

**Evaluation of Outer Retinal Layers
Changes Before and After Intervention in
Diabetic Macular Edema**

Thesis

*Submitted For Partial Fulfillment of MSc.
Degree In Ophthalmology*

By

Radwa Reda Mohamed

M.B.B.Ch

Faculty Of Medicine Cairo University

Supervised By

Prof.Dr. Sherif Zaky Mansour

Professor of Ophthalmology, Ain Shams University

Prof.Dr. Thanaa Helmy Mohamed

Professor of Ophthalmology, Ain Shams University

Dr. Samah Mahmoud Fawzy

Lecturer of Ophthalmology, Ain Shams University

Faculty of Medicine,

Ain Shams University

2018

Cairo-Egypt

Acknowledgement

*First thanks to **ALLAH** to whom I relate any success
in achieving any work in my life.*

*I am forever grateful to **My parents and my husband** for their
ever after love and support.*

*Special thanks are due to **Dr. Sherif Zaky Mansour**
Professor of Ophthalmology, Ain Shams University for his sincere
efforts and fruitful encouragement.*

*I am deeply thankful to **Dr. Thanaa Helmy Mohamed**
Professor of Ophthalmology, Ain Shams University for her help,
support and guidance.*

*I am deeply thankful to **Dr. Samah Mahmoud Fawzy**
Lecturer of Ophthalmology, Ain Shams University for her great help,
active participation, great support and encouragement.*

List of Contents

Title	Page No.
List of Tables	i
List of Figures	ii
List of Abbreviations	iii
Introduction	1
Aim of the Work	3
<u>Review of Literature</u>	
▪ Chapter (1): Prevalence and Pathogenesis of DME	4
▪ Chapter (2): OCT and DME	10
▪ Chapter (3): Argon Laser and Ranibizumab in DME	16
Patients and Methods	20
Results	27
Discussion	42
Conclusion	48
Summary	50
References	52
Arabic Summary	-

List of Tables

Table No.	Title	Page No.
Table (1):	Demographic data of the patients.....	27
Table (2):	Values of CMV in Lucentis and Argon group.....	28
Table (3):	Intra-group comparison of repeated measure ANOVA test of CMV in Lucentis group.....	30
Table (4):	Intra-group comparison of repeated measure ANOVA test of CMV in Argon group.....	30
Table (5):	Mean CMV improvement in the whole follow up period in Lucentis and Argon groups.....	31
Table (6):	Values of CMT in Lucentis and Argon group.....	32
Table (7):	Intra-group comparison in Lucentis group.....	33
Table (8):	Intra-group comparison in Argon group.....	34
Table (9):	Mean CMT improvement in the whole follow up period in Lucentis and Argon groups.....	34
Table (10):	BCVA in Lucentis versus Argon laser groups.....	36
Table (11):	Inter-group comparison of outer layers changes before and after intervention.....	37
Table (12):	BCVA at baseline and after 6 months in Lucentis group.....	39
Table (13):	BCVA at baseline and after 6 months in Argon group.....	40

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Hyperglycemia and its metabolic pathways.....	9
Figure (2):	Normal macular structure – SD OCT representation of retinal layers	13
Figure (3):	Spectral- domain optical coherence tomography that demonstrates the diffuse retinal thickening pattern by the loss of the foveal depression.....	14
Figure (4):	Spectral-domainoptical coherence tomography is emblematic of the cystoid macular edema pattern	14
Figure (5):	Spectral-domain optical coherence tomography that demonstrates the serous retinal detachment pattern	15
Figure (6):	Spectral-domain optical coherence tomography that demonstrates the vitreomacular interface abnormality pattern.....	15
Figure (7):	Optical coherence tomography.....	22
Figure 8(a & b):	Showing cmt and cmv obtained automatically by OCT.....	23
Figure (9):	Photoreceptor is intact (+)	24
Figure (10):	Photoreceptor is interrupted (+,-)	24
Figure (11):	Photoreceptor is absent (-)	24
Figure (12):	CMV in Lucentis versus Argon laser groups	29
Figure (13):	CMT in Lucentis versus Argon laser groups	32
Figure (14):	BCVA in Lucentis versus Argon laser groups	36

List of Abbreviations

Abb.	Meaning
ALP.....	Argon laser photocoagulation
ANOVA.....	Analysis of Variance
BCVA	Best corrected visual acuity
BRB	Blood retinal barrier
CMT	Central macular thickness
CMV	Central macular volume
DCC.....	Diabetes control and complications trial
DME	Diabetic macular edema
DR.....	Diabetic retinopathy
ELM.....	External limiting membrane
ERG	Electroretinography
ETDRS	Early treatment diabetic retinopathy study
FDA	Food and drug administration
GCL.....	Ganglion cell layer
ILs.....	Interleukins
INL	Inner nuclear layer
IOP.....	Intraocular pressure
IPL	Inner plexiform layer
IS/OS	Inner segment outer segment junction
IVTA.....	Intra vitreal triamcinolone
LogMAR.....	Logarithm of the minimum angle of resolution
MMPs	Matrix metalloproteinases
ME.....	Macular Edema
MEM.....	Meropenem

List of Abbreviations

Abb.	Meaning
MHB.....	Muller-Hinton broth
MIC.....	Minimal inhibitory concentration
MLST	Multilocus sequence typing
MRSA.....	Methicillin-resistant Staphylococcus aureus
N	Number
NC	Negative control
NFL.....	Nerve fiber layer
NR.....	Neurosensory retina
OMPs.....	Outer membrane proteins
ONL.....	Outer nuclear layer
OPL.....	Outer plexiform layer
OXA	Oxacillinases
PBPs	Penicillin-binding proteins
PC	Positive control
PCR.....	Polymerase chain reaction
PDRAB.....	Pan-drug resistant Acinetobacter.
PDRGNs	Pan-drug resistant gram negatives
PKC	Protein kinase c
RPE.....	Retinal pigment epithelium
SD OCT	Spectral domain optical coherence tomography
SRD	Serous retinal detachment
TD OCT.....	Time domain optical coherence tomography
TNF.....	Tumor necrosis factor
VA.	Visual acuity
VEGF.....	Vascular endothelial growth factor
VMIA	Vitreomacular interface abnormality
VTDR	Vision threatening diabetic retinopathy

ABSTRACT

Aim of the work: this study aimed to evaluate changes in photoreceptors, inner segment/outer segment (IS/OS) junction and external limiting membrane (ELM) following grid argon laser versus intravitreal injection of antivgef (Ranibizuma) in diabetic type2 patients affected by clinically significant macular edema.

Methods: forty eyes of diabetic type2 patients who were affected by clinically significant macular edema were randomly categorized into two groups (Group treated by argon grid laser and group treated by intravitreal injection of antivgef for the first time) both groups were investigated for best corrected visual acuity (BCVA) and the integrity of the photoreceptors, IS/OS and ELM as being (Continuous(+), interrupted(±) or Absent (-)) by spectral-domain optical coherence tomography (SD-OCT)(CIRRUS HD-OCT) before treatment, month, 2months and 3months after treatment . Other variables, included central macular thickness (CMT) and central macular volume (CMV) were evaluated.

Results: significant difference was found between [photoreceptor, is/os] (+), photoreceptor, is/os (+,-) and photoreceptor, is/os (-) groups in BCVA at baseline; it was 0.61,0.73,and 1 and after 6 months it was 0.48,0.73 and 1 . Results were similar for ELM groups. Signiant difference was found between ELM (+), ELM (+,-) and ELM (-) groups in BCVA at baseline it was 0.59,0.72,and 1 and after 6 months it was 0.46,0.68 and 1 in lucentis group and signiant difference was found between photoreceptor, is/os (+) and photoreceptor ,is/os (+,-) groups in BCVA at baseline was(0.32and 0.3 and after 6 months it was 0.27and 0.3. Results were similar for ELM groups. Signiant difference was found between ELM (+) and ELM (+,-) groups in BCVA at baseline it was 0.29 and 0.5 and after 6 months it was 0.25 and 0.4 in argon laser group

Conclusion: the more damage of outer retinal layers at the time of DME the lower visual acuity following DME resolution and the presence of intact of these layers is an important factor in determining final visual acuity.

Keywords: diabetic macular edema, external limiting membrane, inner segment-outer segment junction, integrity of photoreceptor layer, optical coherence tomography.

Abbreviations:

BCVA: Best corrected visual acuity, **CMT:** Central macular thickness, **CMV:** Central macular volume, **CSME:** Clinically significant macular edema, **DR:** Diabetic retinopathy, **ELM:** External limiting membrane, **ETDRS:** Early treatment diabetic retinopathy study, **IS/OS:** Inner segment outer segment, **ONL:** Outer nuclear layer, **SDOCT:** Spectral domain optical coherence tomography, **VEGF:** Vascular endothelial growth factor.

INTRODUCTION

Diabetic retinopathy (DR) and diabetic macular edema (DME) are the major causes of loss of vision and blindness in diabetic patients. The pathogenesis of DME has not been fully explained since it is caused by complex pathological process with many contributing factors. Dysfunction of the inner and outer retinal barriers leads to accumulation of sub- and intra-retinal fluid in the inner- and outer-plexiform layers. Vascular endothelial growth factor (VEGF) has generally been accepted as the main factor that disrupts the inner blood-retinal barrier (BRB) function, making it an important target for pharmaceutical intervention.^(1,2)

Because of the various clinical presentations of DME, specialized techniques such as spectral-domain optical coherence tomography (SD OCT) have become an important tool and an integral part of the diagnosis and management of this condition. With the improved visualization of retinal architecture offered by OCT, many clinical studies have used this imaging technique to evaluate the effectiveness of various treatment methods and subsequent resolution of macular edema.⁽³⁾

A past study by the Diabetic Retinopathy Clinical Research Network showed only a simple relationship between measured central retinal thickness and visual acuity in patients with DME. The aforesaid observations hint that visual acuity

likely is multifactorial and may be related to damage or disruption of the retinal architecture or direct photoreceptor damage. ⁽⁴⁾

During the past decade, a number of additional pharmacological treatments of DME have been proposed, such as intravitreal injections of anti-vascular endothelial growth factor agents. ⁽⁵⁾

This review outlines treatment strategies, with particular emphasis on the optimal window for a variety of therapeutic DME interventions.

A large body of clinical data has confirmed that early detection and treatment of DME is an effective strategy to prevent vision loss. Effective systemic interventions for DME include control of blood glucose, blood pressure and dyslipidemia. Ophthalmic treatments are laser photocoagulation, surgery and intraocular pharmacotherapy. ⁽⁶⁾

AIM OF THE WORK

To evaluate changes of photoreceptors, inner segment/outer segment (IS/OS) junction and external limiting membrane (ELM) following grid argon laser versus intravitreal injection of anti-VEGF (Ranibizumab) in diabetic (type2) patients affected by clinically significant diabetic macular edema.

Chapter 1

PREVALENCE AND PATHOGENESIS OF DME

DME is the major cause of vision loss associated with DR. There are approximately 93 million people with DR, 17 million with proliferative DR, 21 million with DME and 28 million with vision threatening diabetic retinopathy (VTDR), the overall prevalence of DME is 6.81% (6.74–6.89). According to studies of the natural history of DME, 24% of eyes with DME will lose at least three lines of vision within 3 years. The Diabetes Control and Complications Trial (DCCT) group reported that the incidence of DME in type I diabetes patients with a 9-year diabetic history was 27%. Around 5% of type II diabetes patients had DME when diabetes was diagnosed, gradually increasing to 30% within 25–30 years.⁽⁷⁻¹⁰⁾

Angiogenesis and inflammation have been shown to be involved in the pathogenesis of this disease, but it still needs to be clarified whether angiogenesis following an overexpression of VEGF is a cause or a consequence of inflammation. The retinal macular zone is the target of diabetic macular edema (DME). In order to understand the anatomic changes in DME.⁽¹¹⁾

Retinal anatomy was described with reference to optical coherence tomography (OCT), a technique used currently in the diagnosis and follow-up of DME, which allows us to see the multiple layers of the retina and choroid.⁽¹¹⁾

Retinal Anatomy: The retina is a complex structure of neural tissue made up of different cell types. The retina is divided into the *neurosensory retina (NR)* and *the retinal pigment epithelium (RPE)*. The NR includes all layers from the photoreceptors to ganglion cells, and the RPE is a monolayer formed by a single cell type located in the outermost part of the retina. ⁽¹¹⁾

Non sensory Layer of the Retina

- The *retinal pigment epithelium (RPE)* is a monolayer of cells characterized by a large presence of melanin pigment in the cytoplasm. This allows it to absorb light, which then reaches the retina. RPE is a multifunctional layer. The apical cell of the RPE is closely linked to the photoreceptors, which form a true functional unit. ⁽¹¹⁾

In the other side, the RPE forms a complex with Bruch's membrane seen at optical coherence tomography (OCT) denominated as RPE/Bruch's complex. ⁽¹¹⁾

Neurosensory Retinal Layers

- The *photoreceptor layer* is formed by rods and cones in its internal and outer segments (IS/OS) and includes two layers observable with OCT, both of which are important in visual acuity impairment:
 - i- *Ellipsoid Zone*. This is located just above the RPE and is formed by the union of the IS/OS of the photoreceptors. In

the OCT, the sagittal slice of the retina is shown as a hyper reflective line. In several functional studies of the macula, a link has been found between the integrity of this layer and resulting visual acuity.

- ii- *External Limiting Membrane Layer (ELM)*. This is seen as a discrete line, located just above the ellipsoid zone, which separates the photoreceptor nucleus from its internal segments. It comprises the apical processes of Muller cells and is similar to the ellipsoid zone there is a link between the integrity of this layer and visual acuity.
- The *outer nuclear layer (ONL)* is formed by the cell nuclei of rods and cones. The cones are responsible for visual acuity and colour perception.
- The *outer plexiform layer (OPL)* is established by the synapses of bipolar cells between photoreceptors. In addition, it includes horizontal interneuron cells that adjust vision in extreme environmental light conditions.
- The *inner nuclear layer (INL)* contains the nuclei of bipolar cells. Bipolar cells are the first-neuron cell to process the electrical stimulus coming from the photoreceptors before transmitting it to the ganglion cells. These cells are responsible for the electrical response of the retina as objectified in multifocal electroretinography.
- The *inner plexiform layer (IPL)* is composed of synapses between the bipolar, ganglion, and amacrine cells (responsible for adjusting the retinal image).

- The *ganglion cell layer (GCL)* contains ganglion cells which are the second-neuron cell in visual via and comprises cells that transmit impulses from the photoreceptors through their long axons to the thalamus.
- The *nerve fiber layer (NFL)* is formed by the ganglion cell axons.⁽¹¹⁾

Most pathways lead to increased oxidative stress, inflammation, and vascular dysfunction. Oxidative stress and inflammation result in upregulation of growth factors and cytokines, such as VEGF, angiopoietins, tumor necrosis factor (TNF), interleukins (ILs), and matrix metalloproteinases (MMPs), contribute to breakdown of the BRB and development of DME.⁽¹²⁾

The BRB maintains the tight regulation of the fluid electrolyte balance in the retina. The breakdown of this barrier results in fluid accumulation in the layers of the retina, as seen in DME.⁽¹²⁾

Abnormal capillary permeability results in the leaking of fluid and solutes into the surrounding retinal tissue, which collects around the macula, this is referred to as macular oedema (ME) and it threatens visual acuity.⁽¹³⁾

Breakdown of the outer, especially the inner retinal blood barrier is an early event in the pathogenesis of DME.