulate in the human vitreous and have been shown to decrease with degeneration and disease of the vitreous, we hypothesise that dietary enrichment with targeted exogenous micronutrients will reduce the visual discomfort of patients with symptomatic vitreous degeneration and improve their vision-related quality of life.[14, 78, 223, 224] This study was, therefore, conducted to test the above-stated hypothesis in a randomised controlled trial (RCT) fashion.

5.2 METHODS

5.2.1 Study Design

This interventional study, also referred to as the Floater Intervention Study (FLIES), is a registered (ISRCTN15605916), parallel group, single centre, double-blind, randomised, placebo-controlled clinical trial, designed to investigate the impact of supplementation with an active formulation of antioxidative and antiglycation micronutrients on the visual discomfort experienced by floater sufferers. Inclusion criteria for this study included primary floaters (age-related or myopia-related onset) in at least one eye; 18 years and older; no cataract surgery within the duration of the trial; no neural, developmental and retinal disease (for example, retinal breaks or detachments, age-related macular degeneration (AMD), diabetic retinopathy, and branch retinal vein occlusion); and best corrected visual acuity (BCVA) of 20/40 or better. For visual function assessments conducted as part of the study, one eye was randomly selected as the study eye in patients with bilateral floaters whereas the eye with floaters served as the study eye in unilateral cases.

5.2.2 Randomisation and Intervention

The Consolidated Standards of Reporting Trials (CONSORT) flow diagram of FLIES methodology is shown below (Figure 5.1). A total of 343 patients with vitreous floaters were screened for eligibility to participate in this trial. Out of these, 282 had other ocular co-morbidities and failed to satisfy the inclusion criteria for the study. 61 patients were, therefore, enrolled and randomised in a 50:50 masked fashion to either the active group (n = 31) or the placebo group (n =

30). Randomisation was performed using a customised clinical trial management software program (Trial Controller) developed by NOW-Science Consultancy Ltd, Waterford, Ireland.

The active group received a capsule containing 125mg l-lysine, 40mg vitamin C, 26.3mg *Vitis vinifera* extract, 5mg zinc, and 100mg *Citrus aurantium* (commercially available as VitroCap® N) whereas the placebo group received a placebo capsule containing microcrystalline cellulose. The active and placebo capsules were identical in shape, colour, and packaging. Supplementation was via the oral route; study patients were instructed to take one capsule per day with a meal for 6 months. The study staff and patients remained masked to the group allocations throughout the study. The randomisation sequence for the FLIES study was revealed following completion of the study and masked review of the database.

5.2.3 Ethics approval

The study was approved by the Research Ethics Committee, Health Service Executive, South East, Ireland, and the Waterford Institute of Technology Research Ethics Committee, Waterford, Ireland (WIT2019REC0007). All assessments performed on the study patients enrolled were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all the patients prior to enrolment into the study.

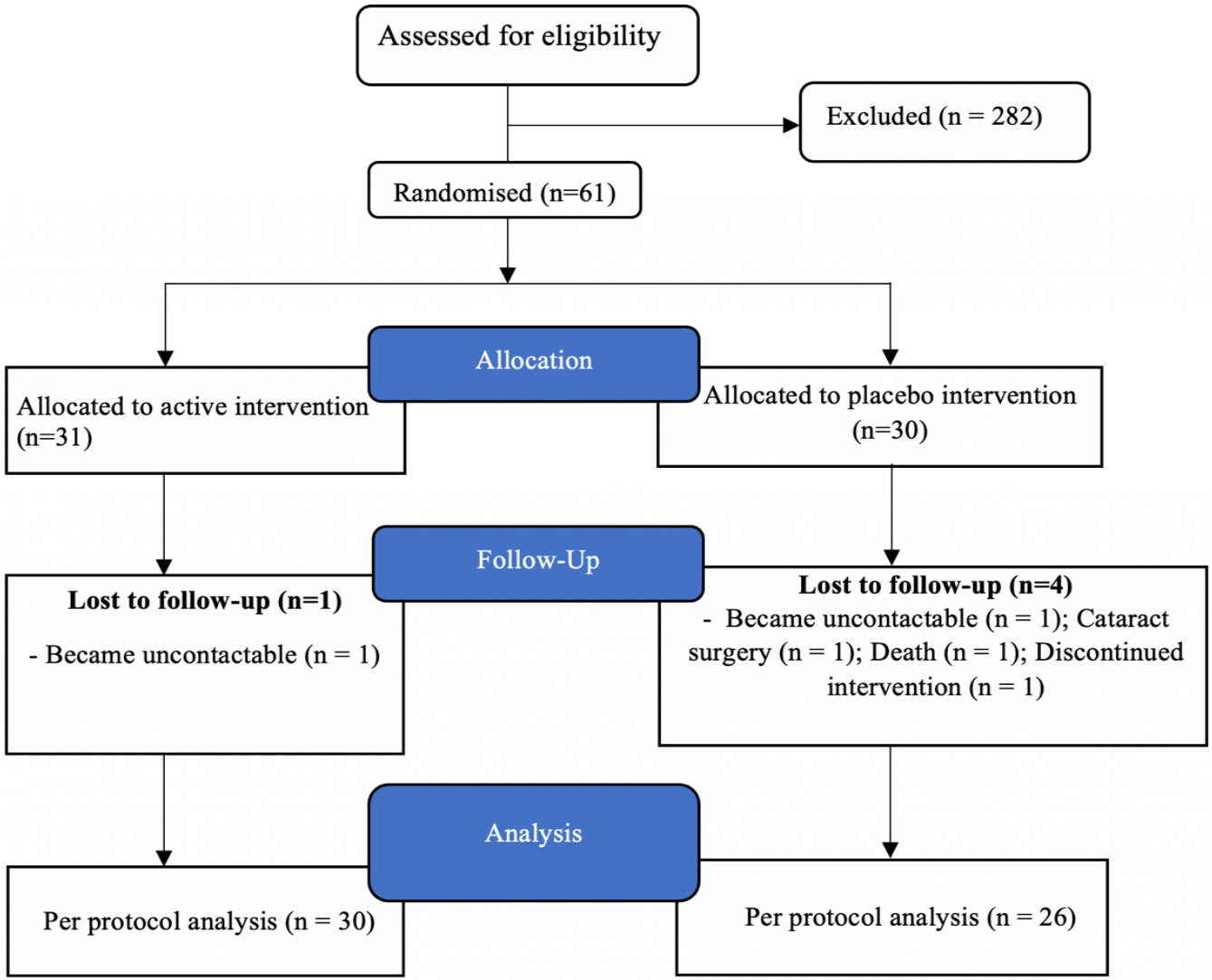


Figure 5.1: *Consolidated Standards of Reporting Trials (CONSORT) Flow Diagram of FLIES.*

5.2.4 Assessments

5.2.4.1 Demographic, Lifestyle and Medical Assessment

Demographic, lifestyle, medical, and ophthalmic data were captured at baseline for all patients. Body mass index (BMI) was calculated from height (m) and weight measurements (kg) recorded using the Leicester Height Measure and SECA weighing scales (SECA, Birmingham, UK), respectively. Smoking status was categorised into current smoker (i.e., smoked ≥ 100 cigarettes in lifetime and at least one cigarette in the last 12 months), ex-smoker (smoked ≥ 100 cigarettes in lifetime and none in the last 12 months) and never smoker (never smoked or smoked ≤ 100 cigarettes in lifetime).

5.2.4.2 Vision-related Quality of Life Assessment: Floater Disturbance Questionnaire

The Floater Disturbance Questionnaire is a short, non-standardised, disease-specific patient-reported outcome measure (PROM) specifically designed to capture the subjective response of patients suffering from vitreous floaters. The questionnaire comprised questions, which employed polytomous response ratings, to assess the visual discomforts associated with floaters. Two questionnaire items which were used to assess the vision-related quality of life in this study, at the baseline and final visits, were *the change in visual discomfort from floaters* and *the effect of floaters on daily life* as shown below:

a. C*hange in visual discomfort (Baseline and Final Visit)*

i. How will you describe your visual discomfort from floaters since you developed floaters? (Baseline only)

OR

ii. Has there been a change in severity of your visual discomfort from floaters following supplementation? (Final Visit only)

☐ *My condition has been stable and I am not bothered by my floaters*

☐ *My floaters have been intermittently and moderately bothersome*

☐ *My floaters have been persistently bothersome*

The question offered a 3-response choice which were rated as follows: My condition has become stable and I have not been bothered by my floaters – 0; My floaters have been intermittently and moderately bothersome – 1; My floaters have been persistently bothersome - 2. Patients' responses were reported as simple frequency distributions.

Given a 6-month follow-up duration, we applied a weighting factor of 3 to all the responses such that a persistent disturbance throughout the 6-month study period could be represented by a score of 6. As a result, the scoring employed for this questionnaire item were 0, 3 and 6 for stable condition, intermittent disturbance, and persistent disturbance, respectively.

The mean score of each intervention group for this questionnaire item was attained by simple summation and averaging of responses of patients in the respective

groups. A mean score of 3 signified a moderate severity of floaters, with increasing severity denoted by a progress towards a score of 6. Paired samples *t*-test was conducted for each intervention group to assess the change in discomfort from floaters following supplementation. The percentage of patients who reported a desired therapeutic effect (of reduction in floater suffering) were also assessed.

b. Effect of floaters on daily life (Baseline and Final Visit)

This questionnaire item employed a 5-point rating scale to assess the effect of floaters on the daily life of patients as shown below:

How would you describe the effect of floaters on your daily life in the past week?

None ☐ *Little* ☐ *Moderate* ☐ *Much* ☐ *Very much* ☐

At baseline, the intent was to assess the effect of floaters on the patient's daily life in the week prior to the study. The question was posed again at the final visit to assess the effect of floaters on the patients' daily life in the week prior to the visit. Patients' responses were reported as simple frequency distributions.

5.2.4.3 Vitreous Opacity Area Quantification

Vitreous opacity areas were imaged and quantified for baseline and final visits by a single masked investigator (E.A), as described previously in Chapter 4. For this study, to assess the reliability of this methodology, some patients were randomly imaged twice at either the baseline visit or final visit under the same testing conditions.

5.2.4.4 Visual Function Assessment

BCVA was measured with a computerised LogMAR Early Treatment Diabetic Retinopathy Study (ETDRS) test chart (Test Chart 2000 Xpert; Thomson Software Solutions, Hatfield, UK).[276, 277] Letter contrast sensitivity (CS) was measured at five different spatial frequencies (1.5, 3.0, 7.5, 12, 18.95 cycles per degree; cpd) using a computerised ETDRS test chart (Test Chart 2000 PRO).[276-279] Both tests employ Sloan optotypes, displayed at 4m, to assess visual performance.

Functional contrast sensitivity (FCS) was assessed using the *Acuity-Plus* test from the Advanced Vision and Optometric Test (AVOT; https://www.city.ac.uk/avot).[247, 280] Flicker sensitivities at photopic luminance of 60 cd/m^2 (cone sensitivity) and mesopic luminance of 0.5 cd/m2 (in conjunction with a spectrally-calibrated, neutral density filters; rod sensitivity) were measured using the *Flicker-Plus* test (AVOT system) which displays, at 1m from the observer, an equiluminant flickering disc modulating sinusoidally at 15 Hz, to assess temporal contrast thresholds at the fovea and four parafoveal locations.[281] These methods have been discussed comprehensively elsewhere and in Chapter 3 above.[247, 276-282]

5.2.4.5 Retinal (Foveal) thickness

Retinal thickness was measured using the Spectralis HRA + OCT Multicolour (Heidelberg Engineering GmbH, Heidelberg, Germany). This instrument combines OCT with scanning laser ophthalmoscopy and produces a reference fundus image. For retinal thickness measurements, 20 × 15 degree raster scans, consisting of 37 high-resolution line scans, were performed. An internal fixation

light was used to centre the scanning area on the fovea. Each scan was separately analysed by using the Spectralis OCT retinal thickness algorithm to generate retinal thickness values in μm.[283]

5.2.4.6 Montreal Cognitive Assessment (MoCA)

The MoCA test was performed at baseline, as described elsewhere, to assess whether the study patients were mentally capable of responding appropriately to the study questions.[284] The decision to perform this test was informed by a recent case report describing self-inflicted ocular injuries in a dementia patient as a result of an altered perception of floaters.[285] A score of 26 and beyond was considered normal and hence, adequate cognitive ability to complete the rest of the study.

5.2.4.7 Posterior Vitreous Detachment (PVD)

PVD status were assessed as discussed in chapter 3 and 4 above as part of baseline assessments.

5.2.5 Outcomes

The change in visual discomfort following 6 months of supplementation, assessed with the Floater Disturbance Questionnaire, represented the primary outcome measure (POM). Secondary outcomes were change in quantitative vitreous opacity areas, BCVA, letter CS, and FCS.

5.2.6 Statistical Analysis

Based on a POM variable with 3-point scale outcome, a large effect size according to Cohen's definition, and a two-tailed test at the 5% level of significance, we estimated that 26 patients would be required in each interventional arm to attain a power of 80% for the comparison of the two groups.[286] Allowing for a maximum primary end point attrition rate of 14%, 30 patients per intervention group was considered appropriate for this study.

The statistical package IBM SPSS® Statistics version 25, Sigma Plot 8, and Microsoft Excel 2016 for Windows were employed for all the statistical analyses. Means ± SDs are presented in the text and tables. Only patients (n = 56) who completed the study were included in the analysis in accordance with the FLIES protocol. Between-group differences in baseline outcome variables were analysed using independent samples *t*-tests for quantitative variables or chi-squared tests for categorical variables as appropriate. Paired samples *t*-tests were used to analyse questionnaire outcomes, objective vitreous opacity areas, and visual function measures in each group for change following supplementation. Test-retest reliability of the vitreous opacity area quantification methodology was assessed by computing intraclass correlation coefficient (ICC) estimates and their 95% confident intervals based on a mean-rating (k = 2), absolute-agreement, 2-way mixed-effects model. An ICC of 0.90 was considered excellent for this methodology**.** The 5% level of significance was used throughout the analyses.

5.3 RESULTS

5.3.1 Baseline

Table 5.1 presents the baseline demographic, visual function, questionnaire characteristics, and vitreous opacity areas for the two groups. The two study groups were comparable for all variables at baseline ($p>0.05$ for all).

***Table 5.1**: Baseline demographic, visual function, vitreous opacity area, and questionnaire characteristics of the placebo and active groups.*

	Placebo (n=26)	**Active (n=30)**	**Sig.**
Age (years)	56.73 ± 14.60	56.67 ± 11.16	0.985
Sex, n (%)			0.453
Male	13 (50)	12 (40)	
Smoking habits, n (%)			0.775
Never smoked	14 (53.85)	15 (50)	
Ex-smoker	11 (42.31)	12 (40)	
Current smoker	1 (3.84)	3 (10)	
BMI (kg/m^2)	27.94 ± 5.03	27.81 ± 4.06	0.919
MOCA Score	26.15 ± 2.59	26.70 ± 2.44	0.420
Refractive error status, n (%)			0.953
Myopia	9 (34.62)	11 (36.67)	
Emmetropia	10 (38.46)	12 (40)	
Hyperopia	7 (26.92)	7 (23.33)	
Laterality of floaters, n (%)			0.414
Unilateral	12 (46.15)	10 (33.33)	
Bilateral	14 (53.85)	20 (66.67)	
PVD status, n (%)			0.180
No PVD	10 (38.46)	17 (56.67)	
PVD	16 (61.54)	13 (43.33)	
Visual function			
BCVA, VAR			
Study Eye	100.77 ± 9.02	103.00 ± 7.83	0.326
Fellow Eye	100.96 ± 9.28	101.03 ± 8.80	0.976
Letter CS, logCS			
1.5 cpd	1.90 ± 0.18	1.96 ± 0.20	0.273

3 cpd	1.87 ± 0.18	1.94 ± 0.19	0.164
7.5 cpd	1.59 ± 0.27	1.66 ± 0.28	0.346
12 cpd	1.27 ± 0.39	1.38 ± 0.36	0.265
18.95 cpd	0.94 ± 0.49	1.07 ± 0.33	0.271
Photopic FCS, logCS			
Positive	0.75 ± 0.25	0.70 ± 0.32	0.565
Negative	0.76 ± 0.30	0.76 ± 0.33	0.962
Rods sensitivity (%)	8.08 ± 3.26	7.75 ± 3.11	0.707
Cones sensitivity (%)	5.86 ± 2.97	4.84 ± 1.88	0.149
Mean foveal thickness (μm)			
Right Eye	285.04 ± 19.94	285.48 ± 21.60	0.940
Left Eye	284.40 ± 18.20	286.03 ± 20.56	0.760
Subjective Questionnaire			
Change in discomfort since onset	3.69 ± 1.54	3.90 ± 1.56	0.797
Effect of floaters on daily life	1.08 ± 0.85	1.37 ± 1.27	0.328
Vitreous opacity area (cm^2)*	125.55 ± 103.20	121.31 ± 90.96	0.882

Data displayed are mean ± SD for interval data and percentages for categorical data; BCVA, Best Corrected Visual Acuity measured with the Test Chart Xpert (Thomson Software Solutions), BCVA was reported in visual acuity rating (VAR); Letter Contrast Sensitivity (CS) measured with the MiQ Contrast 256 test and recorded as log (CS); Photopic functional contrast sensitivity (FCS) were measured with the *Acuity-Plus* test from the Advanced Vision and Optometric Test (AVOT); Flicker sensitivity (for rods and cones sensitivities) measured with the *Flicker-Plus* test from the AVOT Suite and recorded as flicker threshold (%); Foveal thickness measured with Spectralis HRA + OCT Multicolour and recorded as μm; Sig., the statistical difference between the two groups; PVD, Posterior vitreous detachment; Vitreous opacity area measured with the Spectralis HRA + OCT Multicolour and ImageJ, and recorded as cm^2; *, n = 21 and n = 26 for placebo and active groups, respectively.

5.3.2 POM: Questionnaire outcomes

Table 5.2 and Figures 5.2 and 5.3 present the subjective visual discomfort for the two intervention groups at baseline and final visit. Table 5.2 and Figure 5.4 also present the effect of floaters on daily life for the two intervention groups at baseline and final. Patients' responses are presented as summary scores and as frequency distributions.

5.3.2.1 Change in visual discomfort

A. Active group

The active group reported lesser discomfort from floaters at final visit compared to baseline ($p < 0.001$; Figure 5.2). At baseline, 1 patient (3.3%) reported a stable condition, 20 patients (66.7%) reported moderate discomfort, and 9 patients (30%) reported persistent discomfort (Table 5.2; Figure 5.3A). Following supplementation, 11 patients (36.7%) reported a stable condition, 17 patients (57.6%) reported moderate discomfort and 2 patients (6.67%) reported persistent discomfort. In effect, within the active group, report of "stable condition" increased by 33.3%; "moderate disturbance" decreased by 10%; and "persistent disturbance" decreased by 23.3%. That is to say, the desired therapeutic effect was achieved in 66.6% of patients within the active group following the intervention.

B. Placebo group

The visual discomfort from floaters reported by the placebo group did not differ significantly at final visit when compared to their baseline reports ($p = 0.416$; Figure 2). At baseline, 1 patient (3.8%) reported a stable condition, 19 patients (73.1%) reported moderate discomfort, and 6 patients (23.1%) reported that their floaters had been consistently bothersome (Table 5.2; Figure 5.3B). Following supplementation, 4 patients (15.4%) reported a stable condition, 15 (57.7%) had moderate discomfort and 7 patients (26.9%) reported persistent discomfort. In effect, within the placebo group, report of "stable condition" increased by 11.53%; "moderate disturbance" decreased by 15.38%; and "persistent disturbance"

increased by 3.85%. In other words, 26.9% of patients within the placebo group reported a positive placebo effect.

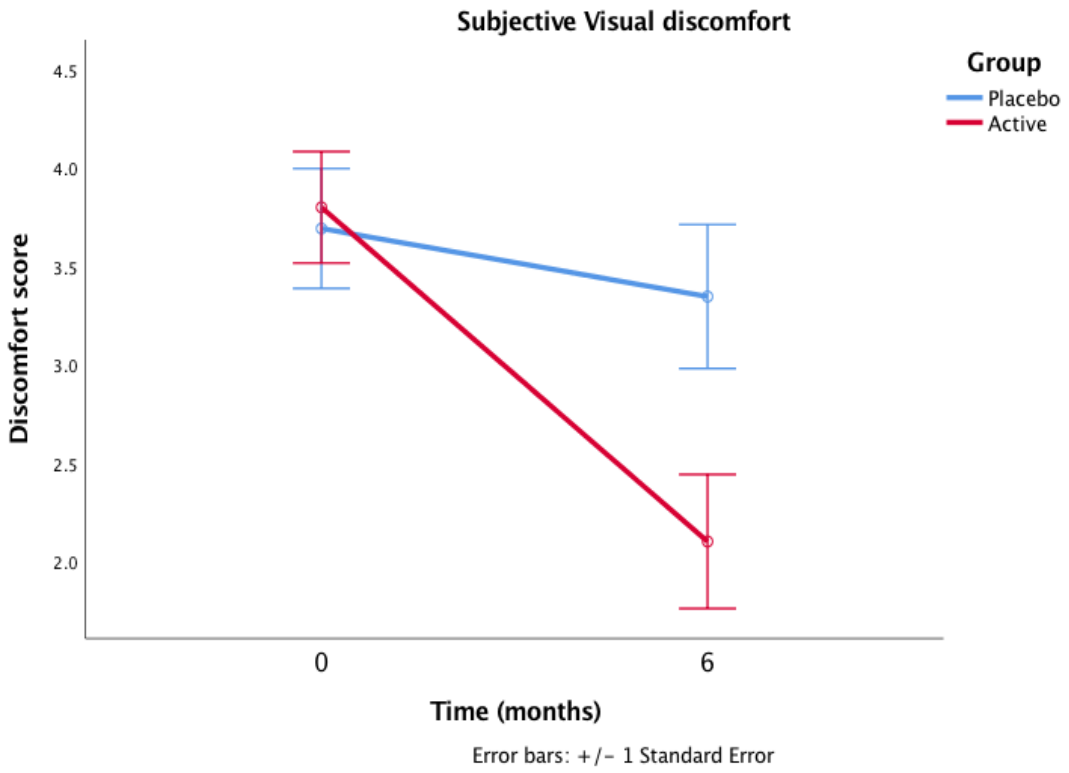
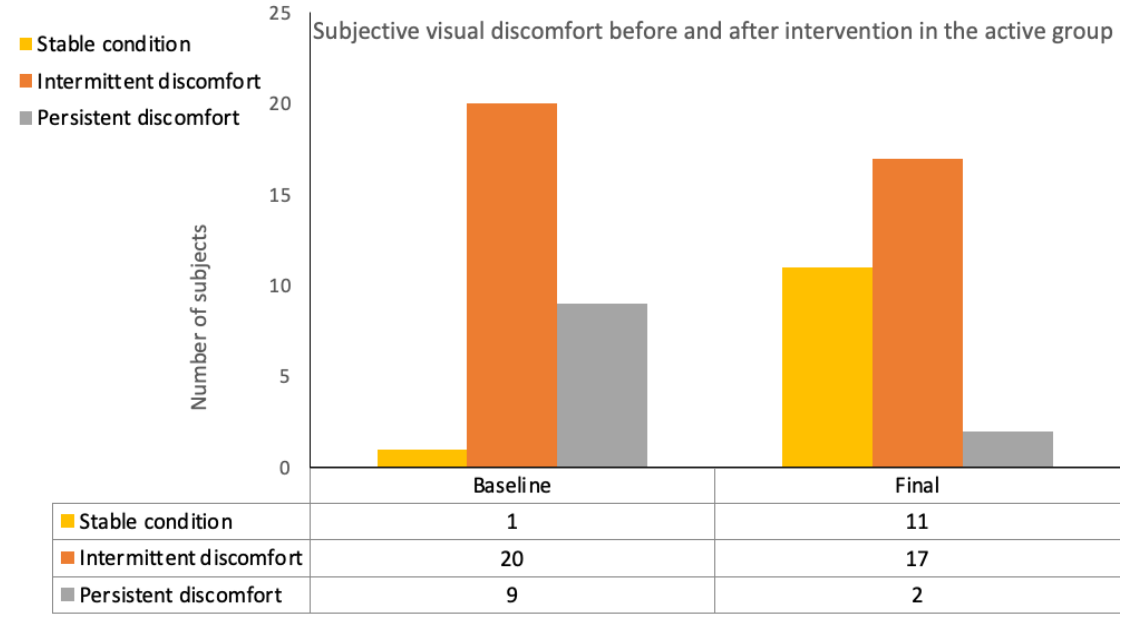


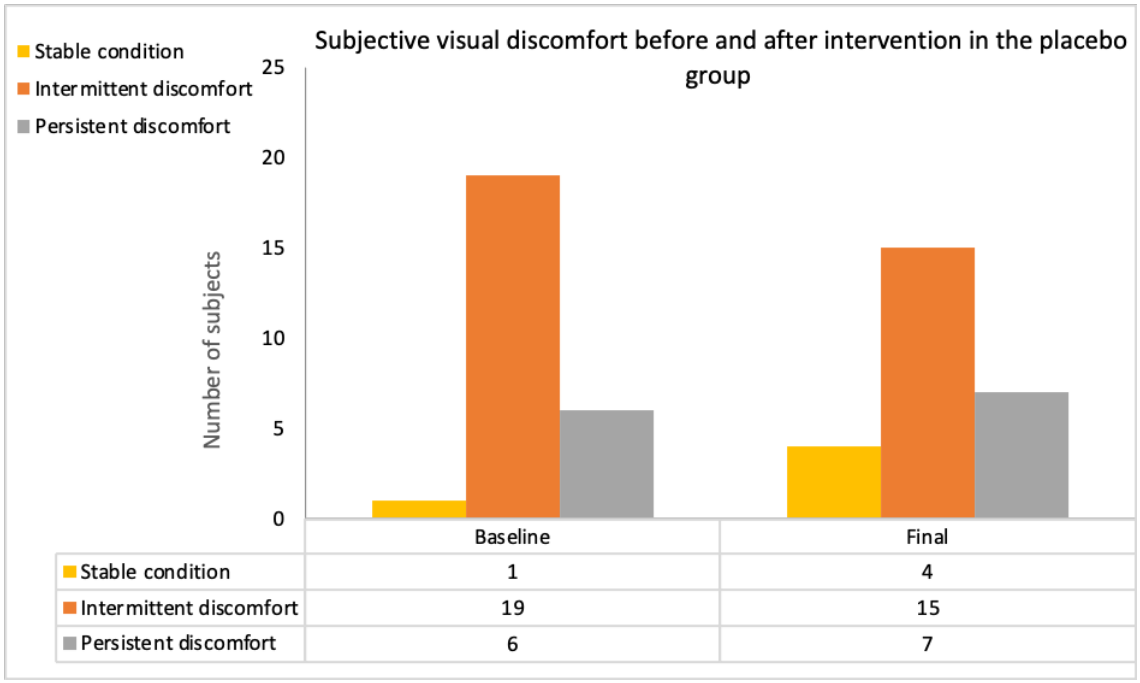
Figure 5.2: *Subjective visual discomfort at baseline and final visit for the active and placebo groups.*

A.



	Baseline	Final
Stable condition	1	11
Intermittent discomfort	20	17
Persistent discomfort	9	2

B.



	Baseline	Final
Stable condition	1	4
Intermittent discomfort	19	15
Persistent discomfort	6	7

Figure 5.3: *Histogram showing the subjective change in floater discomfort at baseline and at 6 months following supplementation for active (A) and placebo (B) groups*

***Table 5.2**: Subjective questionnaire outcomes for the two intervention groups*

Questionnaire Items	**Placebo group (n= 26)**			**Active group (n= 30)**		
	Baseline	**Final**	**p**	**Baseline**	**Final**	**p**
A. **Visual Discomfort**						
Stable condition	1 (3.85)	4 (15.38)		1 (3.33)	11 (36.67)	
Moderate discomfort	19 (73.07)	15 (57.69)		20 (66.67)	17 (56.67)	
Persistent discomfort	6 (23.07)	7 (26.92)		9 (30)	2 (6.67)	
Mean score	3.69 ± 1.54	3.35 ± 1.96	0.416	3.90 ± 1.56	2.10 ± 1.79	**<0.001***
B. **Effect on daily life**						
None	7 (26.92)	10 (38.46)		9 (30)	15 (50)	
Little	11 (42.31)	9 (34.62)		9 (30)	10 (33.33)	
Moderate	7 (26.92)	5 (19.23)		7 (23.33)	4 (13.33)	
Much	1 (3.85)	1 (3.85)		2 (6.67)	1 (3.33)	
Very much	0	1 (3.85)		3 (10)	0	
Mean score	1.08 ± 0.85	1.00 ± 1.06	0.678	1.37 ± 1.27	0.73 ± 0.94	**0.002***

p, difference between baseline and final visit (paired sample t-test); * statistical significance between baseline and final visit

5.3.2.2 Change in effect of floaters on daily life

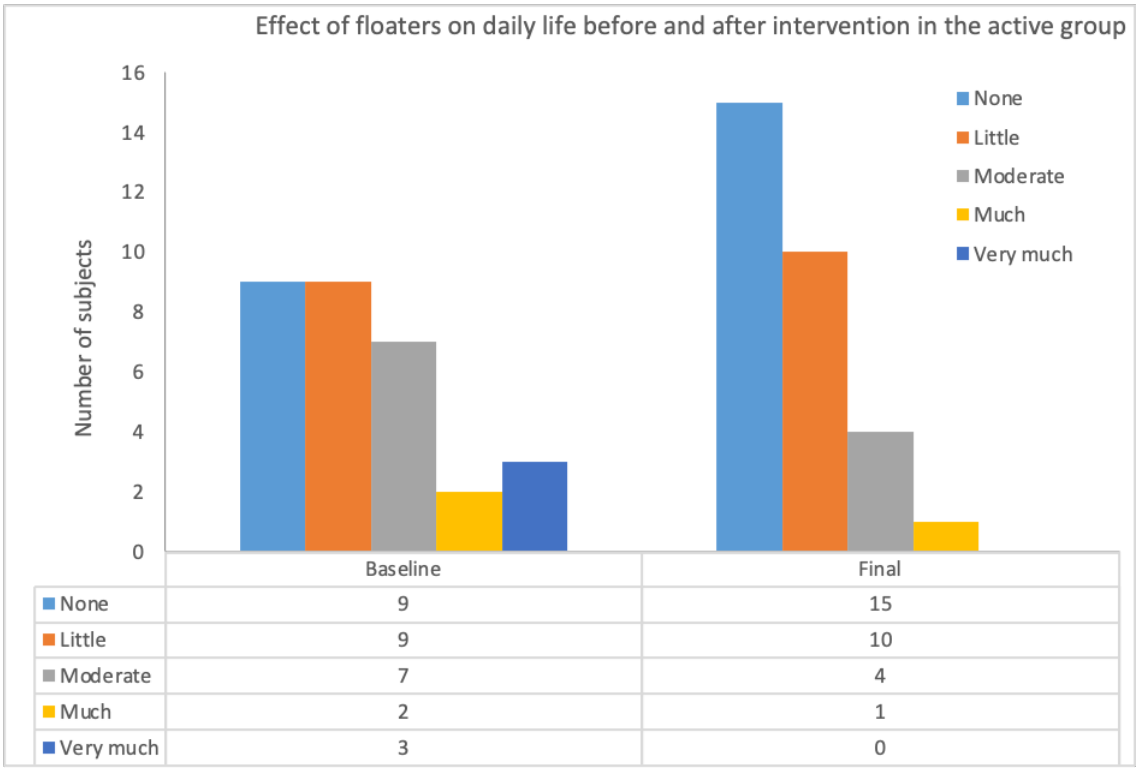
A. Active group

The active group reported lesser effect of floaters on their daily life at final visit compared to baseline (1.37 ± 1.27 and 0.73 ± 0.94 for baseline and final visits, respectively; p = 0.002). At baseline, 9 patients (30%) had no effect; 9 patients (30%) had little effect; 7 patients (23.3%) had moderate effect; 2 patients (6.7%) had much effect; and 3 patients (10%) had very much effect, of their floaters on their daily life (Table 5.2; Figure 5.4A). Following supplementation, 15 patients (50%) reported no effect; 10 patients (33.3%) reported little effect; 4 patients (13.3%) reported moderate effect; and 1 patient (3.3%) reported much effect, of their floaters on their daily life on their daily life and this improvement was statistically significant.

B. Placebo group

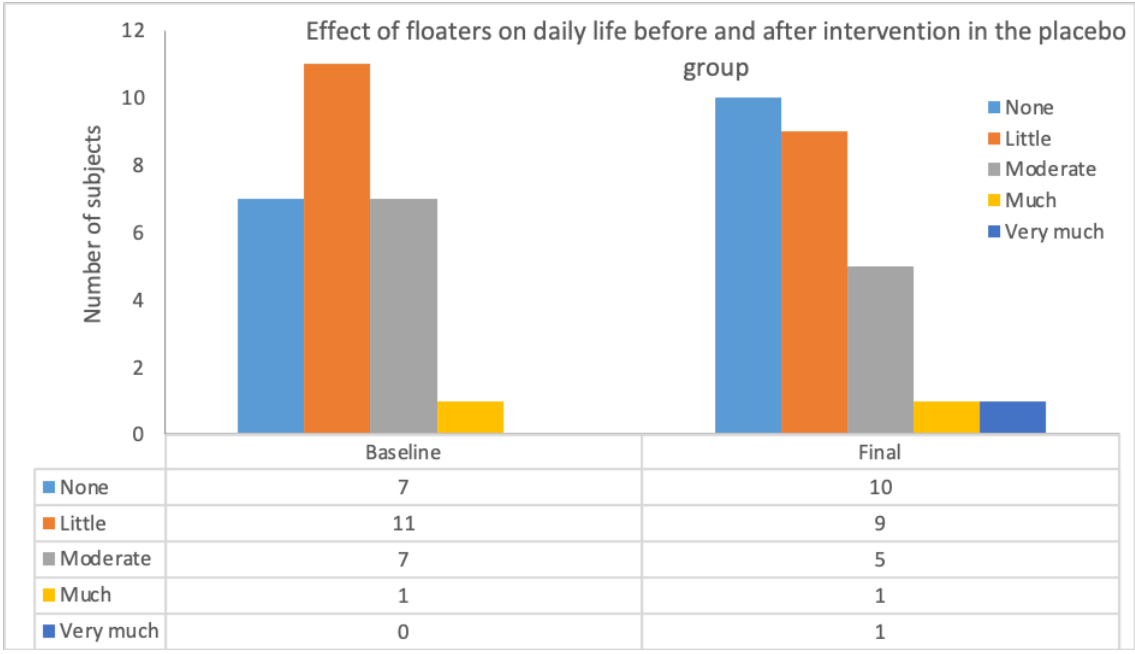
The subjective reports by the placebo group did not differ significantly at final visit when compared to their baseline reports (1.08 ± 0.85 and 1.00 ± 1.06 for baseline and final visits, respectively; p = 0.678). At baseline, 7 patients (26.9%) reported no effect; 11 patients (42.3%) reported little effect; 7 patients (26.9%) reported moderate effect; and 1 patient (3.8%) reported much effect, of their floaters on their daily life (Table 5.2; Figure 5.4B). Following supplementation, 10 patients (38.5%) reported no effect; 9 patients (34.6%) reported little effect; 5 patients (19.2%) reported moderate effect; 1 patient (3.8%) reported much effect; and 1 patient (3.8%) reported very much effect, of their floaters on their daily life.

A.



	Baseline	Final
None	9	15
Little	9	10
Moderate	7	4
Much	2	1
Very much	3	0

B.



	Baseline	Final
None	7	10
Little	11	9
Moderate	7	5
Much	1	1
Very much	0	1

Figure 5.4: *Histogram showing the self-reported effects of floaters on daily life at baseline and at 6 months following supplementation for the active (A) and placebo (B) groups.*

5.3.3 Change in vitreous opacity areas over time

Table 5.3 and Figure 5.5 show the change in vitreous opacity areas of the two groups before and after supplementation. There was a significant reduction in vitreous opacity areas following supplementation in the active group ($p = 0.002$; Figures 5.5 and 5.6). There was an increase in vitreous opacity areas in the placebo group after 6 months of supplementation, but the increase was not significant ($p = 0.081$; Figures 5.5 and 5.7). There was reduction in vitreous opacity areas in 20 out of 26 (76.9%) patients in the active group compared with 6 out of 21 (28.6%) patients in the placebo group.

The test-retest reliability was conducted using 10 eyes of 10 patients (5 males, 5 females) selected at random at baseline and 20 eyes of 17 different patients (4 males, 13 females) selected at random at the final visit. The test revealed an ICC of 0.998 (95% CI: 0.991 – 0.999; $p < 0.001$) at baseline and 0.998 (95% CI: 0.994 – 0.999; $p< 0.001$) at final visit, indicating excellent reliability of the imaging modality.

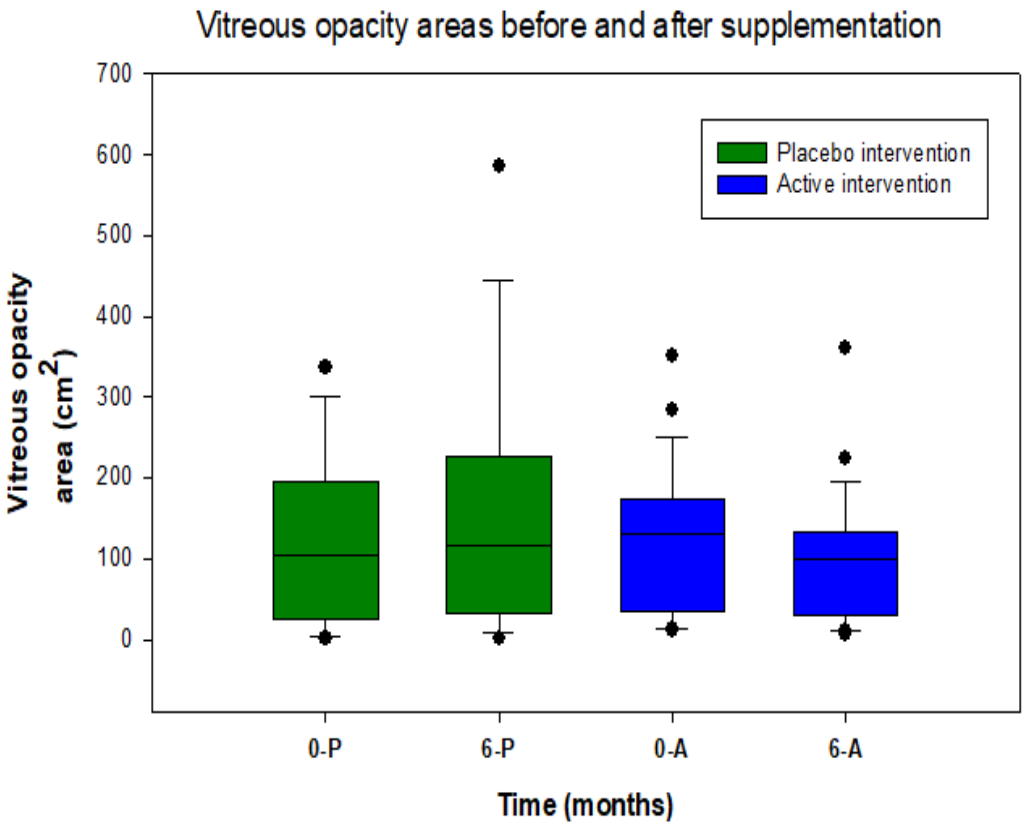
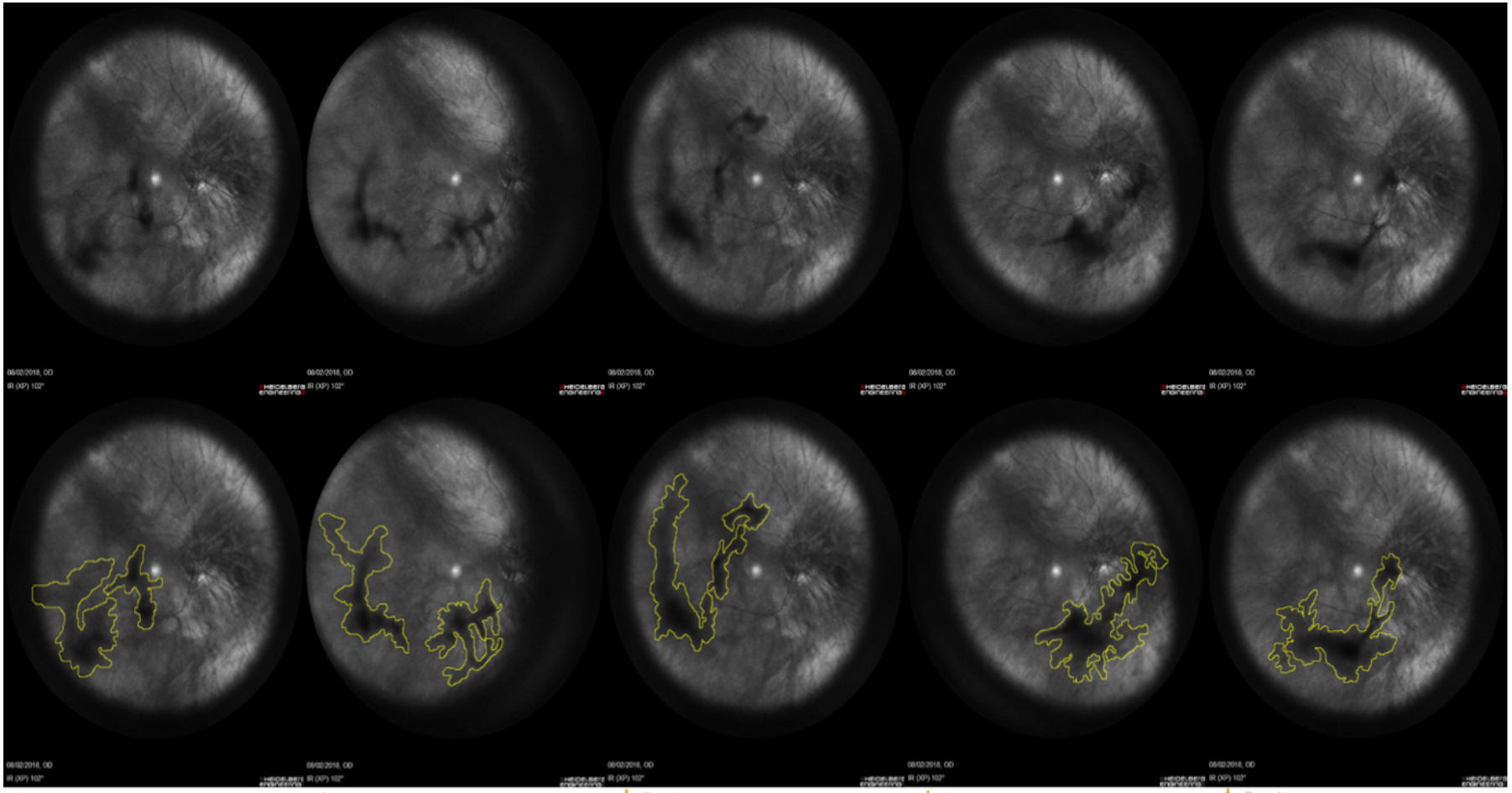


Figure 5.5: *Boxplots illustrating the vitreous opacity areas at baseline and final visit for the active and placebo groups. 0-P, Baseline placebo group; 6-P, 6 months placebo group; 0-A, Baseline active group; 6-A, 6 months active group*

A.



Results

File	Area	Mean	Min	Max
1	37413	48.069	10	101

Results

File	Area	Mean	Min	Max
1	41837	54.236	10	106

Results

File	Area	Mean	Min	Max
1	37547	44.142	4	115

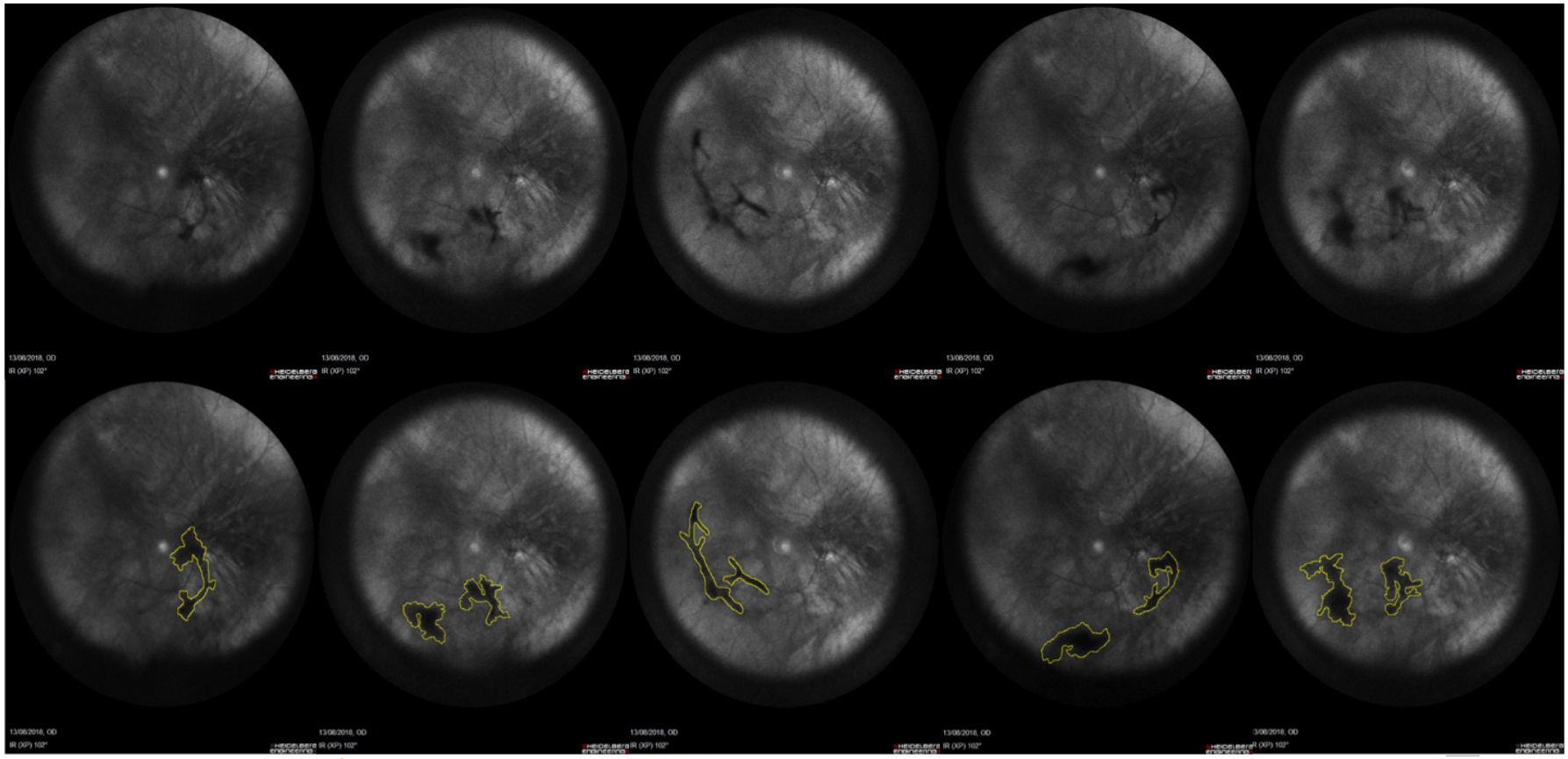
Results

File	Area	Mean	Min	Max
1	34476	49.240	8	129

Results

File	Area	Mean	Min	Max
1	32881	49.357	5	135

B.



Results

File	Edit	Font	Results								
	Area	Mean	Min	Max		Area	Mean	Min	Max
1	7894	37.689	10	98	1	11394	42.094	3	119

Results

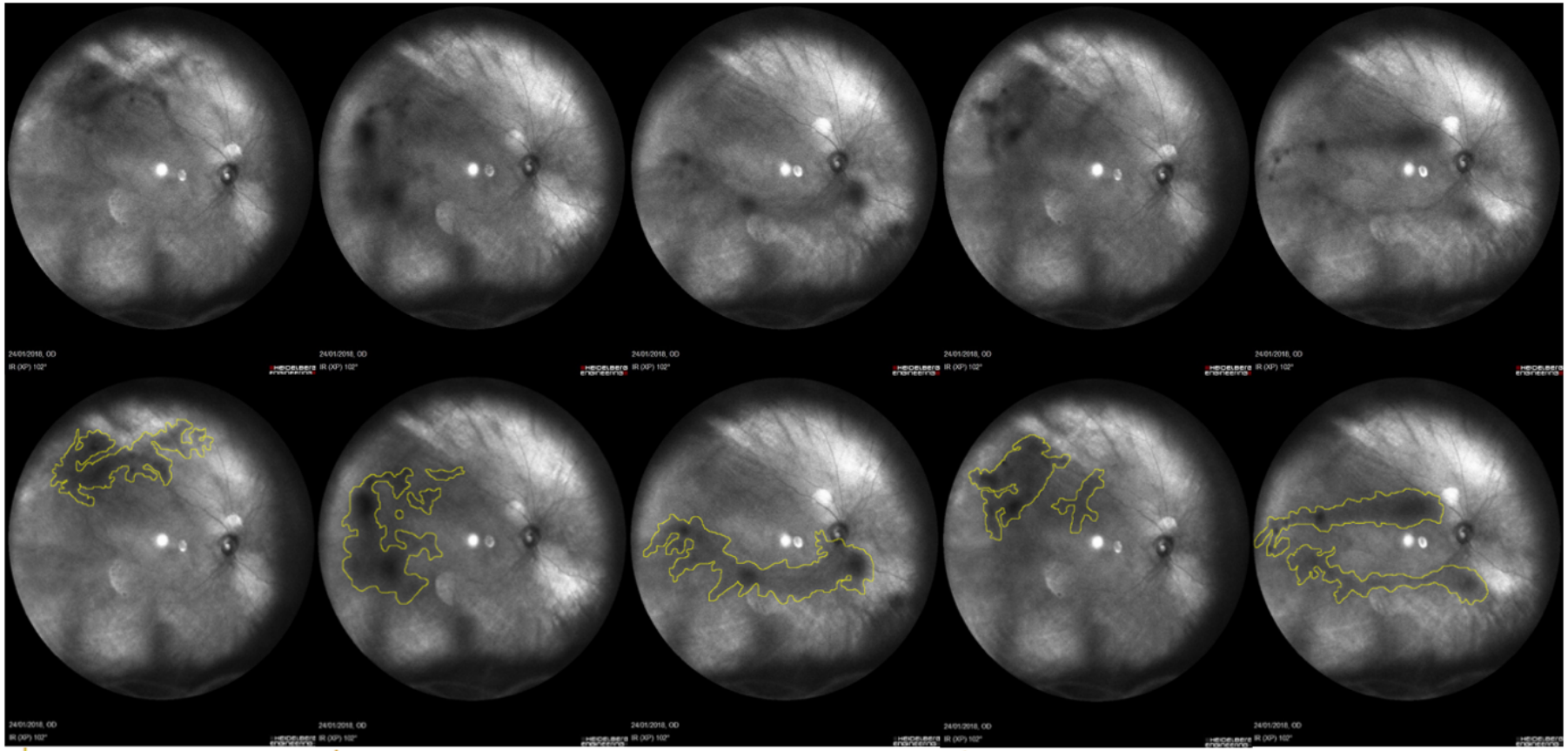
File	Edit	Font	Results								
	Area	Mean	Min	Max		Area	Mean	Min	Max
1	8901	43.133	7	103	1	13006	31.349	2	88

Results

File	Edit	Font	Results								
	Area	Mean	Min	Max		Area	Mean	Min	Max
1	13520	42.373	4	139					

Figure 5.6: *Vitreous opacity quantification for a patient within the active group who reported persistent discomfort at baseline and moderate disturbance at the final visit, showing an average vitreous opacity area of 368.31 cm^2 at baseline (A) and 109.43 cm^2 at final visit (B).*

A.



Results

File	Edit	Font	Results		
	Area	Mean	Min	Max	
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Results

File	Edit	Font	Results		
	Area	Mean	Min	Max	
1	39512	61.243	14	136	

Results

File	Edit	Font	Results		
	Area	Mean	Min	Max	
1	46446	84.306	26	175	

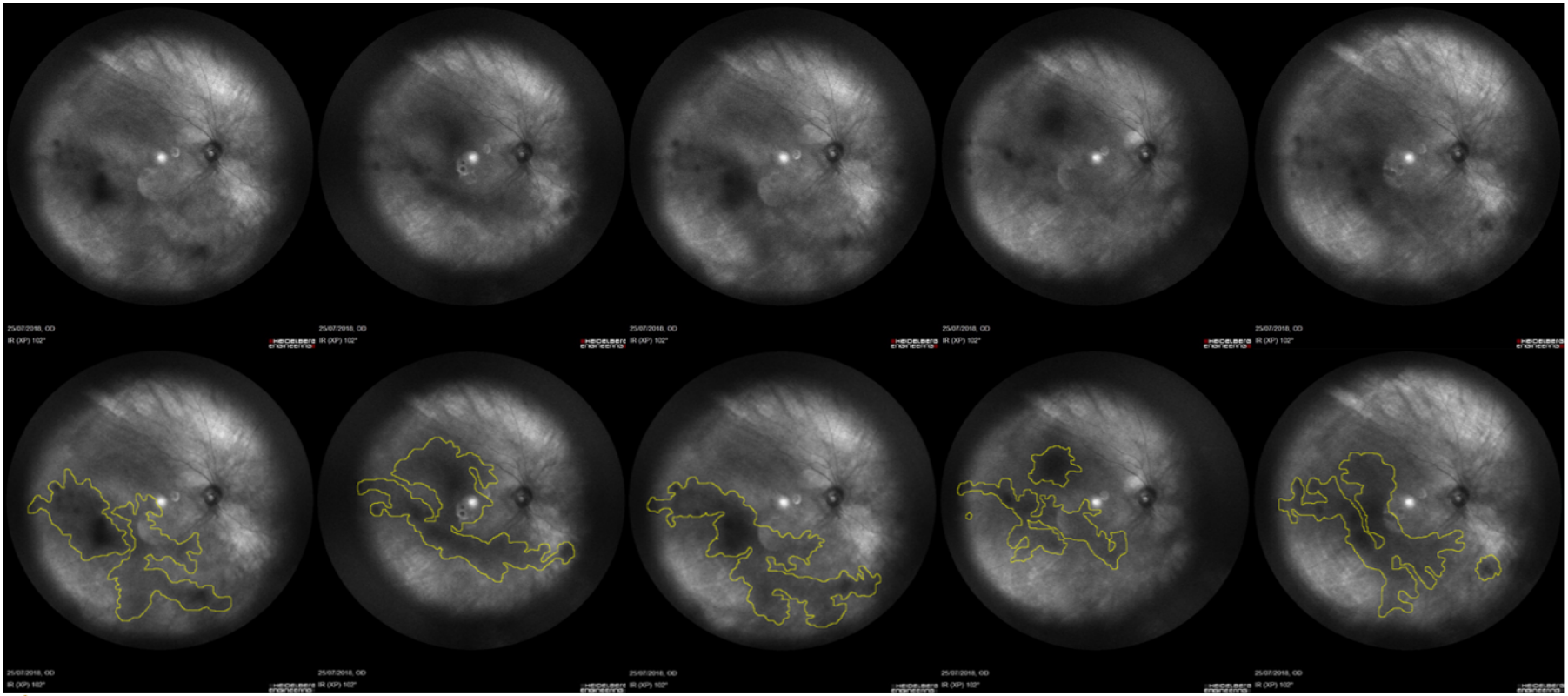
Results

File	Edit	Font	Results		
	Area	Mean	Min	Max	
1	31261	65.153	18	163	

Results

File	Edit	Font	Results		
	Area	Mean	Min	Max	
1	48229	81.559	27	167	

B.



Results

File	Edit	Font	Results																
	Area	Mean	Min	Max		Area	Mean	Min	Max		Area	Mean	Min	Max		Area	Mean	Min	Max
1	65300	68.824	12	157	1	64897	64.472	11	255	1	67668	64.666	14	140	1	39081	64.365	11	145	1	62597	63.056	15	175

Figure 5.7: *Vitreous opacity quantification for a patient within the placebo group who reported persistent disturbance at both baseline and final visits, showing an average vitreous area of 394.12 cm^2 at baseline (A) and 599.09 cm^2 at final visit (B).*

***Table 5.3**: Visual function and vitreous opacity area outcomes from baseline to final study visit for the two study groups.*

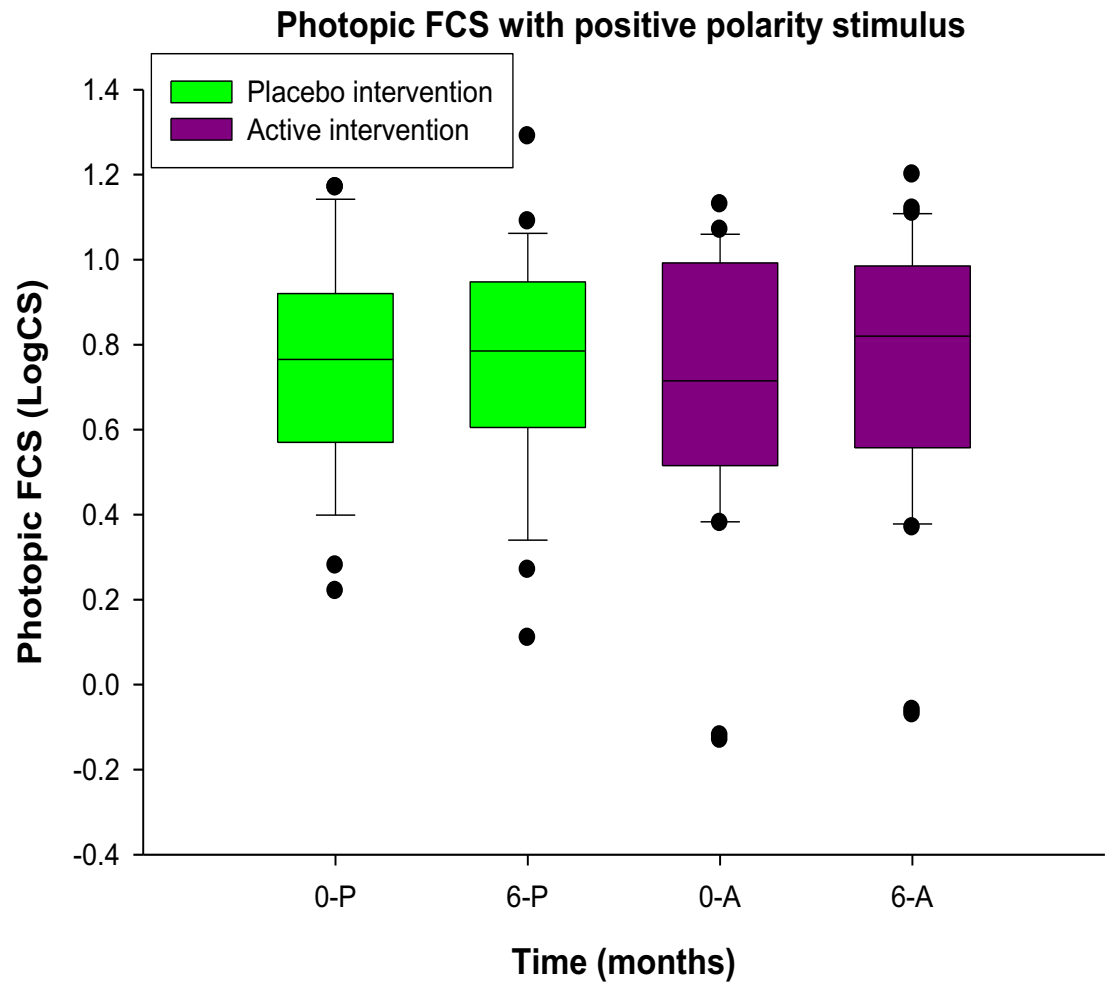
Variables	**Placebo group (n = 26)**			**Active group (n = 30)**		
	Baseline	**6 months**	**Sig.**	**Baseline**	**6 months**	**Sig.**
BCVA	100.77 ± 9.02	103.08 ± 6.01	0.185	103.00 ± 7.83	102.33 ± 7.51	0.502
Letter CS (logCS)						
Test Chart 2000 Pro						
1.5 cpd	1.90 ± 0.18	1.93 ± 0.14	0.354	1.96 ± 0.20	1.95 ± 0.19	0.749
3 cpd	1.87 ± 0.18	1.89 ± 0.17	0.465	1.94 ± 0.19	1.98 ± 0.22	0.109
7.5 cpd	1.59 ± 0.27	1.62 ± 0.23	0.353	1.66 ± 0.28	1.65 ± 0.34	0.746
12 cpd	1.27 ± 0.39	1.39 ± 0.29	0.104	1.38 ± 0.36	1.42 ± 0.40	0.510
18.95 cpd	0.94 ± 0.49	1.02 ± 0.38	0.276	1.07 ± 0.33	1.12 ± 0.33	0.281
MiQ 256	1.67 ± 0.25	1.72 ± 0.25	0.508	1.65 ± 0.29	1.75 ± 0.30	0.059
Photopic FCS (logCS)						
positive	0.75 ± 0.25	0.74 ± 0.27	0.883	0.70 ± 0.32	0.76 ± 0.32	0.047*
negative	0.76 ± 0.30	0.81 ± 0.30	0.223	0.76 ± 0.32	0.79 ± 0.34	0.274
Rod sensitivity (%)	8.08 ± 3.26	7.76 ± 2.85	0.374	7.75 ± 3.11	7.26 ± 2.74	0.110
Cone sensitivity (%)	5.86 ± 2.97	5.37 ± 3.18	0.159	4.84 ± 1.88	4.84 ± 1.85	0.996
Vitreous opacity area (cm^2)[†]	125.55 ± 103.20	155.07 ± 156.87	0.081	121.31 ± 90.96	99.78 ± 79.87	<0.001*

BCVA, Best Corrected Visual Acuity measured with the Test Chart Xpert (Thomson Software Solutions); Letter contrast sensitivity measured with the Test Chart Pro 2000 and the MiQ Contrast 256 test; Photopic functional contrast sensitivity (FCS) were measured with the Acuity-plus test from the Advanced Vision and Optometric Test (AVOT); Sig., the statistical difference between baseline and 6 months (paired samples *t*-test); *, statistically significant difference at the 0.05 level between baseline and 6 months; [†], n = 21 and n = 26 for the placebo and active groups, respectively.

5.3.4 Change in visual function over time

Table 5.3 displays the visual function outcomes of the two groups following supplementation. There was no significant difference in BCVA, letter CS and photopic FCS with negative polarity in either of the study groups ($p > 0.05$ for all). The active group reported statistically significant improvement in photopic FCS with positive polarity following supplementation ($p = 0.047$; Figure 5.8A).

A.



B.

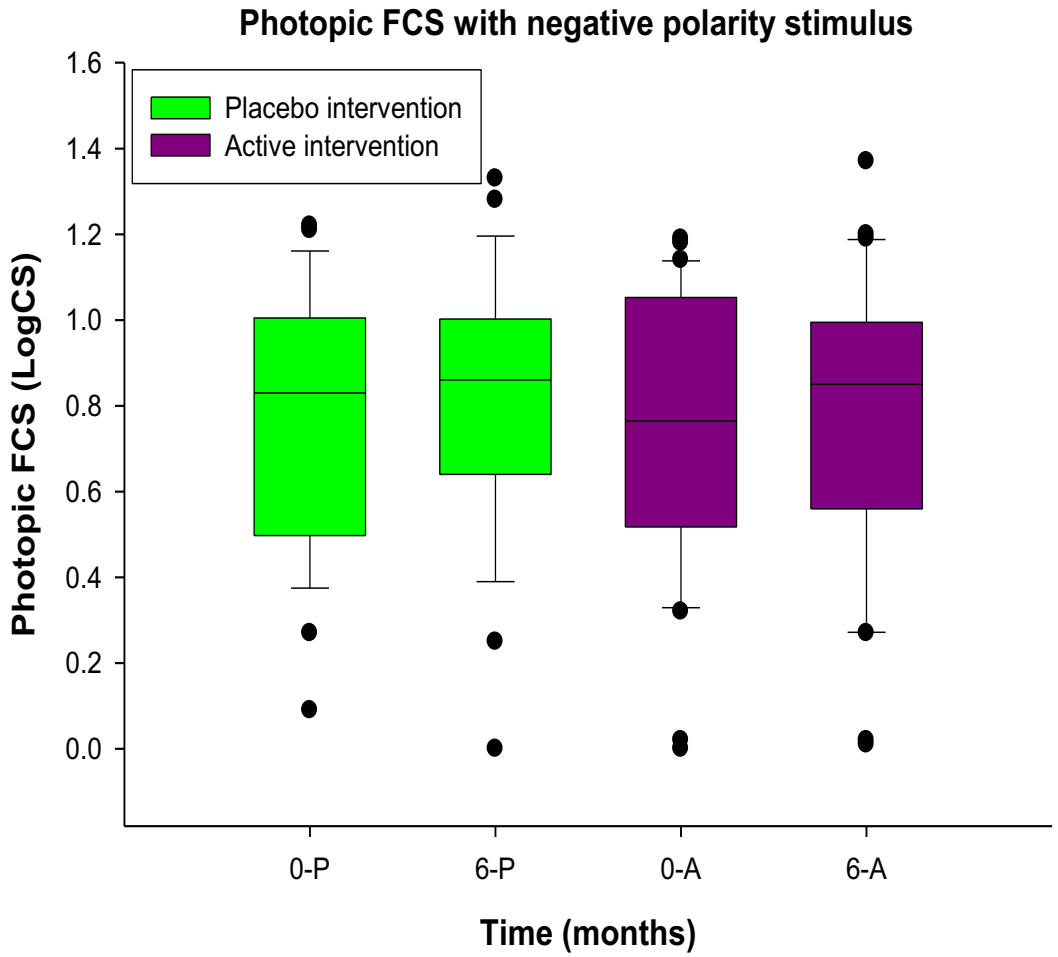


Figure 5.8: *Box plots illustrating photopic FCS with positive polarity stimulus (A) and photopic FCS with negative polarity stimulus (B) at baseline and 6 months by intervention group. 0-P, Baseline placebo group; 6-P, 6 months placebo group; 0-A, Baseline active group; 6-A, 6 months active group*

5.4 DISCUSSION

This is the first study to assess the impact of targeted nutritional supplementation on patient suffering associated with vitreous floaters, in the context of a randomised, double-blind, placebo-controlled clinical trial. Here, we report significant reduction in subjective visual discomfort from floaters, significant reduction in vitreous opacity area, and a significant improvement in contrast sensitivity following 6 months of supplementation with the active formulation. The

observed benefit in the active group confirms our initial hypothesis that dietary intake of a formulation of antioxidative and antiglycation micronutrients could avail relevant micronutrients capable of mitigating the mechanisms underlying vitreous degeneration, thereby reducing the visual discomfort associated with vitreous floaters.

Oxidative stress (secondary to increased intravitreal free radicals), accumulation of non-enzymatic glycation end products, and reduced vitreous antioxidant capacity underpin vitreous degeneration, typified by vitreous collagen aggregation and glycation as well as hyaluronan depolymerisation.[14, 166, 287] The micronutrients within the active supplement exert specific antioxidative and antiglycation activities against the afore-said processes and provide a potentially low-risk and feasible option for managing vitreous degeneration. L-lysine prevents collagen glycation and also acts as a chemical chaperone.[275, 288] Vitamin C guards against intraocular oxidative stress by consuming oxygen released at the vitreo-retinal interface in an ascorbate-dependent fashion.[59] Zinc acts as a stimulus for the synthesis of metallothionein, a metal-binding protein which protects tissues from glycoxidation (a mechanism which leads to vitreous degeneration).[136, 138] Zinc has also been shown to possess antioxidative and antiglycation properties, and zinc supplementation could inhibit formation of advanced glycation end-products (AGE) and AGE-induced oxidative stress.[289] Proanthocyanidin in *Vitis vinifera* exerts an inhibitory effect on protein glycation. Hesperidin prevents oxidative stress by inhibiting the formation and accumulation of cross-linking advanced glycation end-products (AGE) in collagens and tissues.[217, 219]

We have previously postulated that, attaining therapeutic, intravitreal levels of these exogenous micronutrients may require a repeated, long-term administration of the micronutrients.[14] In addition, we have comprehensively reviewed elsewhere the concentrations and potential mode of delivery of exogenous micronutrients into vitreous. In brief, the concentrations of l-lysine, zinc, and vitamin C previously detected in the adult vitreous are 115μM, 1.95μmol/L, and 2mmol/L, respectively.[14, 28, 223] The vitreous gel receives its supply of Vitamin C from the plasma by an active transport from the ciliary process of the ciliary body.[78] Like all soluble intravitreal proteins or amino acids, l-lysine may be sourced via local secretion, filtration from the blood, or diffusion from the surrounding tissues and vasculature.[115] While proanthocyanidins generally enter systemic circulation via passive diffusion, it is only *3,4-dihydroxyphenylacetic acid*, a potent antioxidant as well as a metabolite of proanthocyanidin , that has been detected within the human vitreous.[290-292]

As limited literature exists on the delivery channels for exogenous micronutrients into vitreous, we have previously conjectured based on evidence from fluorometry studies and post-mortem toxicological analysis that transfer of molecules from systemic circulation into vitreous may be mediated by diffusion, hydrostatic and osmotic pressure gradients, convection, and active transport, through the blood-aqueous and blood–retina barriers.[14, 62, 78, 220] As the above-stated micronutrients have been previously detected in the human vitreous, we can theorise that these nutrients utilise the above-mentioned pathways to accumulate in vitreous, in spite of the fact that specific delivery channels have not been isolated for most of these

nutrients.[14] Our findings confirm our prior postulation and suggest that, supplementation with the formulation of antioxidative and antiglycation micronutrients for 6 months delivers therapeutic doses of intravitreal targeted micronutrients, whose collective action against vitreous degenerative mechanisms, result in an improvement in quality of life of patients in the active group. What is not clear from the present study is whether the vitreous degenerative process will commence once intervention has been halted and accumulated intravitreal micronutrients have been used up. Future studies are warranted to estimate the concentrations of these micronutrients within vitreous following supplementation. Further, future studies on targeted nutrition for optimizing vitreous may be also concerned with employing higher doses of the individual micronutrients (not exceeding the upper reference levels of the micronutrients) that will ensure higher intravitreal levels within the therapeutic range.

In the present study, the active and placebo groups were comparable in their visual discomfort score at baseline ($p = 0.797$). However, it is worth mentioning that, there was a slightly high, albeit not statistically significant, difference in PVD rate (61.5% versus 43.3% in the placebo and active groups, respectively) between the two groups at baseline. Given the fact that the densest and most central floaters could have resulted from the detached posterior hyaloid face as part of a PVD, a large (significant) difference in PVD rate between the groups could have potentially influenced our POM, especially when the two groups were compared.[293, 294] As a consequence, a similar representation of such cases in both groups would have been the ideal scenario to allow for comparison between the

two groups. Interestingly, the two study groups had similar objective vitreous opacity area measurements at the onset of the study, implying that the groups were comparable. Coupled to that, the main findings and conclusions herein presented were based on the paired group comparisons for each study group, in which a group's own outcomes at baseline and final visits were compared for change over time, and not on the between-group comparisons.

Following supplementation, the active group reported a significantly lesser visual discomfort score compared to the placebo group ($p = 0.016$). Within the active group, report of "stable condition" increased by 33.3%; "moderate disturbance" decreased by 10%; and "persistent disturbance" decreased by 23.3% ($p < 0.001$). Compared with the placebo group, report of "stable condition" increased by 11.53%; "moderate disturbance" decreased by 15.38%; and "persistent disturbance" increased by 3.85% ($p = 0.416$). In other words, the report of a desired therapeutic effect was 66.6% in the active group compared with 26.9% in the placebo group. These results suggest that the active formulation is an effective intervention which improves the vision-related quality of life of floater sufferers. Given that patients with floaters are typically observed for 6 months before any treatment is considered, it is our view that supplementation with this clinically-tested formulation of antioxidative and antiglycation micronutrients could be considered rather than proffering watchful waiting.

There was a significant reduction in vitreous opacity areas of 76.9% of participants within the active group following supplementation. The findings from this

methodology confirms the subjective report of improvement within the active group. Similar to the observation in chapter 4, the objective improvement observed was higher than the subjective report of improvement (76.9% versus 66.6% for objective and subjective outcomes, respectively). It appears that the same explanation, as highlighted in Chapter 4, is applicable in this case also. Patients who do not experience complete resolution or significant improvement of their symptomatology tend not to notice any difference in their discomfort following an intervention in spite of a reduction in vitreous opacities based on objective metrics.[269] The use of ImageJ software to quantify vitreous opacities was first described by Sun et al, who computed floater shadow areas from 30° or 55°, 768x768 pixel infrared (IR) images for patients who underwent laser vitreolysis for symptomatic floaters.[268] Our methodology of ultra-widefield imaging (102° field) of the vitreous is an improvement on their approach which involved imaging a 30° or 55° field of the retina. In addition, our ICC with 95% confidence interval indicated excellent level of reliability of our methodology, suggesting that the data herein presented are reliable estimates of the vitreous opacity areas of patients at the two time points.

With respect to visual function, the results from the current trial are impressive. Here, we report an improvement of 0.06 log units (equivalent to 2 optotypes on an ETDRS logMAR test chart at 10 cpd), on average, in the active group for photopic FCS with positive polarity stimulus following supplementation ($p = 0.047$). Degenerated vitreous causes intraocular light scattering and degrade both photopic and mesopic contrast sensitivity.[206, 237, 238] The FCS test used in the present study

employs a stimulus reach in high spatial frequencies, which are significantly influenced by optical factors including forward light scatter. Hence, improvement in photopic FCS, with an associated reduction in subjective visual discomfort and objective vitreous opacity areas, suggests a reduction in forward light scatter from vitreous opacities within the active group after supplementation with the active formulation. Further, there was no significant change in rods and cones sensitivity, implying that the improvement in contrast sensitivity observed is not as a result of an improvement in retinal function but the vitreous.

A limitation of this study is that no vitreous biopsies were taken to measure the intravitreal concentrations of the micronutrients following supplementation. The present study was designed to assess the efficacy of a non-invasive, low-risk therapy for floaters. Acquiring vitreous samples via invasive procedures such as vitrectomy or vitreous aspiration needle tap would have violated the protocol of this study.[295] This study, however, provides initial data that paves way for future studies designed to estimate concentrations of the targeted micronutrients (as well as total antioxidant capacity) within vitreous samples from supplemented patients as well as non-supplemented controls.

Another limitation is that the questionnaire employed in this study was developed specifically for the trial and therefore not previously used or validated by other researchers. Therefore, this may increase the tendency to introduce noise into the measurement, and decrease sensitivity to change and correlations with other variables, given that it is a non-validated PROM.[296] However, these errors are

frequent when a composite score is generated based on the individual scores of questions within a non-validated PROM. Our approach of scoring the individual questionnaire items were constructed to limit noise and provide reliable outcomes regarding patients' visual discomfort.

Aside the above, a single masked investigator conducted all the image analysis for the objective vitreous opacity areas quantification. While the investigator manually traced the outlines of the opacity, the actual opacity areas were automatically quantified by the software. Further, all the analysed images were inspected by the entire study team to ensure that all opacities within images had been correctly outlined to ensure accuracy in the methodology. We did not also attempt to distinguish between central and peripheral floaters with our objective methodology. Central floaters may perhaps cause more symptoms so future studies comparing central and peripheral floaters would be useful.

Visual inspection of the raw data from the trial is clear and impressive, indicating a positive impact of supplementation with the active ingredient for patient suffering and visual function. Further, the data presented in this report has provided a proof of concept that targeted nutritional intervention is a promising new approach for managing vitreous degeneration that requires further exploration. What remains to be explored is the duration and dosaging that will elicit the highest therapeutic response with little to no systemic effects.

5.5 CONCLUSION

This study is the first to investigate the impact of targeted nutritional intervention via supplementation for patients suffering from vitreous floaters in a double-blind, placebo-controlled fashion. The findings of this clinical trial indicate improvements in vision-related quality of life and visual function of patients suffering from vitreous floaters following supplementation with a formulation of antioxidative and antiglycation micronutrients. Notably, these improvements were confirmed by the reduction in vitreous opacity areas in the active group. This targeted dietary intervention should be considered to support patients with symptomatic vitreous degeneration.

5.6 CHAPTER SUMMARY

The rationale for this chapter builds on the evidence outlined in the preceding chapters regarding vitreous degeneration as a disease. As a disease underpinned by depletion of vitreous antioxidants and oxidative stress, vitreous degeneration causes deformation of vitreous structure, reduces contrast sensitivity, and impacts negatively on the quality of life of sufferers. The findings of this chapter confirm the hypothesis that an alternative management approach of targeted nutrition with antioxidative and antiglycative micronutrients reduces vitreous floater symptomatology, and this reduction is corroborated by improvements in visual function and objective assessment of vitreous opacities. The findings of this study have been published in Translational Vision Science & Technology (Impact factor = 3.283; manuscript ID, TSVT-21-3596) under the title, '***Dietary intervention with***

a targeted micronutrient formulation reduces the visual discomfort associated with vitreous degeneration' (Appendix F4).

Chapter 6

CONCLUSIONS AND FUTURE RECOMMENDATIONS

"Let us hear the conclusion of the whole matter"

- Ecclesiastes 12:13a, King James Version

6.1 VITREOUS IS A REPOSITORY OF ANTIOXIDANTS

The structures of the human eye are uniquely equipped with different antioxidant defences based on the complexity of each structure, the different sources of ROS threatening each region, and the roles of the specific structures in visual processing.[14] For example, ocular structures concentrate AA differently; AA concentration in the vitreous (2 mmol/L) is 1.4 times higher than in the aqueous (1.4 mmol/L) but 1.75 times lesser than in the natural crystalline lens (3.5 mmol/L).[79, 297] In addition, the concentration of AA within the corneal epithelium has been estimated to be 14 times higher than in the aqueous.[298] Compared with the concentration within plasma (0.06 mmol/L), the above-stated ocular structures accumulate higher concentrations of AA (at least 20 times higher than in plasma) to protect against biological damage secondary to light exposure since these structures function to ensure optical transparency.[299] Further, these structures are

devoid of blood vessels as well as the antioxidant protection that comes with blood supply, hence the need to accumulate higher concentrations of antioxidants. Even within the vitreous, AA concentration at the posterior vitreous is higher than at the core vitreous as vitreous oxygen consumption (a major antioxidant function of the vitreous) is higher at the vitreoretinal interface than within the core vitreous. Interestingly, the retina, which has a rich blood supply as well as the highest oxygen consumption rate per kg of the body, has an AA concentration which is about 100 times higher than plasma concentration due to its critical role in vision.[3, 299] In effect, the functions of the different structures of the eye, the type of injurious events they are exposed to, and the complexity of their structures, influence their antioxidant capacities.

Previous studies have documented the antioxidant molecules within the eye, specifically detailing the antioxidants within the cornea, aqueous, crystalline lens, and the retina.[13] Prior to this thesis, no comprehensive review existed on the antioxidants within the vitreous. This thesis is the first to provide a comprehensive review of vitreous antioxidants, and the relationship between these molecules and vitreoretinal pathophysiology. The literature that I reviewed (as outlined in chapter 1) suggested that the vitreous amasses a vast array of antioxidants (about 17 different molecules) and is suitably positioned between the retina and the natural crystalline lens to provide protection against oxidative stress within the posterior segment. This complex system of vitreous antioxidants comprise non-enzymatic antioxidants such as vitamins, proteins and amino acids, and trace elements, as

well as enzymatic antioxidants including superoxide dismutase, glutathione peroxidase and catalase.

To maintain homeostasis, the eye tightly regulates oxygen distribution intraocularly and ensures a hypoxic environment.[300] In healthy eyes, oxygen from the retinal vasculature in the anterior surface of the retina diffuses into the vitreous.[29] If the oxygen is not quickly eliminated from the intraocular environment, it becomes a precursor for ROS formation and subsequent oxidative stress and ocular neurodegenerative diseases. Evidence points to the vitreous as the structure responsible for oxygen regulation in the eye.[59] The intravitreal antioxidants consume the released oxygen in an ascorbate-dependent fashion and guard against intraocular oxidative stress.[29] This has been demonstrated by previous studies which used fiberoptic optical oxygen sensor (optode) to determine oxygen levels in different regions of the eye and reported that, there is a gradient in oxygen concentration from the retina (~22mmHg) to the posterior lens (~9mmHg), indicating oxygen consumption by the vitreous.[301] This explains why the crystalline lens is mostly predisposed to nuclear cataracts following scenarios where intravitreal antioxidants are depleted and oxygen exposure to the lens has increased such as via full pars plana vitrectomy.[302]

Oxidative stress, reduced/depleted intravitreal antioxidant capacity, and increased proteolytic enzymatic activity have been proposed as the mechanisms that underlie liquefaction and PVD, the broad processes for vitreous degeneration and the entoptic phenomenon, vitreous floaters. The literature review presented in chapter

2 of this thesis highlighted that an age-dependent build-up of free radicals secondary to a life-long irradiation of riboflavin (the naturally present intravitreal photosensitiser molecule) by white light, coupled with reduction/depletion of intravitreal antioxidant capacity, results in molecular alteration of vitreous collagen and HA, a degenerative mechanism referred to as liquefaction.[92] Thus, adequate supply of vitreous antioxidants may be essential for the stability and overall health of the vitreous.

Regrettably, vitreous degeneration has been long considered by eyecare professionals as a 'normal' aspect of the aging process such that there is no emphasis on preventative strategies such as enrichment of the vitreous with antioxidants aimed at optimising vitreous health and retarding the degenerative process. Rather, eye care professionals prefer to monitor patients at the onset of vitreous degeneration and proffer invasive management techniques at the end stage of vitreous degeneration.

As vitreous degeneration has also been shown to be a precursor for a myriad of vitreoretinal pathologies, it may be unreasonable to solely monitor patients with early vitreous degeneration with the intention to treat them should they develop end stage degeneration. Patients may subsequently develop sight-threatening complications following significant vitreous degeneration, which may invariably turn out to be a double whammy of an economic burden as well as a quality of life problem for patients.

6.2 VITREOUS DEGENERATION IMPACTS ON PHOTOPIC AND MESOPIC CONTRAST THRESHOLDS

In explaining health and disease, the biostatistical theory by Christopher Boorse stands out as the current mainstream school of thought in medicine. This theory describes disease as "a type of internal state which impairs health, i.e., reduces one or more functional abilities."[303] Against this backdrop, the present state of medical practice involves the training of clinicians to isolate signs of a disease via proper diagnostics and good medical history to decide whether or not someone under their care has a particular condition and to provide appropriate treatment to curb those diseases.[304] As a result, treating physicians typically define disease parameters based on the 'abnormality' of disease-related indices and/or detection of a structural anomaly or an impaired function, with little to no attention to the patient's subjective reports.

Relating this to Ophthalmology more specifically, eyecare providers are constantly scavenging for presenting structural changes that corroborate the reduction in, impairment of, or loss of, vision. However, in some conditions where the only evidence at the doctor's disposal upon which a diagnosis has to be made is a subjective report and no accompanying loss of visual acuity, such as in vitreous floaters, the question continually posed by doctors is: how do we distinguish properly between real diseases, and human behaviours or characteristics that we just happen to find disturbing?

Originally considered a part of ‘normal’ aging, clinicians in the past failed to describe vitreous degeneration as a disease because the formation of opacities within the vitreous had not been deemed a significant structural alteration that was detrimental to the overall health of the eye. Besides, vitreous floaters are mostly not associated with loss of visual acuity, a clinical ‘abnormality’ that would arouse the treating physician’s attention.

Recently, Professor Sebag and his colleagues have succeeded in raising the awareness of clinicians to consider vitreous degeneration as a disease. For the first time, their studies have shown that an aspect of visual function, specifically mesopic contrast sensitivity, is compromised with vitreous degeneration and needs to be properly addressed to enhance the vision of patients suffering from symptomatic vitreous degeneration.[237, 238] The findings in this thesis are consistent with previous studies that, both vitreous opacities and posterior vitreous detachment reduce mesopic contrast thresholds.[237, 238] However, of particular importance is the new insights the study reported in chapter 3 of this thesis provides regarding the impact of vitreous degeneration on photopic contrast sensitivity.

The findings of chapter 3 of this thesis are consistent with previous research which have shown that optical characteristics of older eyes are largely responsible for older adults’ spatial contrast sensitivity deficits at photopic light levels at high spatial frequencies.[255, 262] The study reported in chapter 3 of this thesis has shown that, compared with healthy eyes, vitreous opacities increase forward light scatter

in the eye and reduce photopic contrast threshold by 37.4%, increasing to 64% when vitreous opacities occur in tandem with posterior vitreous detachment. But the main question to ask from this finding is that: what does a reduction in photopic contrast of 64% imply, and what is its clinical relevance?

Evidence from previous studies on aging and spatial contrast sensitivity have shown that older eyes have an average loss of 0.3 log units (corresponding to a contrast threshold deficit of about 50.1%) at 8 cycles per degree at photopic light levels.[255] In addition, this reduction in contrast, largely caused by cataractous lens changes, causes moderate visual impairment.[255] We also know that there is an increase in contrast threshold loss with increasing spatial frequency at high photopic light levels in the ageing eye.[262] Drawing inference from these previous conclusions, it is reasonable to say that a photopic contrast threshold reduction of about 64% from posterior vitreous detachment at 10 cycles per degree will result in at least a moderate visual impairment owing to the increase in forward light scattering in the eye secondary to vitreous degeneration. While this significant increase in light scattering may not directly affect visual acuity, the corresponding reduction in photopic contrast is what possibly explains the reduction in the quality of life of patients with symptomatic vitreous degeneration and their frequent visits to eye clinics. In effect, there is the need for eyecare providers to incorporate photopic contrast sensitivity assessments into their routine examinations for patients with symptomatic vitreous degeneration, as this test will supplement the regular visual acuity tests with extra information regarding the patients' overall visual function. That aside, the findings of our study imply that the loss of spatial

contrast in the ageing eye should no longer be explained without mention of the contribution of vitreous degeneration since the comparison of our findings with previous research suggests that significant vitreous degeneration can elicit a reduction in photopic contrast sensitivity similar to cataractous lens changes, as discussed above. Of course, a study to compare the contributions of cataractous lens changes and vitreous degeneration to photopic contrast sensitivity deficits within the same study will permit a more direct investigation of this topic.

6.3 NOVEL VITREOUS OPACITY IMAGING

Past efforts to quantify vitreous opacities have relied significantly on B-scan ultrasound imaging. Ketterling and colleagues have developed and patented a quantitative ultrasound method to characterise vitreous inhomogeneities (i.e., changes in acoustic impedance related to local properties and acoustic scatterers) in terms of contrast, size, shape and distribution.[305] This technique, which can be applied to two-dimensional (2D) image planes of the full globe in one embodiment or three-dimensional (3D) volume data assembled from a series of 2D image planes, provides an objective means of characterising the vitreous.

Recently, efforts have been garnered at developing and studying swept-source OCT imaging for vitreous opacities. While initial efforts focussed the applications of OCT imaging on the vitreoretinal interface, current advancements have been focussed on developing a device that allows for imaging of both the anterior and central vitreous.[174-176, 198] Ruminski and associates have recently quantified anterior vitreous and retrolental vitreous opacities using this technology.[198]

This PhD thesis has introduced a novel technique of quantifying vitreous opacities using ultra-widefield infrared imaging technique of the vitreous. This technique is an advancement of an initial idea by Sun et al. which involved the use of an open source software, Image J, to quantify floater shadows on infrared retinal images.[268] The current methodology involved confocal scanning ophthalmoscopy video of the vitreous based on an instruction that allowed patients to change fixation intermittently from the central fixation target within the device to the four cardinal positions of gaze. This method ensured that the vitreous opacities were sufficiently mobile to allow for a more accurate capture of the sizes of the opacities. Five, 102° field images, which were taken after the eye returned to the central fixation target from the positions of gaze, were analysed by Image J as described earlier in chapter 4 and averaged to generate the vitreous opacity area. Compared to Sun et al.'s approach, this technique offers a wider view of the vitreous (102° versus 30° / 55° in Sun et al.) and allows for the capture of the entire opacities within the vitreous body. This thesis reports the data of two studies that have employed this objective metric as an outcome measure. The data from both studies are impressive and highlight the usefulness of this technique as an outcome measure in vitreoretinal research. In addition, the ICC reported in chapter 5 also confirms that the methodology and the data generated thereof are reliable. Image J is an open-source software hence it is easily accessible and is very easy to use. This novel methodology represents a tool that can be employed by clinicians for diagnosing, monitoring and planning the treatment of patients suffering from symptomatic vitreous degeneration.

6.4 RETHINKING THE MANAGEMENT STRATEGIES FOR VITREOUS FLOATERS: THE PLACE OF TARGETED NUTRITION

The work presented in chapter 5 of this thesis has shown the importance of targeted nutrition with selective micronutrients for managing vitreous degeneration. Here, patients on the active supplement in this randomised, double blind, placebo-controlled clinical trial reported lesser floater symptoms, had improved contrast sensitivity as well as reduced vitreous opacities on objective assessment. The next question of interest, based on these findings, is: how does targeted nutrition fit into the armamentarium for vitreous degeneration management, especially for a disease whose main treatments have been mainly vitrectomy and laser vitreolysis?

Previous studies have shown pars plana vitrectomy as an effective treatment modality for vitreous degeneration, alleviating all of patients' symptomatology and improving visual function, specifically mesopic contrast sensitivity.[204, 206] While floater symptoms may not recur, patients may be predisposed to postoperative complications including cataracts, iatrogenic retinal breaks, retinal detachments, transient high postoperative intraocular pressure, transient postoperative hypotony and cystoid macular oedema.[205, 306] As a result, vitrectomy is mostly reserved by vitreoretinal surgeons as the last resort for very severe cases, making the procedure not readily available for all patients with vitreous degeneration.

Work from this thesis, consistent with previous reports, has indicated that laser vitreolysis results in subjective symptomatic improvement for patients (in our case,

an improvement for at least two-thirds of patients) who undergo the procedure.[268, 269, 272] Further, our vitreous opacity areas quantification methodology revealed an objective improvement of 90% following the procedure. Laser vitreolysis is also selective as a treatment; only patients who are deemed the most suitable candidates are offered the procedure. Severe vitreous opacities or specific opacity types such as massive discrete floater(s) or non-discrete vitreous haze may not be amenable to the treatment; laser vitreolysis may rather worsen the condition.

In the conventional scheme of vitreous degeneration management, clinicians typically commence management with watchful waiting (or observation), then progress to laser vitreolysis and finally to pars plana vitrectomy, as the severity of the disease progresses. Of note, majority of retinal surgeons do not offer laser vitreolysis as a treatment option and recommend vitrectomy (floaterectomy) to less than 1% of their patients.[270] What this implies is that the majority of vitreous floater patients are, unfortunately, not offered any treatment. However, the evidence from Wagle's work, as previously discussed, indicates that the utility values for acute and chronic floaters are similar and hence watchful waiting may not be a good management option to proffer to patients in a bid to alleviate their symptoms.[202]

This thesis reports the first clinical trial that successfully validated the use of a clinical formulation for managing symptomatic vitreous degeneration. This formulation holds massive benefits for especially the majority of floater patients who are conventionally observed and not treated. Rather than assigning patients to watchful waiting, clinicians, for the first time, can proffer a safe and effective

nutritional management option to patients, and patients can avail of this clinically-tested formulation to boost their vision-related quality of life. It is also worth mentioning that while this data is novel and impressive, the findings of this clinical trial remain to be validated by other research centres and in different populations.

To answer the question that was posed above regarding the place of targeted nutrition in the management strategies for vitreous degeneration, the evidence from this thesis suggests that nutritional intervention could aptly replace watchful waiting in the management paradigm for symptomatic vitreous degeneration. In doing this, not only are we offering hope to helpless patients who have, for several decades been advised to adapt to their conditions, but also we are adequately arming clinicians with an effective remedy that will forever transform symptomatic vitreous degeneration management.

6.4 SOCIETAL IMPACT

Degeneration of the human vitreous is ubiquitous during life. In fact, according to the National Eye Institute, "almost everyone develops floaters as they get older, but some are at a higher risk."[307] However, the selectivity associated with the current treatment modalities for symptomatic vitreous degeneration precludes majority of sufferers from receiving treatment. Of note, clinicians agree that about 99% of cases should be observed and not treated.[308] That aside, floaters are commonly reported in eyes with myopia.[309] Importantly, myopia is a growing public health concern and 50% of the world's population is estimated to become myopic by 2050.[310] What this implies is that the prevalence of vitreous floaters is

also set to increase in proportion with the increase in the prevalence of myopia, and the current conventional strategy of observation would not suffice to address patients' needs. From a public health perspective, this trajectory will rather result in further frequent visits by these patients to eye clinics, which will invariably put pressure on the already overwhelmed Ophthalmology units which are plagued with time constraints. In effect, any intervention that will reduce the burden of suffering for floater patients and limit the frequency of their visits to eye clinics may invariably benefit the healthcare system tremendously in the long run.

The interventional work performed as part of my PhD has enabled me (and my research colleagues) to develop and clinically test a formulation that responds to the needs of the majority of symptomatic vitreous degeneration patients who are likely to be advised by their clinicians to live with their condition. In light of the findings of this interventional work, this formulation will reduce the burden of illness associated with floaters and reduce the number of cases who would require surgery or laser treatment for their floaters. Since nutritional intervention is comparatively cheaper and safer than laser vitreolysis or vitrectomy, this formulation will also relieve patients of the financial burden associated with surgical or invasive modes of treatment of their floaters. Overall, I believe that this formulation will improve the quality of life of patients and alleviate the burden of this disease on society.

6.5 PERSONAL REFLECTIONS

"To succeed in a PhD, you must work as a scientist, but think like a business man."

John Nolan, 2018

Embarking on this PhD journey to investigate a hitherto, unexplored research area of targeted nutrition for optimising vitreous health has been a daunting, yet fulfilling task right from the onset. This project was daunting, at least in part, due to the lack of previous research that could serve as a foundation for my work. Previous research into the vitreous had been mainly pursued by vitreoretinal surgeons, who were concerned with research for advancing vitreoretinal surgeries; biochemists, who were interested in unravelling the composition and molecular constitution of the vitreous; and vision scientists, who investigated the impact of the vitreous and vitreous disorders on visual perception and visual function. Although this PhD project offered me (and my research colleagues) the opportunity to spearhead an exciting research area to develop and study a unique micronutrient formulation for the vitreous, the potential for failure due to the numerous 'unknowns' surrounding this research area made the whole PhD journey daunting.

Daunting as it seemed, I have come this far in my PhD pursuit because of a heartfelt advice I received from my primary supervisor (Professor John Nolan) on my first official meeting with him at the Nutrition Research Centre Ireland. He advised that, "*To succeed in a PhD, you must work as a scientist, but think like a business*

man." In hindsight, I consider this advice as the game changer in my PhD pursuit as it enabled me to properly focus on my research topic as a scientist whiles developing and applying the requisite core, transferrable skills expected of any PhD, the same skills that are also applicable in the business world. Armed with this advice, I was able to evaluate the actions and strategic decisions that would ensure my success as a PhD as well as the potential risks or pitfalls that may befall me in the PhD process and how to navigate my way out of them. Further, I had to consider the collaborations and creative approaches that were necessary to get my PhD done, sounds like '*thinking like a business man*,' doesn't it?

With no previous work to rely on, my PhD taught me to be creative, innovative, and inventive. As part of my PhD, I received specialised training in methodologies employed in conducting vitreoretinal research such as confocal scanning laser ophthalmoscopy video imaging of the vitreous; OCT scanning and interpretation of the vitreoretinal interface; and visual function assessments (BCVA, CS and FCS) with different testing devices including Thomson Solutions software, M&S Clinical Trial Suite, and AVOT Suite. However, my PhD also allowed me to be creative in developing methodologies that were integral for the success of my project. I successfully developed a questionnaire for assessing the subjective discomforts of patients with symptomatic vitreous degeneration. Although not validated yet, this questionnaire provided a disease-specific instrument to capture the quality of life of symptomatic vitreous degeneration patients. Further, I developed a novel methodology for objectively quantifying vitreous opacities.

These methodologies were essential in carrying out and gathering data for the interventional work that has been reported in this thesis.

The interventional work I conducted as part of my PhD offered me the opportunity to harness my project management and organisational skills. In thinking like a business man, I considered this interventional work as my primary project and myself as the project manager. As the main researcher on the project, I managed the various phases or tasks of the FLIES project including organising study-related documentation, patient scheduling, patient management, and data collection and management. I harnessed my skills on setting realistic goals and timelines for the project tasks and ensured that all deadlines were met successfully. I was also able to communicate effectively with all the stakeholders of the project during its execution phase to manage their expectations. All these experiences have consolidated my ability to effectively manage projects and coordinate project-related activities.

Within this same period, I have had the opportunity to disseminate the outcomes of my research via written and oral communication channels. I have published four first-author manuscripts and submitted another paper for peer-review at high impact factor journals in ophthalmology. I have also presented aspects of my work at four international conferences as either conference lectures or poster presentations. Of note, I was awarded a travel grant by the Institute of Eye Surgery to deliver a conference lecture at the 2018 British and Eire Vitreoretinal Surgeons (BEAVRS) conference in Liverpool, United Kingdom. I was also awarded a travel

grant by ebiga-VISION GmbH to present aspects of my PhD at the Kongress der Deutsche Ophthalmologische Gesselschaft 2019 in Berlin. All these experiences and exposures allowed me to meet and interact with clinicians and researchers who were able to comment and give feedback on my research. That aside, some aspects of the data reported in this thesis have been prepared and published as part of a patent application in a bid to protect the intellectual property related to the interventional study (see Appendix E). I have also had the opportunity to serve as a reviewer for PLoS One journal where I have reviewed three papers to date. All these activities have helped shape my writing and presentation skills.

In summary, my PhD has been a fulfilling journey of learning to adapt to life in a new country and at the same time availing myself to the rigorous discipline and training of the PhD curriculum. All these have contributed to my development into the independent researcher and lead scientific investigator (in the area of vitreous nutrition) I am today. All in all, I have enjoyed being a scientist at heart and a business man in my thinking. And for this, I will be eternally grateful to Professor Nolan for showing me the way.

6.6 FUTURE RECOMMENDATIONS

Although this thesis has made significant contributions to vitreous research in the context of targeted nutrition for optimising vitreous health, contrast threshold loss with vitreous degeneration, and vitreous imaging, there still remains a lot to learn about these thematic areas and further research is warranted in the following areas:

6.6.1 Targeted nutrition for the vitreous

This PhD thesis has provided proof that targeted nutrition is a plausible option for managing vitreous degeneration. This has opened up a new and exciting area of research where future explorations should be geared at identifying more putative molecules with antioxidative and antiglycation properties that can guard against vitreous degeneration mechanisms. Further, future studies to quantify the concentrations of the various micronutrients within the vitreous are warranted to enhance our understanding of the vitreous antioxidant capacity, especially following supplementation. Another research worth pursuing is to examine the impact of higher concentrations of the present formulation on symptomatic vitreous degeneration. We know that myopic vitreopathy occurs 5 to 10 years before the fourth decade and with the global prevalence of myopia predicted to reach 50% in 2050, it is expected that myopic vitreopathy will increase accordingly.[38, 310] Thus, future studies to investigate the impact of nutritional supplementation on the onset of myopia-related vitreous degeneration will be useful. Aside these, it is evident that a common post-operative side effect of cataract surgeries is the development of vitreous floaters. It will be interesting to study the impact of prior nutritional supplementation with vitreous antioxidative and antiglycation micronutrients on the development of floaters following cataract surgery.

6.6.2 Vitreous degeneration and contrast thresholds

This thesis has shown that vitreous degeneration reduces spatial contrast at photopic and mesopic luminance at high spatial frequencies. It will be interesting to investigate the impact of vitreous degeneration on low to mid spatial frequencies, if any, at photopic and mesopic light levels. Another future research relates to contrast threshold comparisons between patients with cataract, vitreous degeneration and healthy controls. This will enhance our understanding of the contribution of vitreous degeneration as a forward scatter source to the overall photopic contrast loss caused by intraocular forward light scattering.

6.6.3 Vitreous imaging

This thesis has shown that quantitative assessment of vitreous opacities with ultra-widefield infrared imaging provides reliable objective data that can enhance diagnosis and treatment planning for symptomatic vitreous degeneration. Future studies should be aimed at correlating this technique with other important clinical indices used for assessing vitreous degeneration such as photopic and mesopic contrast sensitivity, visual acuity, subjective questionnaires and intraocular straylight. Future studies should also be directed at employing this imaging modality to distinguish between central and peripheral vitreous opacities.

6.7 CONCLUDING STATEMENT

The principal aim of this thesis was to investigate the impact of targeted nutrition on vitreous health. It is apparent from this thesis that targeted nutrition with selected antioxidative and antiglycation micronutrients reduce vitreous opacity areas, improve contrast sensitivity, and reduce visual discomforts associated with symptomatic vitreous degeneration. Further research is warranted in this new and exciting area to enhance our understanding of targeted nutrition for optimising vitreous health.

7

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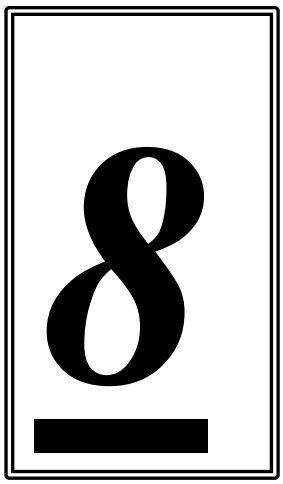

APPENDICES

Appendix A: Ethical Approval

A1: University Hospital Waterford - FLIES

Ospidéal Ollscoile Phort Láirge
University Hospital Waterford
Regional Cancer Centre South East

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Research Ethics Office
Old School of Nursing
University Hospital Waterford

Tel: 051- 842026/051-842391

24th April 2017

Professor John M. Nolan,
Carriganore House,
WIT West Campus,
Carriganore,
Waterford.

STUDY TITLE: "Floater Intervention Study (FLIES)"

STUDY STATUS: *APPROVED*

Dear Professor Nolan,

The Research Ethics Committee, HSE, South East reviewed the above Study and are happy to grant you Full Ethical Approval.

The following documents were reviewed and approved:

1. Ethics Submission Form.
2. FLIES: Floater Intervention Study.
3. Floater Intervention Study (FLIES) Supplementation Trial.
4. The Floater Intervention Study (FLIES) Case Report Form (CRF).
5. Floater Intervention Study (FLIES): Supplementation trial Information Leaflet.

The following documents were received:

1. GP Letter.
2. Certificate of Insurance - Newline.
3. CV – Professor John M Nolan.
4. Signed Hard Copy of Declaration form posted, e-mailed, scanned or faxed to Research Ethics Office.

Please notify the Research Ethics Committee Office, Old School of Nursing, University Hospital Waterford on completion of Research.

Yours sincerely,

Caroline Lamb

Ms Caroline Lamb
Research Ethics Committee Coordinator
Health Service Executive, South Eastern Area

C.C. Rachel Moran, Carriganore House, WIT West Campus, Carriganore, Waterford.

The Research Ethics Committee, HSE, South East is a recognized Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human use) Regulations 2004 and as such is authorized to undertake ethical review of clinical trials of all descriptions and classes for the Republic of Ireland.

The Research Ethics Committee, HSE South East issues ethical approval on the basis of information provided. It is the responsibility of the researcher to notify the Research Ethics Office of any changes to a study to ensure that the approval is still relevant.

A2: Waterford Institute of Technology - FLIES

Institiúid Teicneolaíochta Phort Láirge Waterford Institute *of* Technology

Phort Láirge, Éire.
T: +353-51-302000
info@wit.ie

Waterford, Ireland.
T: +353-51-302000
www.wit.ie

REF: WIT2019REC0007

26th March, 2019.

Mr. Emmanuel Ankamah,
Nutrition Research Centre Ireland,
WIT,
West Campus Carriganore,
Waterford.

Dear Emmanuel,

Thank you for bringing your project *'Enhancement of self-reported quality of life of patients with vitreous floaters following supplementation with VitroCap NEM: Floater Intervention Study (FLIES)'* to the attention of the WIT Research Ethics Committee.

Based on a review of your application and supporting documentation I am pleased to inform you that we now fully approve the conduct of this project.

We will convey this decision to Academic Council.

We wish you well in the work ahead.

Yours sincerely,

Prof. John Wells,
Chairperson,
WIT Institute Ethics Committee

cc: Prof. John Nolan
Dr. Rachel Moran
Dr. Eugene Ng

A3: Clinical Research Ethics Committee of the Cork Teaching Hospitals – Laser Study

COISTE EITICE UM THAIGHDE CLINICIÚIL

Clinical Research Ethics Committee of the Cork Teaching Hospitals

Tel: +353-21-4901901
Email: crec@ucc.ie

University College Cork
Lancaster Hall
6 Little Hanover Street
Cork
Ireland

CREC Review Reference Number: ECM 4 (z) 10/03/2020

Date: 24th March 2020

Mr Eugene Ng
Consultant Ophthalmologist
Institute of Eye Surgery
UPMC Whitfield Hospital (Suite 14)
Butlerstown North
Cork Road
Waterford, X91 DH9W
Ireland

Study Title: The long-term safety and efficacy of laser vitreolysis for managing symptomatic floaters.

Approval is granted to carry out the above study.

The following documents have been approved:

Document	Approved	Version	Date
Application Form	Yes		5th February 2020 (received 7th February 2020)
CV for Chief Investigator	Yes		
Proof of Insurance	Yes		Expiry 17th January 2021
Sample Consent Form signed by patient for use of data			
Data Collection Sheet	Yes		

We note that the co-investigator(s) involved in this project will be:

Name	Occupation
Emmanuel Ankamah	Research Fellow.

Please keep a copy of this signed approval letter in your study master file for audit purposes.

You should note that ethical approval will lapse if you do not adhere to the following conditions:

1. Submission of an Annual Progress Report/Annual Renewal Survey (due annually from the date of this approval letter)
2. Report unexpected adverse events, serious adverse events or any event that may affect ethical acceptability of the study
3. Submit any change to study documentation (minor or major) to CREC for review and approval. Amendments must be submitted on an amendment application form and revised study documents must clearly highlight the changes and contain a new version number and date. Amendments cannot be implemented without written approval from CREC.
4. Notify CREC of discontinuation of the study

5. Submit an End of Trial Declaration Form and Final Study Report/Study Synopsis when the study has been completed.

Yours sincerely

Professor David Kerins
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice.

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Ref: WIT2019REC0026

Date 22nd June 2021

Mr. Emmanuel Ankamah,
PhD Researcher,
WIT

Dear Emmanuel,

Thank you for bringing your project **'The safety and efficacy of laser vitreolysis for managing symptomatic floaters'** to the attention of the WIT Research Ethics Committee on the 5th of December 2019.

I am pleased to inform you that we fully approve WIT's participation in this project and we will convey this to Academic Council.

We wish you well in the work ahead.

Yours sincerely,

_____

Prof. John Wells,
Chairperson,
Research Ethics Committee.

Appendix B: Patient Eligibility - FLIES

Floater Intervention Study (FLIES): supplementation trial
Patient Eligibility

Patient Name: _____ **Date of birth:** _____

Contact number: _____

Eligible = Yes to questions 1, 2 and 8
Ineligible = Yes to questions 3-5

Questions:	**Yes**	**No**
1. Has the patient proactively reported or complained of floaters?	☐	☐
2. Does the patient have a history of posterior vitreous detachment [PVD] or during the patient's standard eye examination was PVD detected? Explain in comments below (e.g. PVD both eyes or one eye).	☐	☐
3. Does the patient present with secondary floaters?	☐	☐
4. Does the patient have a history of any retinal disease or during the patient's standard eye examination was retinal pathology detected? *(e.g. retinal scars, retinal detachment, glaucoma, age-related macular degeneration, macular oedema)*	☐	☐
5. Is the patient scheduled for cataract surgery in the next 9 months?	☐	☐
6. Is the patient interested in taking part of the FLIES trial? (Give patient leaflet)	☐	☐

Comments:

_____

_____

_____

Optician/Ophthalmologist: _____ Date: _____

Appendix C: Patient Information Leaflet & Consent Form – FLIES

Floater Intervention Study (FLIES): supplementation trial Information Leaflet

Aim

The aim of the Floater Intervention Study (FLIES) is to investigate if supplementation with VitroCap NEM reduces visual disturbances associated with the degeneration of the vitreous body of the eye (eye floaters disturbances).

Background Information

Within the eye is a clear, jelly-like fluid called the vitreous, containing 98% water. The vitreous structure is perfectly stabilised by a loose network of collagen fibres, arranged in parallel, with some crosslinking and wide spacing in between. The vitreous is light-transparent, so images formed at the back of the eye are usually undisturbed.

Floaters may result from a slow degeneration process of the vitreous body, which leads initially to division and changes of the collagen fibres and later on to a breakdown of the gel structure and liquefaction of the vitreous. As the fibres lose their surface coating or are partially divided, they tend to clump. The clumps of collagen fibres may move within the vitreous body, and the shadows they cast tumble and turn. Because of the way the visual system works, we see these shadows as something moving across our visual field, however, as explained, these visible particles exist within the eye. The visual system can misinterpret them as moving objects; floating cobwebs, flies or even small birds. When they move into our line of vision, they can interfere with many everyday tasks such as reading or driving.

VitroCap NEM is a clinically tested dietary food for special medical purposes used to help treat vitreous floaters, or simply floaters. Floaters result from changes in the vitreous body, a transparent meshwork of connective tissue (collagen) that is able to bind a large amount of water.

Study Design

FLIES is a double-blind, randomised, placebo-controlled trial. This means that neither the volunteers enrolled nor the study investigators will know which patients are consuming the dietary supplement containing the active ingredient, and which patients are consuming the placebo containing no active ingredient. This study aims to recruit 60 volunteers. Each volunteer will attend the Nutrition Research Centre Ireland, Waterford Institute of Technology, West Campus, Carriganore, Waterford on two occasions over a 6-month period (at month one and month 6). Each study visit will last approximately two and a half hours. The volunteers will be asked to take either the active (VitroCap NEM, containing the active ingredients: 125mg of L-lysine, 40 mg of vitamin C, 25 mg of *Vitis vinifera* extract [procyanidines], 5 mg of zinc and 60 mg of *Citrus aurantinium* flavonoids) or a placebo (a capsule containing excipients but no active ingredients) once a day with a meal for 6 months. The supplements will be provided free of charge by the study investigators.

Risks and/or Discomforts

We foresee no risks to subjects participating in this research. We will also inform your G.P. that you will be participating in our research study. Participation in this trial is not a substitute for their standard medical care or eye care.

Study Visit

Informed consent

The study investigator will explain all aspects of the study to you, in addition to this information leaflet. If you would like to volunteer, you will be asked to sign an informed consent document which states that you are happy to participate in the study and that all aspects of the study have been explained to you by the study investigator.

Blood sample

A blood sample will be taken at baseline and at 6 months for an occupational health profile.

Demographic information
You will be asked to complete a brief questionnaire to gather information on your demographics and lifestyle. This questionnaire will collect your contact and lifestyle details, and medical history, for analysis.

Assessment of the change in floater disturbance
The primary outcome measure of this interventional trial will be the completion of a subjective (questionnaire) assessment of the change in floater disturbance following the 6-month intervention.

Vision and cognition tests
Various aspects of your vision will be tested using the following tests: visual acuity, contrast sensitivity, rod and cone sensitivity, colour vision and macular pigment levels. It is important to note that all the vision tests are non-invasive. These tests will measure the overall visual quality of your retina. All tests will be performed using specialised optical devices, and the results will allow the investigators to assess the functional status of the macula and identify changes over time (both eyes will be dilated). Feedback will be given regarding your vision status and macular pigment levels. Cognitive function (how well the brain is working) will be assessed using specialised equipment designed for this intended purpose. This is similar to an aptitude test as it will assess brain function, including memory, reaction speed etc. This test has been designed especially for this project by a company called CANTAB UK.

Study Participation
This study is entirely voluntary. You will not be paid for your participation in this study. If you decide to take part you are free to withdraw at any time and without giving a reason, and you can request that data already collected from you is not used by the investigators. This will not affect the standard of care you receive. A person who does not wish to participate will not be discriminated against in any way. Participation in this study is not intended to replace standard medical care, and is therefore for research purposes only.

Benefits
It is anticipated that society may benefit from the results of this study. At the end of the study, the investigators will provide you with information on the measurements performed on you during your study visits. General information on general health and eye health will be provided at the study centre for your interest.

Data Confidentiality
All the data collected in this study will be treated as strictly confidential and will be obtained and processed in keeping with the Data Protection Act 1988 and the amended Data Protection Act of 2003. All data will be analysed collectively as a group and coded by data link to ensure volunteers' confidentiality.

Compensation
The study and its investigators are covered by an insurance, which protects you in case of problems directly caused by this study.

Organisers and Sponsors
Researchers at Nutrition Research Centre Ireland (NRCI), under the direction of Professor John Nolan, Principal Investigator, will be conducting and managing this study. This study is sponsored by ebiga-VISION GmbH, Berlin, Germany.

Ethical Approval
Ethical approval for this study has been obtained from the Research Ethics Committee, HSE, South East.

Questions
A member of the study research team will be available to answer any further questions you may have concerning the study, and any outcomes that may appear to be related to the research. The support number is **051 302153**.

We hope that this information has answered most of your questions. Should you have further questions or do not fully understand the information given, please feel free to ask us. The doctors and researchers who are carrying out this research would like to thank you for taking the time to read this information.

Floater Intervention Study (FLIES): supplementation trial

Date: _____ **Subject Number:** _____

- I confirm I have received, read and understand the Patient Information Leaflet for this study. I have had sufficient time to review the information, consider my participation and all relevant information has been discussed fully in non-technical terms, and all my questions have been answered satisfactorily. ☐
- I understand that my participation is voluntary and that I am free to withdraw at any time, without my medical care or legal rights being affected. ☐
- I understand that my data concerning this study will be entered on a computer in order to be analysed together with the data obtained from other patients. My identity will always be protected. I give permission for this analysis. ☐
- I understand that responsible authorities within the Nutrition Research Centre Ireland (NRCI) and Nutrasight Consultancy Ltd (NCL) may look at my data collected for this study where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
- I agree to take part in the above study and hereby give my consent to have a blood sample collected for occupational blood profile analysis. ☐
- I agree for my blood sample to be stored until time of analysis. My identity will always be protected. ☐
- I voluntarily agree to take part in this study. ☐

_____	_____	_____
Name of Volunteer (PRINT)	Date	Signature of Volunteer
_____	_____	_____
Name of Witness (PRINT)	Date	Signature of Witness

Appendix D: Case Report Form – FLIES
D1: Baseline

Subject number: _____

The Floater Intervention Study (FLIES)

Case Report Form (CRF)

CRF Code: .....
(e.g. FLSV1001)

Investigator check

Signature ..... Date .....

Subject number: _____

FLIES Study Procedures

Description	Approx. time (minutes)
A. Eligibility	2
B. Informed Consent	5
C. Demographic, lifestyle and medical history questionnaires	20
D. Subjective assessment	5
E. Cognition test	5
F. Acuity Plus	5
G. CAD	10
H. Flicker Plus	10
I. Visual acuity	10
J. Letter Contrast Sensitivity	10
K. Multiquity	15
L. Dilation	3
M. Blood Sample	5
N. Ocular Coherence Tomography	5
O. Measurement of Macular Pigment: Autofluorescence	5
P. Fundus Photography	5
Total study visit time:	**2 hours**

A. Informed consent

Was the patient given a copy of his/her consent? yes ☐ no ☐

If yes,

Date of informed consent: _____ Obtained by: _____

(DD/MM/YYYY)

B. Demographic, medical history, and lifestyle questionnaires

Patient Name: _____

GP Name: _____

GP Address: _____

_____

_____

Date of birth: _____ **Age:** _____(years)

Subject number: _____

Please circle number corresponding to correct answer. All questions must be answered unless otherwise specified.

1. Sex

Male ……. 1

Female ……. 2

2. Smoking

2.1. Which best describes your smoking habits (whether cigarette, cigar, pipe etc.)?

Never smoker (smoked < 100 cigs in lifetime) ……. 1

Ex-smoker (smoked ≥ 100 cigs in lifetime and none in past year) …… 2

Current smoker (smoked ≥ 100 cigs in lifetime and at least 1 cig in last year) …. 3

2.2. Have you smoked at least 100 cigarettes in your life? yes ☐ no ☐

If no skip to question **2.6.**

2.3. How long has it been since you last smoked?

Less than 1 day……...	1	Less than 6 months…….	5
Less than 7 days…….	2	6 months to a year……...	6
Less than 1 month……..	3	Greater than 1 year……	7
Less than 3 months……	4		

2.4. What is the average number of cigarettes you smoke (or smoked) daily? _____

2.5. For how many years have you smoked (or did you smoke)? _____

2.6. Are you commonly exposed to second-hand smoke at home or in the work place?

yes ☐ no ☐

Subject number: _____

3. Alcohol

3.2. Regarding alcohol, which of the following statements best describes the way you drink?

I never drink……..	1
I drink only on special occasions…….....	2
I drink once or twice a month…….....	3
I drink once or twice a week……..	4
I drink every day……	5
I drink twice a day or more……	6

3.3. What is your average alcohol consumption on a weekly basis?

0 units a week……	1
1 unit a week……	2
2-5 units a week……..	3
6-10 units a week……	4
> 10 units a week……	5

Initial: _____

Subject number: _____

4. Exercise

4.1. Do you perform any physical activity? yes ☐ no ☐

4.2. If yes:

Type of physical activity	**Duration (min/week)**

5. Medical History

Medical history including surgical procedures (Body)	**Yes**	**No**	**Date of Diagnosis**	**Ongoing?**
Cardiovascular disease				☐ No ☐ Yes
Hypertension				☐ No ☐ Yes
Angina				☐ No ☐ Yes
Stroke				☐ No ☐ Yes
Peripheral vascular disease				☐ No ☐ Yes
Diabetes				☐ No ☐ Yes
Malabsorption				☐ No ☐ Yes
Ocular disease				☐ No ☐ Yes
Other (please specify)				☐ No ☐ Yes

7. <u>Supplementation</u>

Is the patient taking any dietary supplements?

e.g. Omegas, vitamin C, carotenoids

Yes / No

Comments: _____

_____

_____

Subject number: _____

Patient Name: DOB: Visit:

C. Subject assessment **Month/Season: _____**

Item 1: Eyes with floaters
Question: Which of your eyes has vitreous floaters: right eye, left eye or both? Tick the appropriate:

1. Right eye ☐
2. Left eye ☐
3. Both eyes ☐

Skip to Item 3 if floaters are in one eye only.

Item 2: The eye with more suffering

Question: Which of your two eyes bothers you the more: the right or the left? Tick the appropriate:

1. Right eye ☐
2. Left eye ☐

Item 3: Frequency of floater disturbance per day
Question: What time or times in the day have you been mostly affected by your floaters in ***the past week***: In the morning, afternoon, evening or not at all? Tick the appropriate:

1. Not at all
2. Once in a day (Either morning, afternoon or evening)
3. Twice in a day (When two specific times are mentioned)
4. All the time (When patient is affected in the morning, afternoon and evening)

Not at all	Once in a day	Twice in a day	All the time
☐	☐	☐	☐

Subject number: _____

Item 4: Effect of floaters on daily life
Question: How would you describe the effect of your floaters on your daily life in ***the past week***: no effect, little effect, moderate effect, much effect or very much effect? Tick the appropriate:

No effect	Little effect	Moderate effect	Much effect	Very much effect
☐	☐	☐	☐	☐

Item 5: Activities affected by floaters
Tick either yes or no, as appropriate, for each of the following activities if your floaters bother you while performing the activity:

	Yes	No
a. When reading small print, such as cosmetics or drug labels	☐	☐
b. When reading a newspaper or a book	☐	☐
c. When driving a car	☐	☐
d. When using a computer or mobile phone	☐	☐
e. When watching TV	☐	☐
f. In any other situations	☐	☐

If you ticked "yes" to "other situations", please give details in the box provided:

In which situation (a-f) in Item 5 above were you ***most*** affected by eye-floaters? Insert one of a – f in the box provided: ☐

Subject number: _____

Item 6: Severity of floater disturbance over the last 6 months

Question: How would you describe the severity of the disturbance from your floaters over the ***last 6 months***?

My condition has been stable and I have not been bothered by my floaters ☐

My floaters have been intermittently and moderately bothersome ☐

My floaters have been persistently bothersome ☐

Initial: _____

Subject number: _____

D. Cognition test

MoCA □

Reaction time (RTI) □

Comments:

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Initial: _____

Subject number: _____

E. Acuity Plus- Binocular

Binocular Photopic

Visual acuity	Mean ±SD
+	
-	

Functional Contrast Sensitivity	Mean ±SD
+	
-	

Binocular Mesopic

Visual acuity	Mean ±SD
+	
-	

Functional Contrast Sensitivity	Mean ±SD
+	
-	

F. Flicker Plus- Binocular

Photopic – cones	Mean ±SD	Mesopic – rods	Mean ±SD
5°, 60°, -135	±	5°, 90°, -135	±
5°, 60°, -45	±	5°, 90°, -45	±
0°, 30°, 0	±	0°, 45°, 0	±
5°, 60°, 45	±	5°, 90°, 45	±
5°, 60°, 135	±	5°, 90°, 135	±

Subject number: _____

Patient Name: **DOB:** **Visit:**

Floaters: RE LE Both **PVD: RE LE Both**

G. Visual Acuity- The eye with the best visual acuity will used for MP assessment.

Right Eye		Left Eye	
6/24	_____	6/24	_____
6/19	_____	6/19	_____
6/15	_____	6/15	_____
6/12	_____	6/12	_____
6/9.5	_____	6/9.5	_____
6/7.6	_____	6/7.6	_____
6/6	_____	6/6	_____
6/4.8	_____	6/4.8	_____
6/3.8	_____	6/3.8	_____
6/3	_____	6/3	_____

	Eye	UA=1 CA=2	Snellen	1st	2nd	3rd	Average of 3	Extra letters	LogMAR	VAR	Total Score
Right eye			6/	/5	/5	/5	/5	+			
Left eye			6/	/5	/5	/5	/5	+			

Subject number: _____

H. Letter Contrast Sensitivity

6/120 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	
6/60 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	

Initial: _____

Subject number: _____

6/24 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	
6/15 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	

Initial: _____

Subject number: _____

6/9.5 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	

Initial: _____

Subject number: _____

I. Multiquity

Visual acuity by MultiQuity

	Right Eye (RE)	Left Eye (LE)
Acuity score		
Exact logMar		
Nearest Snellen (US)		
Nearest Snellen (metric)		

Contrast sensitivity by MultiQuity

	Right Eye (RE)	Left Eye (LE)
Contrast score		
LogCS score		

Health Information

Height	cm	Body mass index (kg/m^2) _____
Weight	Kg	
Blood pressure	_____/_____ mmHg Systolic/Diastolic	

Subject number: _____

J. Dilation-Both eyes ☐

Comments: _____

_____

K. Blood Extraction

Was 4 blood sample (1 x serum [yellow top], 1 x glucose [grey top], 1 x heparin [green top] and 1 x EDTA whole blood [purple]) taken from the subject?

Yes ☐ No ☐

Comments: _____

_____

If yes:
Time of blood extraction: _____ Time of subject's last meal: _____

Was the blood sample centrifuged, the serum extracted and stored in duplicate at -70˚C?
Yes / No

If yes:
Time of centrifugation: .....
Name of person obtaining blood sample: .....
Signature of person obtaining blood sample: .....

Supplement pack and instructions given ☐

L. Ocular coherence tomography-Both eyes

Right eye	Left eye
1: min foveal thickness: ……	1: min foveal thickness: ……
2: mean foveal thickness: ……	2: mean foveal thickness: ……
3: max foveal thickness: …….	3: max foveal thickness: …….

Subject number: _____

M. SLO Video Acquisition

RE ☐
LE ☐

N. Measurement of macular pigment by autofluorescence

Circle one: RE or LE

Eccentricities	MPOD
0.23	
0.51	
0.98	
1.76	
Volume	

O. Fundus photography

Was a fundus photograph taken of each eye? ..... Yes / No

Does fundus look normal? Yes / No

Do fundus photographs require further assessment? ..... Yes / No
If yes, refer patient to ophthalmologist

N.B. Code for fundus photograph to correspond to subject number, visit and study eye (e.g. FLSV1001R)

Comments:

_____

_____

Subject number: _____

The Floater Intervention Study (FLIES)

6 month visit

Case Report Form (CRF)

CRF Code: .....
(e.g. FLSV1001)

Investigator check

Signature ..... Date .....

Subject number: _____
Patient Name: **DOB:** **Visit:**

A. Subject assessment **Month/Season: _____**

Item 1: Eyes with floaters
Question: Which of your eyes has vitreous floaters: right eye, left eye or both? Tick the appropriate:

1. Right eye ☐
2. Left eye ☐
3. Both eyes ☐

Skip to Item 3 if floaters are in one eye only.

Item 2: The eye with more suffering

Question: Which of your two eyes bothers you the more: the right or the left? Tick the appropriate:

1. Right eye ☐
2. Left eye ☐

Item 3: Frequency of floater disturbance per day
Question: What time or times in the day have you been mostly affected by your floaters in ***the past week***: In the morning, afternoon, evening or not at all? Tick the appropriate:

1. Not at all
2. Once in a day (Either morning, afternoon or evening)
3. Twice in a day (When two specific times are mentioned)
4. All the time (When patient is affected in the morning, afternoon and evening)

Not at all	Once in a day	Twice in a day	All the time
☐	☐	☐	☐

Subject number: _____

Item 4: Effect of floaters on daily life
Question: How would you describe the effect of your floaters on your daily life in ***the past week***: no effect, little effect, moderate effect, much effect or very much effect? Tick the appropriate:

No effect	Little effect	Moderate effect	Much effect	Very much effect
☐	☐	☐	☐	☐

Item 5: Activities affected by floaters
Tick either yes or no, as appropriate, for each of the following activities if your floaters bother you while performing the activity:

	Yes	No
a. When reading small print, such as cosmetics or drug labels	☐	☐
b. When reading a newspaper or a book	☐	☐
c. When driving a car	☐	☐
d. When using a computer or mobile phone	☐	☐
e. When watching TV	☐	☐
f. In any other situations	☐	☐

If you ticked "yes" to "other situations", please give details in the box provided:

☐

In which situation (a-f) in Item 5 above were you ***most*** affected by eye-floaters? Insert one of a – f in the box provided: ☐

Subject number: _____

Item 6: Severity of floater disturbance over the last 6 months

Question: How would you describe the severity of the disturbance from your floaters over the ***last 6 months***?

My condition has been stable and I have not been bothered by my floaters ☐

My floaters have been intermittently and moderately bothersome ☐

My floaters have been persistently bothersome ☐

Initial: _____

Subject number: _____

B. Acuity Plus- Binocular

Binocular Photopic

Visual acuity	Mean ±SD
+	
-	

Functional Contrast Sensitivity	Mean ±SD
+	
-	

Binocular Mesopic

Visual acuity	Mean ±SD
+	
-	

Functional Contrast Sensitivity	Mean ±SD
+	
-	

C. Flicker Plus- Binocular

Photopic – cones	Mean ±SD	Mesopic – rods	Mean ±SD
±	**5°, 60°, -135**	±	**5°, 90°, -135**
±	**5°, 60°, -45**	±	**5°, 90°, -45**
±	**0°, 30°, 0**	±	**0°, 45°, 0**
±	**5°, 60°, 45**	±	**5°, 90°, 45**
±	**5°, 60°, 135**	±	**5°, 90°, 135**

Initial: _____

Subject number: _____

Patient Name: **DOB:** **Visit:**

Floaters: RE LE Both PVD: RE LE Both

D. Visual Acuity- The eye with the best visual acuity will used for MP assessment.

Right Eye		Left Eye	
6/24	_____	6/24	_____
6/19	_____	6/19	_____
6/15	_____	6/15	_____
6/12	_____	6/12	_____
6/9.5	_____	6/9.5	_____
6/7.6	_____	6/7.6	_____
6/6	_____	6/6	_____
6/4.8	_____	6/4.8	_____
6/3.8	_____	6/3.8	_____
6/3	_____	6/3	_____

	Eye	UA=1 CA=2	Snellen	1st	2nd	3rd	Average of 3	Extra letters	LogMAR	VAR	Total Score
Right eye			6/	/5	/5	/5	/5	+			
Left eye			6/	/5	/5	/5	/5	+			

6

Subject number: _____

E. Letter Contrast Sensitivity

6/120 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	
6/60 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	

Initial: _____

Subject number: _____

6/24 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	
6/15 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	

Initial: _____

Subject number: _____

6/9.5 spatial frequency							
% Contrast	**LogCS**	**1**	**2**	**3**	**4**	**5**	**Extra letters**
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
			CS score:	RE	/	LE	

Initial: _____

Subject number: _____

F. Multiquity

Visual acuity by MultiQuity

	Right Eye (RE)	**Left Eye (LE)**
Acuity score		
Exact logMar		
Nearest Snellen (US)		
Nearest Snellen (metric)		

Contrast sensitivity by MultiQuity

	Right Eye (RE)	**Left Eye (LE)**
Contrast score		
LogCS score		

Initial: _____

Subject number: _____

G. Dilation-Both eyes ☐

Comments: _____

_____

H. Blood Extraction

Was 4 blood sample (1 x serum [yellow top], 1 x glucose [grey top], 1 x lithium heparin [green top] and 1 x EDTA whole blood [purple]) taken from the subject?

Yes ☐ No ☐

Comments: _____

_____

If yes:
Time of blood extraction: _____ Time of subject's last meal: _____

Was the blood sample centrifuged, the serum extracted and stored in duplicate at -70˚C?
Yes / No

If yes:
Time of centrifugation: .....
Name of person obtaining blood sample: .....
Signature of person obtaining blood sample: .....

Thank you supplement pack given ☐

I. Ocular coherence tomography-Both eyes

Right eye	Left eye
1: min foveal thickness: ……	1: min foveal thickness: ……
2: mean foveal thickness: ……	2: mean foveal thickness: ……
3: max foveal thickness: …….	3: max foveal thickness: …….

Subject number: _____

J. SLO Video Acquisition

RE ☐
LE ☐

K. Measurement of macular pigment by autofluorescence

Circle one: RE or LE

Eccentricities	MPOD
0.23	
0.51	
0.98	
1.76	
Volume	

L. Fundus photography

Was a fundus photograph taken of each eye? ..... Yes / No

Does fundus look normal? Yes / No

Do fundus photographs require further assessment? ..... Yes / No
If yes, refer patient to ophthalmologist

N.B. Code for fundus photograph to correspond to subject number, visit and study eye (e.g. FLSV1001R)

Comments:

_____

_____

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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) **Title:** COMPOSITION OF A DIETARY SUPPLEMENT AND/OR A NUTRITIONAL ADDITIVE FOR FOOD, A UNITARY DOSAGE FORM OF SAID COMPOSITION, AND THEIR USE FOR IMPROVEMENT OF THE QUALITY OF VISUAL PERFORMANCE INCLUDING CONTRAST SENSITIVITY IN PERSONS IN NEED OF SUCH AN IMPROVEMENT, INCLUDING PERSONS SUFFERING FROM AT LEAST ONE EYE DISEASE, PARTICULARLY VITREOUS FLOATERS

(57) **Abstract:** The subject-matter of the invention is a composition of a dietary supplement and/or a nutritional additive for food, a unitary dosage form of said composition, and their use for the improvement of the quality of visual performance including contrast sensitivity in persons in need of such an improvement, including persons suffering from at least one eye disease, particularly vitreous floaters. The composition of the dietary supplement and/or of nutritional additive for food according to the invention comprises as active substances L-lysine, vitamin C, zinc, grape extract and bitter orange extract in therapeutically effective quantities, and optionally at least one excipient. In the preferred embodiment of said composition, the percentages of the individual active substances relative to the sum of the percentages of all active substances are about 50% by weight of L-lysine, about 15% by weight of vitamin C, about 2% by weight of zinc, about 10% by weight of the grape extract and about 23% by weight of the bitter orange extract, with all percentages of the individual active substances adding up to 100% by weight of all active substances.

WO 2021/061000 A1

Appendix F: First Author Publication
F1: Antioxidants

antioxidants

MDPI

Review

Vitreous Antioxidants, Degeneration, and Vitreo-Retinopathy: Exploring the Links

Emmanuel Ankamah [1,2,*], J. Sebag [3], Eugene Ng [1,2] and John M. Nolan [1,*]

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check for updates

Abstract: The transparent vitreous body, which occupies about 80% of the eye's volume, is laden with numerous enzymatic and non-enzymatic antioxidants that could protect the eye from oxidative stress and disease. Aging is associated with degeneration of vitreous structure as well as a reduction in its antioxidant capacity. A growing body of evidence suggests these age-related changes may be the precursor of numerous oxidative stress-induced vitreo-retinopathies, including vision degrading myodesopsia, the clinically significant entoptic phenomena that can result from advanced vitreous degeneration. Adequate intravitreal antioxidant levels may be protective against vitreous degeneration, possibly preventing and even improving vision degrading myodesopsia as well as mitigating various other vitreo-retinopathies. The present article is, therefore, a review of the different antioxidant molecules within vitreous and the inter-relationships between vitreous antioxidant capacity and degeneration.

Keywords: antioxidants; vitreous; oxidative stress; vitreous degeneration; floaters; vision degrading myodesopsia

1. Introduction

Ocular Antioxidants—Protection against Oxidative Damage and Disease

Vision relies on the coordinated roles played by various structures of the visual system, from the tear film on the ocular surface to the visual centers within the brain. Visual perception commences with sensory information organization, the process by which the highly specialized neurosensory retina of the eye captures photons from the environment and converts them into neural signals for visual processing and transmission to the higher visual centers within the brain [1]. Concurrently, the eye is exposed to exogenous, potentially injury-precipitating factors including visible light, ultraviolet light, ionizing radiation, and environmental toxins; as well as endogenous stress-inducing influences generated by the mitochondria within ocular tissues during the eye's physiological functions [2]. These endogenous and exogenous oxidants produce unstable reactive oxygen species (ROS) characterized by one or two unpaired electrons within their external orbit [3].

While normal concentrations of ROS are a physiological response to stress and are an integral part of normal ocular metabolic activity, excess levels could be debilitating to the eye [4]. To remain functional, the eye is replete with an assortment of antioxidants (substances that, when present in low concentrations compared to that of an oxidizable substrate, significantly delay or inhibit the oxidation of the substrate) by which it mitigates the damaging effects of ROS [5]. Over-production or inadequate elimination of ROS beyond the counteracting ability of the eye's antioxidant system can cause ocular

INTERNAL DRAINAGE OF SUBRETINAL FLUID DURING CHANDELIER-ASSISTED SCLERAL BUCKLING

Emmanuel Ankamah, OD,*† Martin J. Siemerink, MD,‡ Philip J. Polkinghorne, MD,‡ John M. Nolan, PhD,† Eugene Ng, MD*†

Purpose: To describe the surgical technique of internal drainage of subretinal fluid as an adjunct to chandelier-assisted scleral buckling for the repair of rhegmatogenous retinal detachment.

Methods: The technique of internal drainage with a sharp needle or cannula through a trocar is described and shown in a Supplemental Digital Content 1 (see Video, http://links.lww.com/ICB/A87).

Results: Three patients (3 eyes) underwent scleral buckling for rhegmatogenous retinal detachment repair. Subretinal fluid was drained using the internal drainage approach in all cases. All three patients had successful reattachment of retina with improvement in visual function. No complications were reported related to vitreous loss, retinal incarceration, or redetachment following primary surgery.

Conclusion: Internal drainage of subretinal fluid during chandelier-assisted scleral buckling is a useful technique that can be considered for repairing rhegmatogenous retinal detachment.

RETINAL CASES & BRIEF REPORTS 00:1–4, 2019

*From the *Institute of Eye Surgery, UPMC Whitfield, Butlerstown Co, Waterford, Ireland; †Nutrition Research Centre Ireland, School of Health Science, Waterford Institute of Technology, West Campus, Carriganore House, Waterford, Ireland; and ‡Department of Ophthalmology, University of Auckland, Auckland, New Zealand.*

Despite the advent of vitrectomy and advances of surgical instrumentation, scleral buckling remains a successful technique to manage rhegmatogenous retinal detachment. The characteristics of retinal detachments most suited for scleral buckling differ from that of vitrectomy. Young (phakic) patients with detachments from inferior breaks without posterior vitreous detachment and dialysis detachments are the most compelling cases to treat with a buckle. Unlike vitrectomy, scleral buckling does not interfere with a patient's lens and vitreous detachment status. A vitrectomy set up is also associated with a higher cost because it includes a vitrector and associated infusion lines connected to a vitrectomy machine's cassette.[1]

Scleral buckling surgery aims to reestablish anatomical adhesion of the neurosensory retina to the retinal pigment epithelium by indenting the sclera at the site of the primary break, through accurate placement of a silicon or sponge explant. This reduces the ocular circumference, reducing traction and preventing further subretinal fluid (SRF) accumulation. Using chandelier endoillumination and a wide-angle visualization system during the procedure improves visualization of the peripheral retina during training, localization of retinal tears, and positioning of the scleral buckle, thereby possibly reducing the rate of retinal redetachment.[2]

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Impact of symptomatic vitreous degeneration on photopic and mesopic contrast thresholds

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ABSTRACT

Clinical relevance: Contrast thresholds under photopic and mesopic luminance conditions are compromised in subjects with vitreous degeneration. A plausible explanation is needed for the visual discomfort expressed by patients suffering from symptomatic vitreous degeneration.

Background: The current study investigates the effect of symptomatic vitreous degeneration on photopic and mesopic contrast at high spatial frequencies.

Methods: An age-matched sample of 115 subjects, comprising 30 subjects with symptomatic vitreous floaters (cases) and 85 healthy subjects (controls), was included in this study. Visual acuity and flicker thresholds were measured for all participants. Photopic and mesopic functional contrast thresholds at 10 cycles per degree were measured for all participants to assess the effect of floaters on contrast. Further, to determine the effect of posterior vitreous detachment on contrast, the sample was divided into three groups: cases with posterior vitreous detachment (n = 12); cases without posterior vitreous detachment (n = 18); and controls (n = 85), and their contrast thresholds were compared.

Results: Photopic and mesopic contrast thresholds were lower by 37.4% and 27.5%, respectively, when the cases were compared with the controls ($p = 0.028$ and $p < 0.001$ for photopic and mesopic contrast thresholds, respectively). Photopic contrast was lower by 64.0% in cases with posterior vitreous detachment compared with controls ($p = 0.001$). Compared with controls, mesopic contrast was lower in cases with posterior vitreous detachment and in cases without posterior vitreous detachment by 30.3% and 25.6%, respectively ($p = 0.014$ and $p = 0.017$ for cases with and without posterior vitreous detachment, respectively).

Conclusion: : Subjects with vitreous degeneration have diminished photopic and mesopic contrast thresholds compared with controls. This finding highlights the negative impact of vitreous degeneration on the quality of vision.

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Contrast sensitivity; floaters; Posterior vitreous detachment; Visual function; vitreous

Introduction

The ultrastructure of the human vitreous gel is composed mainly of water, collagen and hyaluronan. Ageing and disease cause this fine structure to destabilise and even disintegrate.[1] The disintegration of the vitreous gel results in the formation of aggregated collagen bundles and liquid pools of vitreous, culminating in an entoptic phenomenon termed vitreous floaters.[2] Sufferers of symptomatic floaters attend eye clinics with characteristic presentations of the perception of dark grey spots, amorphous clouds, and moving objects within their central visual field.[3]

Further degeneration of the vitreous gel results in the weakening of the vitreoretinal adhesions and the separation of the posterior vitreous cortex from the inner limiting membrane of the retina, at the vitreoretinal interface, a phenomenon referred to as posterior vitreous detachment (PVD).[4] PVD has been described as the principal underlying phenomenon for the sudden onset of primary floaters (that is, vitreous opacities that arise from structures endogenous to the vitreous body).[2] That notwithstanding, primary floaters can occur asynchronously from PVD, especially when sufferers are myopic.

High contrast visual acuity (VA) tests provide an effective way of assessing spatial vision in a clinical setting. The task of the subject in a conventional VA test is to correctly name small letters that are close to 100% contrast and have spatial features that approach the resolving power of the eye.[5] The difficulty of the task varies across letters and subjects often achieve VA values within 'normal limits', even when the contrast of the retinal image is lowered as a result of aberrations and scattered light.

Contrast thresholds for either spatially periodic patterns or single optotypes such as Landolt rings can provide a sensitive measure of spatial vision that can be used to detect changes in retinal image contrast even when individual observers manage to achieve VA values within the normal range.[6,7] The size of the optotype employed is usually fixed at three times the mean acuity limit of 5 min arc (6/6) and the reciprocal of the contrast threshold needed to resolve the gap is usually described as functional contrast sensitivity (FCS).[8] When a contrast test is conducted in conjunction with a conventional VA test, the combined results provide a more informative assessment of spatial vision.[9]

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Clinical Trials

Dietary Intervention With a Targeted Micronutrient Formulation Reduces the Visual Discomfort Associated With Vitreous Degeneration

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Purpose: To investigate the impact of supplementation with a targeted micronutrient formulation on the visual discomfort associated with vitreous degeneration.

Methods: In this clinical trial, 61 patients with symptomatic vitreous floaters were randomized to consume daily, the active supplement consisting of 125 mg L-lysine, 40 mg vitamin C, 26.3 mg *Vitis vinifera* extract, 5 mg zinc, and 100 mg *Citrus aurantium* or placebo for 6 months. Change in visual discomfort from floaters, assessed with the Floater Disturbance Questionnaire, was the primary outcome measure. Secondary outcome measures included best-corrected visual acuity, letter contrast sensitivity, photopic functional contrast sensitivity with positive and negative contrast polarity, and quantitative vitreous opacity areas.

Results: After supplementation, the active group reported a significant decrease in their visual discomfort from floaters ($P < 0.001$), whereas the placebo group had no significant change in their visual discomfort ($P = 0.416$). At 6 months, there was a significant decrease in vitreous opacity areas in the active group ($P < 0.001$) and an insignificant increase in vitreous opacity areas in the placebo group ($P = 0.081$). Also, there was a significant improvement in photopic functional contrast sensitivity with positive contrast polarity in the active group after supplementation ($P = 0.047$).

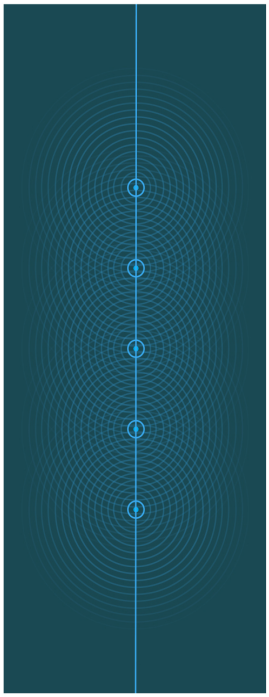
Conclusions: The findings of this study indicate improvements in vision-related quality of life and visual function of patients suffering from vitreous floaters after supplementation with a formulation of antioxidative and antiglycation micronutrients. Notably, these improvements were confirmed by the decrease in vitreous opacity areas in the active group.

Translational Relevance: This targeted dietary intervention should be considered to support patients with symptomatic vitreous degeneration.

Introduction

Vitreous fills the posterior segment of the eye and contributes to optical transparency. Degeneration of this exquisite gel is, nonetheless, ubiquitous during life, mainly resulting from aging or disease. Two principal and inter-related processes, liquefaction (synchisis senilis) and posterior vitreous detachment (PVD), account for vitreous degeneration.[1] Oxidative stress, increased intravitreal proteolytic enzymes, and a decrease in vitreous antioxidant capacity have been proposed as the underlying mechanisms for these degenerative processes.[2–4] Aging aside, high myopia, menopause, and hereditary extracellular matrix syndromes such as Stickler syndrome and

Appendix G: Certificates



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2nd October 2020

University College Cork

Ruben E. Keane

Ruben E. Keane

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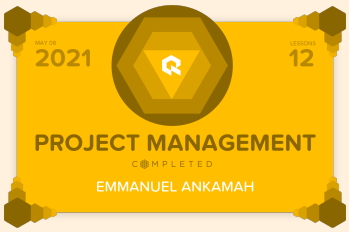

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