"ROLE OF SERUM AND AQUEOUS HUMOR MAGNESIUM LEVELS AS A MARKER IN ASSESSMENT OF DIABETIC RETINOPATHY IN TYPE 2 DIABETIC AND NON DIABETIC PATIENTS."

By

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Under the guidance of

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The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the Synopsis entitled **"Role of Serum and Aqueous Humor Magnesium Levels as a Marker in Assessment of Diabetic Retinopathy in Type 2 Diabetic and Non Diabetic Patients"** being investigated by Dr.AASTHA GARG & Dr. H Mohan Kumar[1] in the Department of Ophthalmology at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study**.

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LIST OF ABBREVIATIONS

SL NO.	ABBREVIATION	FULL FORM
1.	DM	Diabetes Mellitus
2.	GFAP	Glial Fibrillary Acidic Protein
3.	AQP	Aquaporin
4.	WHO	World Health Organization
5.	cAMP	Cyclic Adenosine Monophosphate
6.	ATP	Adenosine triphosphate
7.	ADP	Adenosine diphosphate
8.	NPDR	Non proliferative diabetic retinopathy
9.	PDR	Proliferative diabetic retinopathy
10.	IL	Interleukin
11.	ETDR	Early Treatment Diabetic Retinopathy
12.	IRMA	Intra retinal microaneurysm
13.	NVD	Neovascularization at the disc
14.	NVE	Neovascularization elsewhere
15.	CSME	Clinically Significant Macular Edema
16.	OCT	Optical Coherence Tomography
17.	AGE	Advanced Glycation end products
18.	VEGF	Vascular endothelial growth factor
19.	AH	Aqueous humor
20.	CA	Carbonic anhydrase
21.	PE	Pigmented epithelium
22.	NPE	Non pigmented epithelium
23.	Mg^{2+}	Magnesium

ABSTRACT

Background:

The burden of Type 2 Diabetes Mellitus related morbidity is increasing worldwide. Longer duration of the disease is associated with greater complications as well. Diabetic retinopathy is a microvascular complication of diabetes which is a significant cause of visual impairment. Various etiologies have been postulated for the same. Serum hypomagnesemia is one such cause of increased progression of diabetic retinopathy changes. This novel study aims to establish a relationship of aqueous humor magnesium concentration and its use as biomarker for the assessment of diabetic retinopathy changes.

Methods:

This case control study recruited a total of 54 patients fulfilling the inclusion criteria from December 2019 to May 2021. Group A consisted of diabetic cases with senile cataract and Group B included non-diabetic controls with senile cataract. All patients underwent standard cataract and fundus evaluation. HbA1c levels and serum magnesium levels were assessed in the blood samples. Intraoperatively, aqueous humor sample was aspirated and tested for magnesium levels. All the samples were analysed at Central Diagnostic Laboratory Services (CDLS), a unit of R.L. Jalappa Hospital & Research Centre attached to Sri Devaraj Urs Medical College, Kolar.

Results:

The mean serum magnesium concentration in cases and controls were 1.70 mg/dL and 1.90 mg/dL respectively. The mean aqueous humor magnesium levels among cases and controls were 1.70 mg/dL and 1.80 mg/dL respectively. The mean HbA1c concentration among the diabetics and non-diabetics controls was 7.5% and 5.70% respectively. Observed values were

statistically significant with a p value of <0.05. Majority of the diabetic cases with HbA1c >7% had serum and aqueous humor magnesium in the range of 1.5-1.7 mg/dL. Mild non proliferative diabetic retinopathy (NPDR) was the most common retinopathy change observed. The mean serum and aqueous humor magnesium among these mild NPDR cases was 1.7 mg/dL and 1.67 mg/dL. A strong positive, statistically significant correlation was observed between serum and aqueous humor magnesium.

Conclusion:

Hypomagnesemia is associated with diabetes and associated retinopathy changes. Aqueous humor magnesium level can be used as a novel biomarker to assess diabetic retinopathy changes.

Key Words: Diabetes mellitus, aqueous humor, diabetic retinopathy, HbA1c, serum magnesium, aqueous humor magnesium

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INTRODUCTION

INTRODUCTION

Diabetes is identified as one of the four priority based non-communicable diseases around the world for targeted action.[1] The burden of diabetes specially among type 2 diabetic patients has vastly increased over the past two decades in India with an estimated burden of 1.3 billion people suffering with it.[2]

In recent years it has been observed that the occurrence of diabetes in younger adults is increasing which is due to early onset of disease. This early onset increases the chance of development of complications of diabetes mellitus due to longer extent of the disease. Diabetes control is found to worsen with longer duration of the disease causing development of neuropathic complications in 24.6%, cardiovascular complications in 23.6%, renal issues in 21.1%, retinopathy in 16.6% and foot ulcers in 5.5% of diabetic patients.[3] Majority of diabetic patients in India have poor glycaemic control which causes micro and macrovascular changes leading to greater percentage of them developing diabetic complications like myonecrosis and muscle infarction.[4,5]

The burden of the disease in rural areas of India is lesser than that in urban areas, but it is slowly increasing in rural areas as well.[6] Type 2 diabetes mellitus epidemic is firmly established in India. The main concern is the burden of type 2 diabetes gaining entry into rural low income households who cannot afford continuous monitoring and treatment.[7]

The occurrence of retinopathy associated with diabetes, as one of the serious complications of type 2 diabetes resulting in irreversible visual loss if not treated early is increasing in India. The chances of development of diabetic retinopathy increases with increased diabetes duration, ineffective glucose control, increased blood lipid levels and blood pressure.[8] Diabetic retinopathy remains asymptomatic in most of the cases until development of

complications like vitreous haemorrhage, tractional retinal detachment or diabetic macular edema.[9] Systematic screening, early diagnosis and timely treatment has reduced the burden of diabetes associated blindness in the developed countries.[10] Hypomagnesemia caused by poor glycaemic control leads to development of diabetic complications like diabetic retinopathy. Magnesium deficiency has been implicated in insulin resistance, carbohydrate intolerance, dyslipidaemia, and complications of diabetes.[11]

Diabetic retinopathy has multifactorial pathophysiology including all retinal components. Hyperglycaemia is found to cause neuronal cell degeneration, glial cell dysfunction and microvascular damage of retina.[12] Muller cells are the main retinal glial cells. They are the neuronal structural support cells of retina. They regulate neuronal nutrition, development and metabolism by acting as connective elements between neurons and vascular cells. The Muller cells are key elements in onset and progression of diabetic retinopathy. Aquaporins (AQP1 and AQP4) are low molecular weight integral membrane proteins that help in maintaining ion and osmotic equilibrium of neuroretina by facilitating bidirectional flow of free water and modulating neuronal excitability. Muller cells are found to react to hyperglycaemia by three non-specific responses, one of which results in increase of glial fibrillary acidic protein (GFAP) levels.[13]

Aqueous humor sampling has been performed previously in rare instances to understand the electrolyte and protein concentration of aqueous humor affecting the pathology of various diseases. A study done previously studied the human aqueous humor for the quantification of GFAP, AQP1 and AQP4 and their use as a biomarker for identification of early Muller cell activation which in turn indicates onset of diabetic retinopathy.[13]

Aqueous humor sampling has also been performed in glaucoma patients to get an insight into the various electrolytes affecting and predicting the prognosis of this condition.[14]

Diabetic retinopathy is a chronic disease with a long latent phase. 67% of type 2 diabetes patients develop diabetic retinopathy after 10 years and 10% among them will have proliferative diabetic retinopathy.[15] Effective screening can help avoid diabetic retinopathy associated blindness in 90% of the patients, thus decreasing the cost associated with disability loss.[16,17]

Studies done previously show that there is an inverse relationship between serum magnesium and diabetic retinopathy changes. In a study by Kumar P et al association of serum magnesium with Type 2 Diabetes mellitus and diabetic retinopathy was evaluated and it was reported that magnesium deficiency is associated with increased risk of diabetic retinopathy.[18] In another study by Kundu, D et al it was seen that hypomagnesemia and albuminuria individually or in conjunction, serve as indicators for dysglycemia and could be used as a marker for the risk of development of diabetic retinopathy.[19]

However, role of aqueous humor magnesium with retinopathy changes has not been established yet. Thus, we undertake this study to establish the role of aqueous humor magnesium as a biomarker for diabetic retinopathy.

OBJECTIVES

AIMS AND OBJECTVES OF STUDY

1. To estimate serum magnesium concentration in the study groups.
2. To estimate aqueous humor magnesium concentration in the study groups.
3. Correlation of serum and aqueous humor magnesium concentration in relation to severity of diabetic retinopathy.
4. Establish a relationship, if any, between aqueous humor and serum magnesium with HbA1c levels.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

DIABETES MELLITUS

Epidemiology of type 2 diabetes mellitus:

According to estimates of International Diabetes Federation there were 4.2 million diabetes related deaths in 2019 and 463 million adults were suffering from diabetes. This number is estimated to rise to 700 million people suffering, by the end of 2045. Type 2 diabetes mellitus is underdiagnosed, with one in three diabetic people remaining undiagnosed. Hence the true burden is much more than reported, since more than 80% of type 2 diabetes patients live in low and middle income countries.[20]

Half of the diabetic population of world is estimated to be from South-East Asian countries. Diabetes has reached an epidemic stage in China with 11.6% of the adults being confirmed diabetic cases and one-third of entire diabetic population being from this country. In India there are approximately 67 million cases of diabetes accounting to 8.6% of adult population.[21]

Classification of diabetes:

The following is the classification of diabetes given by WHO[22]

- Type 1 Diabetes Mellitus (T1DM): occurs frequently in childhood but onset can occur in adults and 84% of people living with T1DM are adults.
- Type 2 Diabetes Mellitus (T2DM): accounts for 90 to 95% of diabetic cases.

Hybrid forms of diabetes

- Slowly evolving immune-mediated diabetes of adults: This is slowly evolving form of diabetes resembling type 2 diabetes mellitus, but these patients have pancreatic autoantibodies reacting with non-specific cytoplasmic antigens in islet cells, glutamic

acid decarboxylase, protein tyrosine phosphatase, insulin or zinc transporter 8 (ZnT8).[23]

- Ketosis prone type 2 diabetes: This is an unusual form of diabetes presenting with ketosis and severe insulin deficiency. This goes into remission later and does not require insulin treatment. The restoration after insulin therapy causes prolonged improvement in beta cell insulin secretory function. [24]

Other specific types of diabetes:

- Monogenic diabetes: due to gene mutations.
- Monogenic defects of β-cell function: This form does not require insulin treatment and clinical manifestations include maturity onset diabetes of young (MODY), permanent neonatal diabetes mellitus (PNDM), transient neonatal diabetes (TND), and genetic syndromes. [25]
- Monogenic defects in insulin action: caused due to mutations in insulin receptors.[26]
- Diseases of the exocrine pancreas: Pancreatitis, trauma, infection, pancreatic cancer and pancreatectomy causing damage to pancreas result in this form of diabetes.[27]
- Endocrine disorders: This is caused by excess secretion of hormones antagonizing insulin action.[28]
- Drug or chemical-induced: Many drugs like corticosteroids, thiazide diuretics, beta blockers, statins etc. impair the insulin secretion or action and induce a risk for development of type 2 diabetes mellitus.[29]
- Infections: Some viruses like Coxsackie-B-virus cause beta cell destruction resulting in type 1 diabetes.[30]
- Uncommon specific forms of immune-mediated diabetes: This includes forms of diabetes associated with particular immunological diseases with different pathogenesis.[31]

- Unclassified diabetes: This category is used temporarily when there is no clear diagnostic category, especially close to the time of diagnosis of diabetes.[32]
- Hyperglycaemia first detected during pregnancy:
    - Diabetes mellitus in pregnancy
    - Gestational diabetes mellitus[32]

Indian Scenario:

The National Health policy of India 2017 aims to reduce premature diabetes related deaths by 25% by the year 2025.[33] The age adjusted mortality rates among diabetics is found to be 1.5 to 2.5% times higher than general population.[34]

WHO predicts that by 2030 majority of diabetics will be from India, China and United States of America. There are many factors attributing to increased prevalence of diabetes in India which include population growth, aging, industrialization, urbanization, changing food habits and sedentary lifestyle.[35] The prevalence of diabetes is estimated to double globally from 171 million in 2000 to 366 million in 2030 and India is predicted to be a major contributor to this increase.[36]

Diabetes being a chronic disorder causes continuous economic burden to individuals and to the country as a whole.[37] The susceptibility of diabetic individuals to development of associated complications increases the treatment cost posing enormous financial burden to affected families.[38] It is estimated that the cost of diabetes is 1.8 times higher for complicated non-hospitalized patients and 2.4 times higher for complicated hospitalized patients.[39]

People developing diabetes at an early age as is evident in Indian population have double risk of developing diabetic retinopathy.[40] The central obesity prevalent in Indians is found to increase the risk of development of diabetic retinopathy by two times. People having suboptimal or low glycaemic control, anaemia and early nephropathy all of which are found

to be more in Indian population, increase the risk of development of diabetic retinopathy.[41,42] It is estimated that in accordance with increased prevalence of type 2 diabetes in India, the prevalence of diabetic retinopathy would increase to 22.4 million in two decades.[43] The awareness of diabetic retinopathy is low in India attributing to low screening and diagnosis of the condition. Proliferative diabetic retinopathy is treatable when diagnosed early. [44]

Type 2 diabetes is the most common form of diabetes prevalent in India. There are 69.2 million type 2 diabetics in India making it the country with second highest number of people with diabetes following China.[45] Asia is found to be the epicentre for diabetic epidemics accounting for 60% of global diabetes burden.[46] It is observed that type 2 diabetes mellitus occurs in Asian Indian population at a younger age, in those with low BMI levels and the susceptibility to development of complications is also more in them.[47] The main reason for this is that the 'Asian Indian phenotype' is a collection of clinical biochemical features which are responsible for susceptibility of Asian Indian population to development of type 2 diabetes and its related complications.[48] In this phenotype though individuals have low BMI they have higher waist circumference, higher waist-hip ratios, more subcutaneous and visceral fat, more insulin resistance and higher prevalence of metabolic syndrome.[49,50] The presence of this phenotype may be due to specific genetic factors. It is established by studies that certain genetic factors predispose the Asian Indian population to type 2 diabetes mellitus. However, increased prevalence of this disease cannot be attributed to genetic factors alone because a major change in genetic makeup of the population cannot occur in a short span of time. Hence it is thought that environmental factors also contribute to the development and propagation of type 2 diabetic epidemic in Asia and India. Among these environmental factors, early life factors are considered to exert a greater influence on the development of type 2 diabetes.[50]

Maternal malnutrition which is widely prevalent in India, is accompanied by rapid weight gain in children after 2 years of age and increases the risk of development of impaired glucose tolerance.[51] Infants born to malnourished mothers have been found to have higher levels of adiposity in comparison to normal mothers. Maternal malnourishment is also found to be associated with diminished beta cell mass in pancreas in animals and its implication in humans is not yet established.[52]

Pathophysiology of Diabetes Mellitus:

Improper insulin action and insulin secretion due to β cell dysfunction are two main mechanisms leading to disturbance of glucose homeostasis.[53] Beta (β) cell dysfunction results in reduced secretion of insulin and insulin resistance caused by increased glucose production in liver and decreased glucose uptake. The two processes may occur simultaneously from early stage of disease, thus increasing hyperglycaemia and progression of disease.[54]

Reduced insulin secretion: Insulin is synthesized as pre-proinsulin by the β cells of pancreas which is converted to proinsulin by the proteins of endoplasmic reticulum.[55] Proinsulin moves from endoplasmic reticulum immature secretory vesicles of Golgi complex where it is broken down into c-peptide and insulin.[56] Insulin is stored in matured secretory granules until its release is triggered by high glucose concentration. Amino acids, fatty acids and hormones also trigger insulin release.[57] Glucose circulating in blood enters into β cells through glucose transporter-2 (GLUT-2) solute carrier protein. After glucose enters into the β cells, intracellular ATP/ADP ratio is increased closing ATP- dependant potassium channels in plasma membrane. This causes depolarization of the membrane and opening up of calcium channels which results in entry of calcium into the cells. Secretory granules with insulin fuse with plasma membrane on rise in intracellular calcium concentration leading in exocytosis of insulin.[58]

Cyclic AMP (cAMP) pathway and extracellular ATP also induce insulin secretion. cAMP pathway induces mobilization of insulin containing secretory vesicles by depleting intracellular calcium reservoirs and increasing intracellular calcium concentration.[59]

Beta (β) cell dysfunction: Excessive nutritional states including obesity, hyperglycaemia and hyperlipidemia cause inflammation, inflammatory stress, endoplasmic reticulum stress, metabolic/oxidative stress, amyloid stress of β cells due to differences in their genetic susceptibility. This will lead to loss of islet integrity.[60] Hyperglycaemia and increased levels of free fatty acids activate apoptotic unfolded protein response pathways which induce endoplasmic reticulum stress.[61] Sustained hyperglycaemia also increases proinsulin biosynthesis and islet amyloid polypeptides in β cells causing accumulation of misfolded insulin and increased production of oxidative protein folding, mediated reactive oxygen species. All these lead to islet inflammation by alteration of endoplasmic reticulum calcium mobilization, proapoptotic signals, proinsulin mRNA degradation and interleukin-1 β release. Disruption of islet integrity dysregulates insulin secretion and glucagon release leading to hyperglycaemia.[62]

Insulin resistance:

In this condition the action of insulin is not proportional to its concentration and is caused due to genetic and environmental factors. [63]

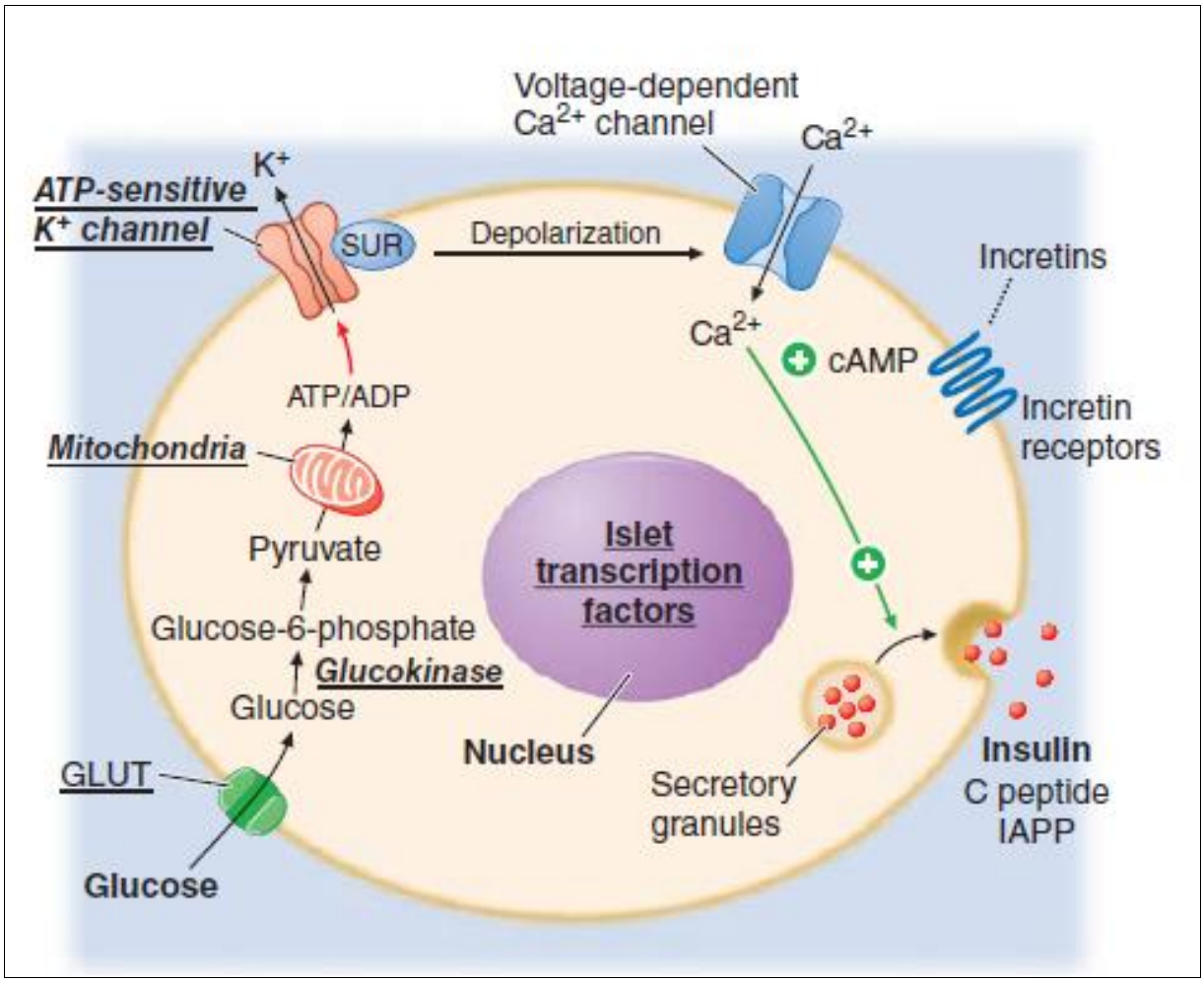


Figure 1: Mechanism of glucose stimulated insulin secretion and abnormalities in diabetes. (GLUT: glucose transporter)

Source: Harison's principles of internal medicine, Chapter: Endocrinology and Metabolism

Mutations in the proteins- ADP: adenosine diphosphate; ATP: adenosine triphosphate, cAMP: cyclic adenosine monophosphate, IAPP: islet amyloid polypeptide or amylin, SUR: Sulfonylurea receptors are the cause for monogenic form of diabetes.

Risk factors for type 2 diabetes:

Genetic, environmental and metabolic risk factors are interrelated and contribute to development of type 2 diabetes.[64]

1. Lifestyle factors: These are modifiable risk factors and include a sedentary lifestyle, physical inactivity, smoking, alcohol consumption, which contribute to risk of development of type 2 diabetes. Obesity is another risk factor which leads to development of insulin resistance and disease progression.[65-69]

2. Diet: This is also a modifiable risk factor. Low fibre and high fat diet affecting insulin resistance.[66]

3. Gut microbial dysbiosis: with decrease in butyrate producing bacteria and increase in opportunistic pathogens, the risk of type 2 diabetes mellitus increases.

4. Vitamin D deficiency: causes negative effects on glucose tolerance, insulin secretion either directly through vitamin D receptors. The indirect effect is through vitamin D receptors or through calcium hormones and inflammation leading to development of type 2 diabetes.[70]

Complications:

Type 2 diabetes leads to several micro and macrovascular complications. Macrovascular diseases include hypertension, hyperlipidaemia, heart attacks, coronary artery disease, strokes, cerebral vascular disease and peripheral vascular disease. Microvascular diseases include retinopathy, nephropathy and neuropathy.

Diabetic neuropathy: neuropathy causes loss of protective sensation of feet leading to callous formation, ulceration, infection of skin and bones of foot and gangrene.

Diabetic nephropathy: It is manifested as microalbuminuria and progression can be prevented with early diagnosis.[71]

Diabetic retinopathy: Chronic hyperglycaemia causes microvascular damage to retinal vessels leading edema and haemorrhage in retina or vitreous humor.[72]

Management of Diabetes Mellitus:

Dietary intake modifications and physical exercise in combination with adequate amount of sleep are considered as two basic treatment options for type 2 diabetes.[73]

Dietary modifications like limiting caloric intake, taking carbohydrates with low glycaemic index along with increase in dietary fibre will help to achieve and maintain desired blood glucose levels, blood pressure, lipid profile and weight.[74]

Dietary modifications also help in improving sleep, reducing depression and hence improve overall health related quality of life. Sugary drinks must be avoided.[75]

Physical exercise: increases insulin sensitivity in tissues which helps in improving glycaemic control. Individuals with type 2 diabetes can do both aerobic and resistance exercises.[76]

Pharmacological management: Metformin is used as a first-line therapy because of its glucose lowering efficacy, safety profile, weight neutrality and less cost.[77]

Incretin based therapies like injectable glucagon like peptide receptor agonists and dipeptidyl peptidase 4 inhibitors augment glucose dependent insulin secretion, decrease islet glucagon secretion, slow gastric emptying and increase satiety.[78]

Sodium glucose transporter 2 inhibitors decrease renal absorption of glucose, lower HbA1c levels but they have potential adverse effects like polyuria, diuresis, blood pressure lowering effect, weight loss, ketoacidosis and genital infections.[79]

Insulin therapy: Insulin therapy is required for patients with HbA1c levels greater than 9%. A low dose of long-acting insulin at bedtime is given initially with insulin titration up to 30 to 40 units is recommended as safe and effective regimen for insulin therapy.[80]

DIABETIC RETINOPATHY

Epidemiology:

Diabetic retinopathy is the most common microvascular complication of diabetes with 34.6% of global prevalence.[81] It is estimated that more than 60% of type 2 diabetic patients will have some form of diabetic retinopathy.[82] Diabetic retinopathy is ranked as fifth among most common cause of preventable blindness.[83] It was estimated in 2010 that among 285 million people with diabetes, one-third had signs of diabetic retinopathy and a third of these developed severe non-proliferative diabetic retinopathy or proliferative diabetic retinopathy. The prevalence of diabetic retinopathy is more in western countries than Asian countries. In United States of America about 28.5 to 40.3% of diabetic patients develop diabetic retinopathy whereas about 12.1% to 23% of diabetic patients develop diabetic retinopathy in Asia.[84,85] In Asian countries, India and China there is difference in prevalence of diabetic retinopathy between urban and rural areas. In India, diabetic retinopathy prevalence is 18% in urban areas and 10.8% in rural areas.[8, 40]

Risk factors:

Risk factors include non modifiable and modifiable factors

Non-modifiable factors are

- Duration of diabetes
- Puberty and pregnancy

Modifiable risk factors are

- Hyper glycaemia
- Hypertension
- Dyslipidaemia
- Obesity [86]

Pathophysiology:

Diabetic retinopathy (DR) is broadly classified into two stages- non-proliferative phase and proliferative phase. Non-proliferative diabetic retinopathy (NPDR) is usually asymptomatic, characterized by changes in retinal vessels. This stage, if untreated, will progress to proliferative diabetic retinopathy (PDR) which is characterized by proliferation of new vessels triggered by retinal ischaemia. These unstable blood vessels cause tissue alterations due to bleeding and leakage. This will result in fibrovascular epiretinal membranes, vitreous haemorrhage and retinal detachment. Macular edema is a frequent complication of diabetic retinopathy (DR) causing vision loss.[87]

DR is a complex multifactorial process causing increased oxidative stress, increase pro-inflammatory mediators and increased secretion of vascular endothelial growth factors brought about by various interrelated pathways. Diabetes in very early stages itself impairs normal regulatory mechanisms in the neurovascular complex of retina. The insulin receptors of retina stimulate neuronal development, growth, survival and anabolic synthesis apart from mediation of glucose transport. The rays of light passing also stimulate the insulin receptors present in rods and cones. The insulin receptors also mediate their survival. Thus, ganglion cells among the retinal neurons undergo early apoptosis of onset of diabetes.[88]

The various inter regulated pathways result in neurodegeneration, increased vascular permeability, vascular occlusion and dysregulated angiogenesis. Neurodegeneration attributes

to vision loss. Initial trigger of DR is hyperglycaemia. Subsequent progression of pathology is caused by neuronal toxins entering through damaged blood retinal barrier and ischaemic damage due to impaired blood supply. Ischaemia caused by vascular occlusion result in neuronal damage and death. Angiogenesis which is the defining character of PDR is caused by multitude of growth factors triggered by tissue non-perfusion.[89]

Oxidative stress of DR: Hyperglycaemia increases the flux of glucose through glycolytic and tricarboxylic acid pathways, thereby flooding the mitochondria with electrons and leading to increased production of reactive oxygen species (ROS). This increases the oxidative stress in the retinal capillary cells which become more vulnerable to oxidative damage and undergo accelerated apoptosis. [88]

Advanced Glycated End products (AGEs): are formed when the glucose molecules bind with amine residues on proteins, lipids, or nucleic acids. They then interact with the receptor for AGE (RAGE). This upregulates the AGE and glial fibrillary acidic protein (GFAP) in the Müller cells. Stimulation of Müller cell causes release of inflammatory cytokines such as VEGF and monocyte chemoattractant protein-1 (MCP-1), further causing retinopathy changes.[88]

Inflammation: Chronic low grade inflammation is found in different stages of DR. There is an increased adherence of leukocytes in retinal vasculature due to increased expression of intracellular adhesion molecule -1 (ICAM-1). Leukocyte-endothelium adhesion mediated by adhesion molecules is caused by leukostasis. Chemokines such as monocyte chemotactic protein-1, macrophage inflammatory protein (MIP)-1α and MIP- 1β are elevated in diabetic patients. Inflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukin(IL)-6, IL-8, IL-1β are upregulated and their expression is correlated with severity of diabetic retinopathy.[90] Retinal inflammation is amplified by retinal glial cells

dysfunction.[91] T helper-17 cells producing interleukin (IL)-17 A, IL-17F, IL-21 and IL-22 are responsible for inflammatory responses. IL-17A induces production of proinflammatory cytokines and its level in aqueous humour increases in patients with PDR.[92]

Retinal neurodegeneration: Retinal neurodegeneration occurs early during progression of DR due to mitochondrial dysfunction, increased retinal expression of pro-apoptotic mitochondrial proteins like cytochrome c and apoptosis inducing factor. [93, 94]

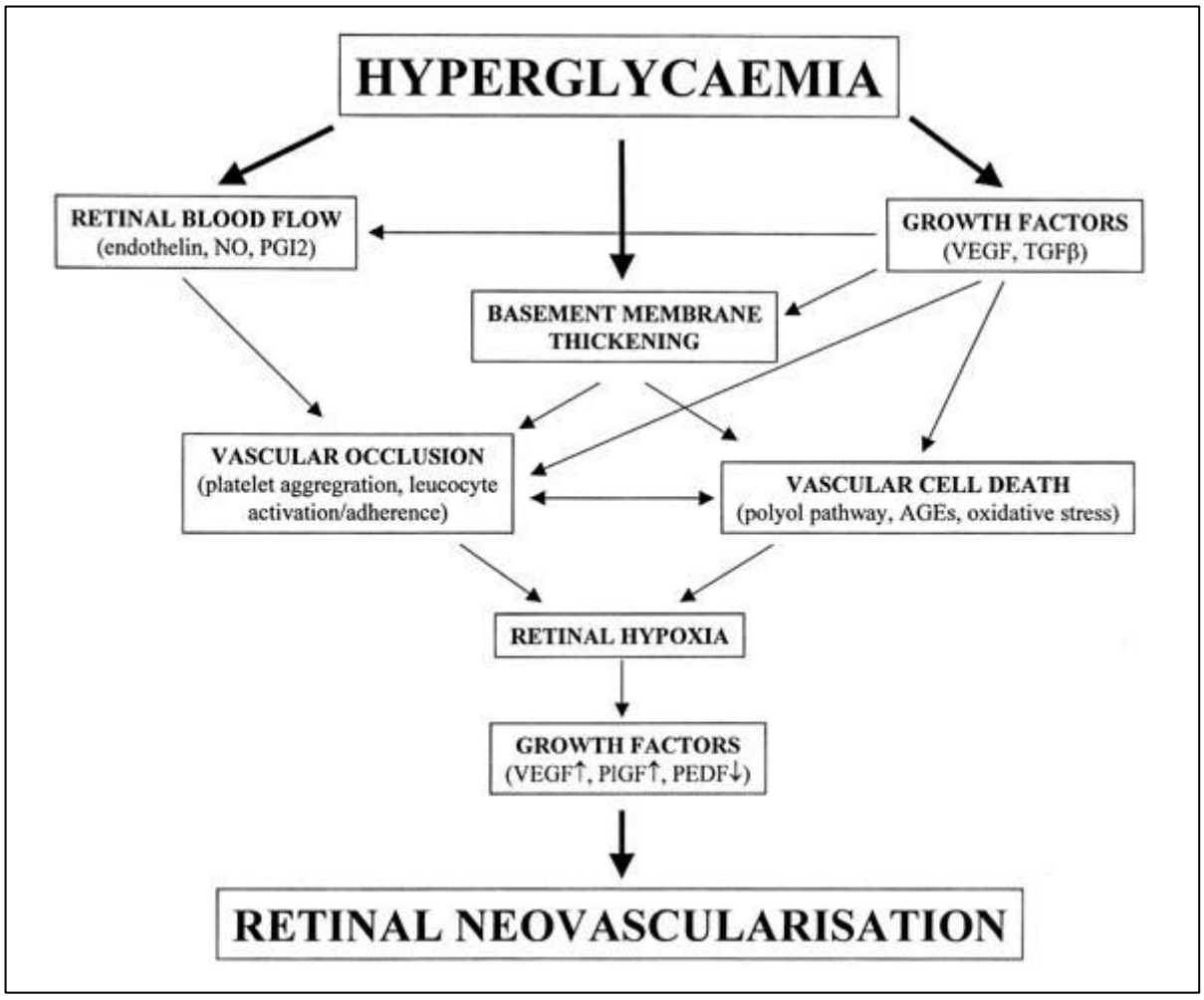


Figure 2: Schematic diagram of the pathogenesis of diabetic retinopathy.

Source: Cai J, Boulton M. The pathogenesis of diabetic retinopathy: old concepts and new questions. Eye. 2002;16:242-60.

(NO: nitric oxide; PGI2: prostacyclin; VEGF: vascular endothelial growth factor; TGF: transforming growth factor; AGEs: advanced glycation end products; PIGF: placenta growth factor; PEDF: pigment epithelium-derived factor)

Fundus signs seen in diabetic retinopathy:

Changes of diabetic retinopathy include microaneurysms, intra-retinal haemorrhages, intraretinal micro-vascular abnormalities and cotton wool spots.

- Microaneurysms: These are pathognomonic lesions of diabetic retinopathy seen in the inner nuclear layer of the retina. These are hypercellular saccular outpouchings of capillary wall which either form by focal dilatation due to absence of pericytes in the capillary wall or by fusion of the two arms of capillary loop. [95]

  The number and size of microaneurysms is important to predict the progression of diabetic retinopathy. High number of microaneurysms and small size predict progression of diabetic retinopathy.[96] Smaller microaneurysms are prone to leakage and cause retinal thickening.[97] Microaneurysms are classified into six morphological groups- focal bulge, saccular, fusiform, mixed, pedunculated and irregular.[98]
- Exudates: usually referred to as 'hard' exudates are caused by chronic localized retinal oedema. They are seen at the junction of normal and oedematous retina and are lipoproteinaceous and lipid-filled macrophages in composition. They are located mainly within the outer plexiform layer of the retina. They appear as waxy yellow lesions with distinct margins, often surrounding leaking microaneurysms.
- Cotton wool spots: are composed of accumulations of neuronal debris within the nerve fibre layer. They are formed from the ischaemic of disruption of nerve axons.
- Retinal haemorrhages: arise from the large superficial pre-capillary arterioles. They assume the characteristic shape because of the architecture of the retinal nerve fibre layer. Intraretinal haemorrhages arise from the venous end of capillaries and are located in the compact middle layers of the retina with a resultant red dot and blot configuration. Deeper dark round haemorrhages represent haemorrhagic retinal infarcts and are located within the middle retinal layers.

- Intra-retinal micro-vascular abnormalities (IRMA): arteriolar–venular shunts that run from retinal arterioles to venules, thus bypassing the capillary bed and are therefore often seen adjacent to areas of marked capillary hypoperfusion they appear as fine, irregular, red intraretinal lines that run from arterioles to venules, without crossing major blood vessels.[95]

Classification of Diabetic Retinopathy:

The Early Treatment Diabetic Retinopathy Study (ETDRS) classification system is used to assess and grade severity of diabetic retinopathy. [99]

Non Proliferative Diabetic Retinopathy (NPDR)

Very mild NPDR

- Microaneurysms only

Mild NPDR

- Any or all of: microaneurysms, retinal haemorrhages, exudates, cotton wool spots, up to the level of moderate NPDR. No intraretinal microvascular anomalies (IRMA) or significant beading

Moderate NPDR

- Severe retinal haemorrhages (more than ETDRS high-risk PDR in up to 8% standard photograph 2A: about 20 medium–large per quadrant) in 1–3 quadrants or mild IRMA
- Significant venous beading can be present in no more than 1 quadrant
- Cotton wool spots commonly present

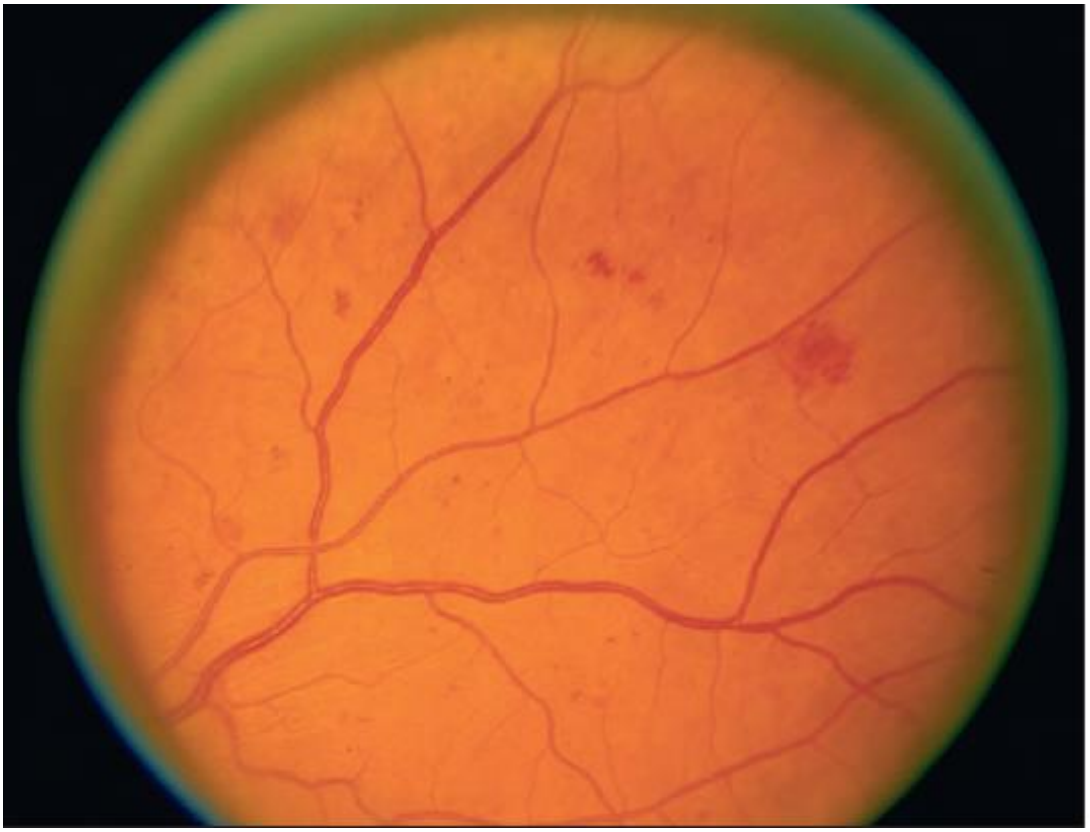


Figure 3: Moderate NPDR

Source: Kanski's Clinical Ophthalmology, A systemic approach; Chapter: Retinal Vascular Disease

Severe NPDR

The 4–2–1 rule; one or more of:

- Severe haemorrhages in all 4 quadrants
- Significant venous beading in 2 or more quadrants
- Moderate IRMA in 1 or more quadrants

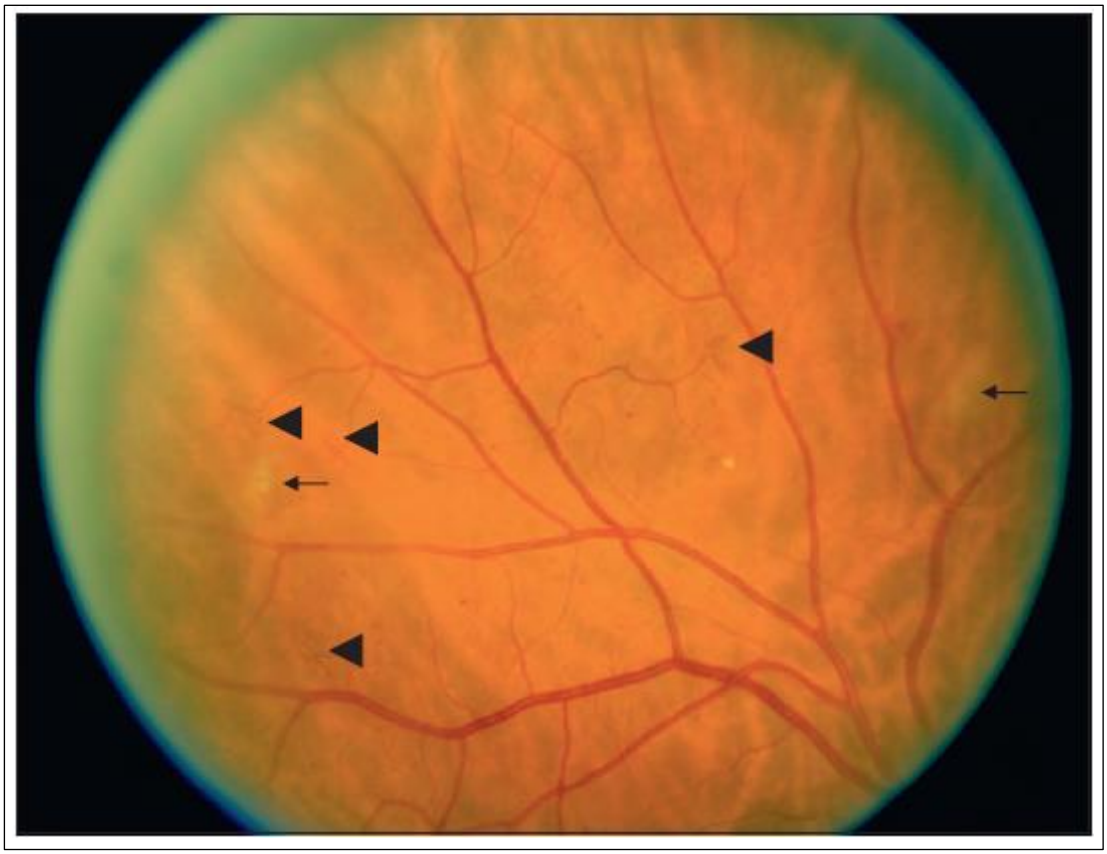


Figure 4: Severe NPDR (Arrows: cotton wool spots, arrow heads: IRMA: intraretinal retinal microvascular aneurysm)

Source: Kanski's Clinical Ophthalmology, A systemic approach; Chapter: Retinal Vascular Disease

Very severe NPDR

- Two or more of the criteria for severe NPDR

Proliferative diabetic retinopathy

Mild to moderate PDR

- New vessels on the disc (NVD) or new vessels elsewhere (NVE), but extent
- insufficient to meet the high-risk criteria

High-risk PDR

- New vessels on the disc (NVD) greater than ETDRS standard photograph 10A (about 1/3 disc area)

- Any NVD with vitreous haemorrhage
- NVE greater than 1/2 disc area with vitreous haemorrhage

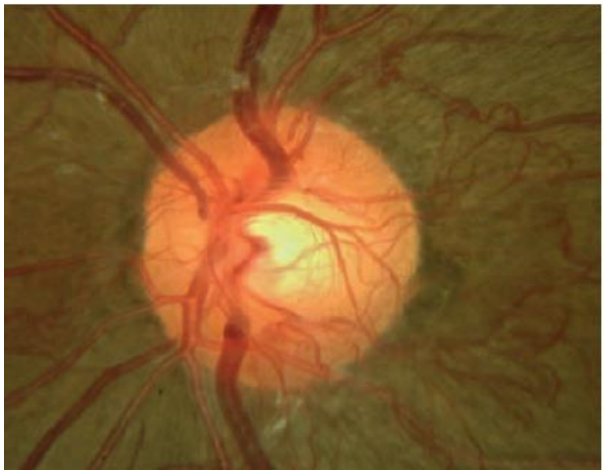


Figure 5: Neovascularisation of the disc

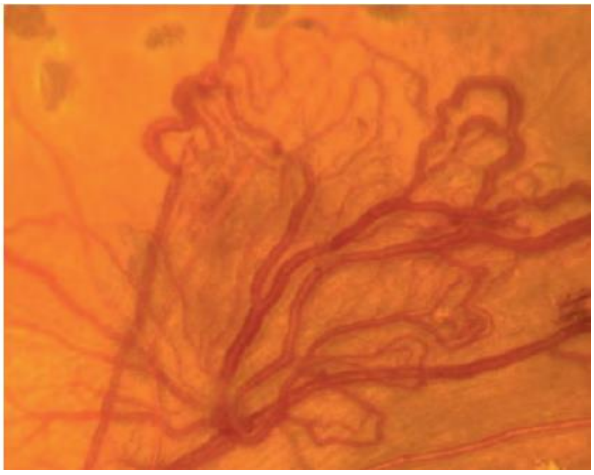


Figure 6: Neovascularisation elsewhere

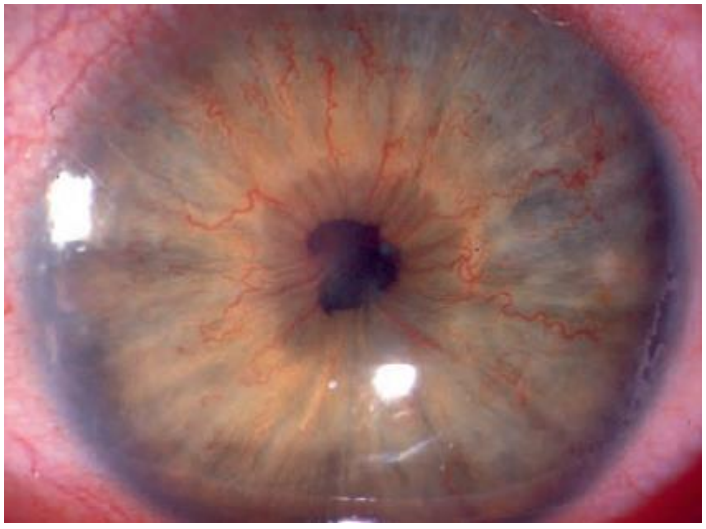


Figure 7: Neovascularisation of the Iris

Source: Kanski's Clinical Ophthalmology, A systemic approach;
Chapter: Retinal Vascular Disease

Advanced diabetic eye disease

It is serious vision threatening complication of diabetic retinopathy consisting of following complications:

- Persistent vitreous haemorrhage
- Tractional retinal detachment
- Neovascular glaucoma

Figure 8: Advanced diabetic eye disease: retrohyaloid and intragel bleeding

Figure 9: Advanced diabetic eye disease: tractional retinal detachment

Source: Kanski's Clinical Ophthalmology, A systemic approach;
Chapter: Retinal Vascular Disease

Diabetic Macular Edema[99]

Diabetic maculopathy (foveal, exudative or ischemic) is one of predominant cause of decreased vision in a diabetic patient. Diffuse edema is caused by capillary leakage and focal edema is caused by microaneurysms fluid being present between the outer plexiform layer and the inner nuclear layers.

Clinically significant macular edema (CSME) is defined as diabetic macular edema consisting of one of the following findings as defined by ETDRS, on clinical examination:

1. Retinal thickening within 500μm of the centre of the macula.

2. Exudates within 500μm of the centre of the macula, if associated with retinal thickening

3. Retinal thickening one optic disc area (1500 μm) or larger any part of which is within one disc diameter of the centre of the macula.

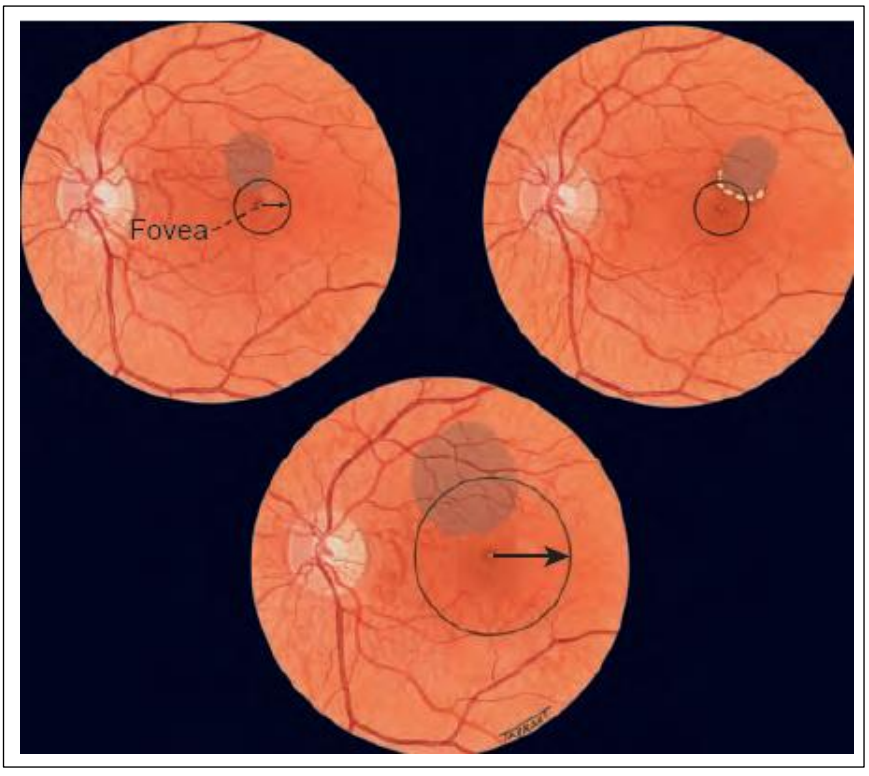


Figure 10: Criteria for Clinically Significant Macular Edema

Source: Kanski's Clinical Ophthalmology, A systemic approach;
Chapter: Retinal Vascular Disease

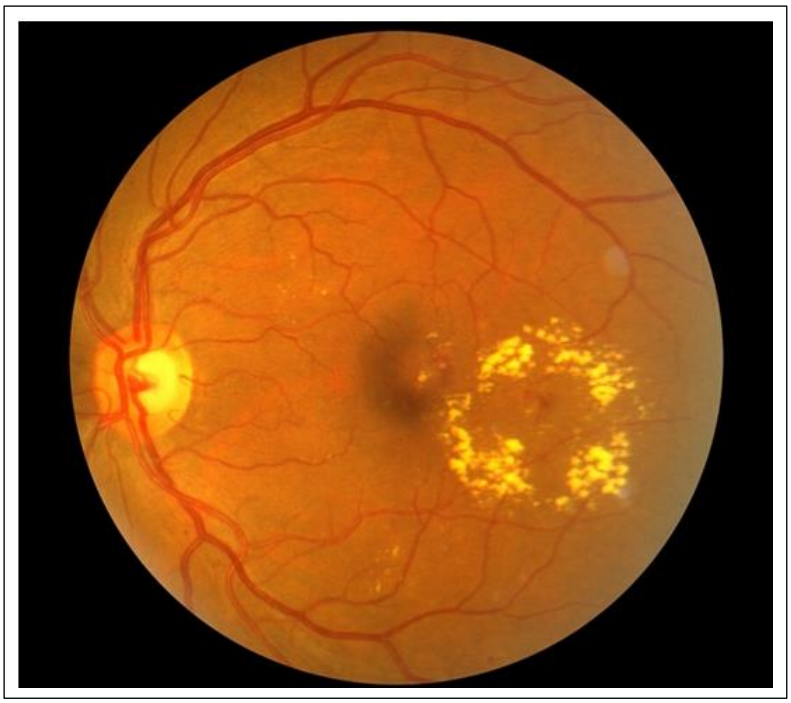


Figure 11: Clinically Significant Macular Edema

Source: Kanski's Clinical Ophthalmology, A systemic approach;
Chapter: Retinal Vascular Disease

Investigations:

The following are investigations for diabetic retinopathy:

- **Vision:** Visual acuity and visual fields help to assess the severity of diabetic retinopathy but they become abnormal only in advanced stages of the disease, thus it is not a very reliable predictor.
- **Blood flow changes:** Altered retinal blood flow and loss of normal autoregulatory capacity are the early markers that help in predicting the progression of diabetic retinopathy. Early changes in blood flow can be identified using Doppler flow velocity wave form analysis.[100]
- **Retinal vessel flicker response:** it is found to be reduced in type 2 diabetes and worsens with severe diabetic retinopathy.[101]

- **Oxygen saturation in retinal vasculature:** Oxygenation of blood in retinal vessels can be non-invasively quantified based on differential light absorbance by oxyhaemoglobin and deoxyhaemoglobin.[102] Oxygen saturation is higher in retinal venules of diabetic patients and it increases with increasing severity of diabetic retinopathy.[103]
- **Retinal vessel calibre:** Retinal arterial and venous calibre are early predictors of diabetic retinopathy.[104] Diabetic retinopathy induces venular widening and reduces retinal arteriole calibre.[82]
- **Retinal vessel geometry:** Larger arteriolar branching angles, increased arteriolar optimality deviation, increased arteriolar tortuosity, decreased arteriolar length to diameter ratio are seen in diabetic retinopathy.
- **Ocular coherence tomography:** This provides images of multiple retinal layers and measures thickness of various layers which help to identify and quantify macular edema. Early changes in disorganization of retinal inner layer can be also detected by OCT and used for prognosis of visual acuity and diabetic retinopathy.[105]
- **Corneal confocal microscopy (CCM):** CCM can quantify corneal nerve fibre density, branch density and length and detect nerve damage related to diabetic retinopathy.[106]

BIOMARKERS

Biomarkers in determining severity of diabetic retinopathy:

Biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[107] Biomarkers help in early diagnosis and establish an early treatment choice for diabetic retinopathy, earlier than the appearance of clinical symptoms. Diabetic retinopathy is a slow developing condition and by the time there are clinically visible symptoms, the retinal vascular and neural damage is greater. The clinically visible signs of diabetic retinopathy can be detected by ophthalmoscopy or retinal photography evaluation. Biomarkers facilitate early detection even when diabetic retinopathy changes are not visualised clinically.[108]

The following are different types of biomarkers used for detection of diabetic retinopathy:

- **Glucose related biomarkers:**

  HbA1c: Diabetic retinopathy development and progression is related to glycaemic control and glycaemic control can be assessed from HbA1c levels.[109]

  Hypoglycaemia: it has been stipulated that hypoglycaemia is also related to vascular complications of diabetes. But it cannot accurately predict diabetic retinopathy.[110]

- **Advanced glycation end product (AGE) biomarkers:** High levels of circulating AGEs are sometimes associated with diabetic retinopathy but it depends on method used to assay AGEs and site of AGE presence.[111]

- **Lipid and lipoprotein related biomarkers:** Lipoproteins identified in retinal circulation are associated with diabetic retinopathy.[108]

- **Inflammatory biomarkers:** There are multiple biomarkers related to inflammatory response associated with diabetic retinopathy.[112]

Table 1: Inflammatory biomarkers in diabetic retinopathy: [112]

Inflammatory biomarker group	Example
Vascular adhesion molecules	Vascular cell adhesion molecule 1 (VCAM-1), Intercellular adhesion molecule-1 (ICAM-1), E-selectin
Cytokines • Inflammatory • Anti-inflammatory	 Tumour necrosis factor alpha (TNFα) & IL-(1α,1β,6,8) Interleukin-10 (IL-10)
Chemokines • Pro-inflammatory/angiogenic • Anti-inflammatory/antiangiogenic	 Monocyte Chemoattractant Protein-1 (MCP-1), Macrophage migration inhibitory factor (MIF), Stromal Cell-Derived Factor 1 (SDF-1), fractalkine Interferon gamma-induced protein 10 (IP-10)
Transcription factors	Hypoxia-inducible factor-1 alpha (HIF-1α)
Growth/ angiogenesis related • Pro-inflammatory/angiogenic • Anti-inflammatory/antiangiogenic • Anti-inflammatory/proangiogenic	 Vascular endothelial growth factor (VEGF), Insulin growth factor (IGF1), Connective tissue growth factor (CTGF), stem cell factor Pigment epithelium-derived factor (PEDF) Erythropoietin (EPO)
Innate immune response cells	Retinal endothelial cells with toll like receptors.

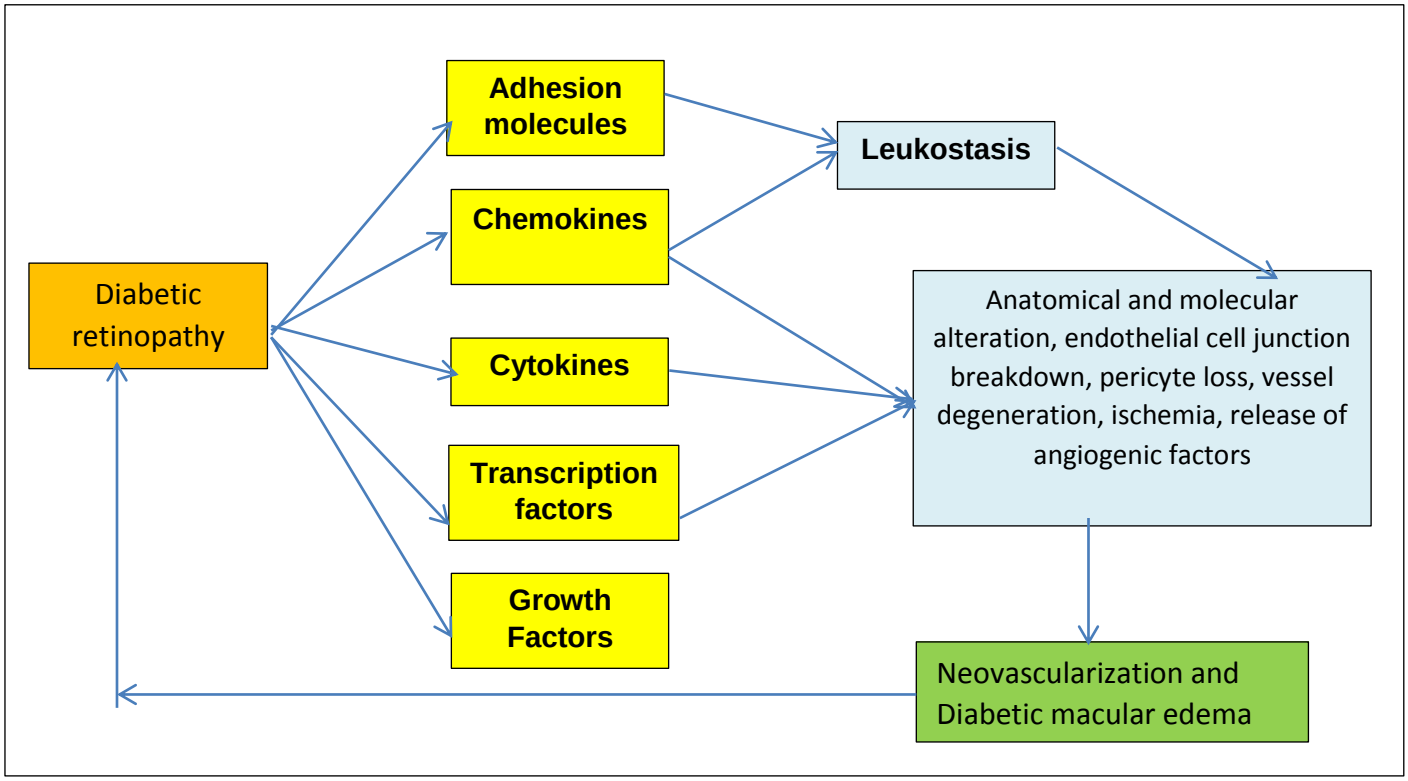


Figure 12: Role of vitreous mediators in DR progression

Source: Semeraro F, Cancarini A, Dell'Omo R, Rezzola S, Romano MR, Costagliola C. Diabetic retinopathy: Vascular and inflammatory disease. J Diabetes Res. 2015;2015.

- **Thrombosis related biomarkers**: Plasminogen activator inhibitor-1 (PAI-1) is associated diabetic retinopathy.[113]
- **Angiogenesis related biomarkers:** VEGF, Fibroblast growth factor 21, Hepatic growth factor, Connective tissue growth factor are found to be associated with diabetic retinopathy.[114]
- **Oxidative stress markers:** Oxidative stress markers (OSM) are short lived and difficult to measure. Higher levels of OSM and lower levels of superoxide dismutase in vitreous fluid are associated with diabetic retinopathy.[115]
- **Nutrition related biomarkers:** Deficiency of vitamin D, high levels of homocysteine are associated with diabetic retinopathy.[116]

- **Molecular biomarkers:** Genes related to VEGF, aldose reductase, RAAS, lipoproteins, EPO, inflammation, vitamin D receptor, are associated with diabetic retinopathy.[117]
- **Telomeres:** Shorter telomeres are associated with diabetic complications.[118]

Novel biomarkers in diabetes:

The following are novel biomarkers have been used for detection of diabetes: [119]

- Adiponectin: Low levels are associated with an increase in insulin resistance and obesity, while high levels are associated with a debilitating lifestyle as seen in diabetes prevention trials. They are inversely related to the risk of prediabetes, racial or gender differences. Adiponectin levels are directly and indirectly associated with insulin sensitivity and secretion, respectively.
- FetA: It correlates with increased risk of T2DM development and its complications.
- L-GPC: is a negative predictor of T2DM progression.
- Lp(a): has an inverse relationship with prevalence of prediabetes and T2DM.
- Triglycerides: are associated with β-cell dysfunction and reduced insulin secretion in subjects with prediabetes.
- Ferritin and transferrin: High levels have been strongly tested for the increased risk of prediabetes and diabetes mellitus. Iron functions by contributing to resistant to insulin, including production of highly active radical formation, damage to DNA and cell membrane integrity, β-cell oxidative stress leading to decreased insulin secretion capacity, and interference with glucose uptake in skeletal muscles and adipocytes. Moreover, catalytic iron stimulates the formation of reactive oxidative species, hepatic dysfunction, and β-cell apoptosis, all of which contribute to insulin resistance.

- MBL -associated serine proteases: shown to be positively correlated with prediabetes, diabetes, and CVD
- Acyl-carnitine: Elevated levels of acyl-carnitine found in individuals with prediabetes Associated with inflammation and IR.
- THBS1: THBS1 positively associated with:
    - Higher prediabetes prevalence
    - Increased IR
    - Increased 2-hour glucose
    - Adipose inflammation and metabolic dysregulation in obesity and type 2 diabetes.

Association of serum magnesium levels and diabetic retinopathy:

Hypomagnesemia caused by poor glycaemic control leads to development of diabetic complications like diabetic retinopathy.[120]

Magnesium, which is the fourth most abundant cation in human body, plays an important role in phosphorylation reactions of glucose and its metabolism. Deficiency of magnesium causes insulin resistance, carbohydrate intolerance, dyslipidaemia and diabetic complications.[11] Magnesium is a cofactor for more than 300 cellular enzymatic systems. It is distributed in body in three major compartments- 65% in mineral phase of bones, 34% in intracellular space and 1% in extracellular fluid.[121]

Magnesium deficiency is categorized into two types- magnesium deficiency and magnesium depletion. Magnesium deficiency is caused due to inadequate dietary intake. Magnesium depletion occurs due to dysregulation of factors controlling magnesium status, intestinal hypo-absorption of magnesium, reduced uptake and mobilization of bone magnesium, urinary leakage or hyperadrenoglucocorticism, insulin resistance and adrenergic hypo receptivity.[122]

Mechanism of action of diabetic retinopathy with hypomagnesemia:

Magnesium plays a significant role in carbohydrate metabolism. It regulates GLUT-4 transporter for glucose uptake by adipose tissue and maintains insulin sensitivity. Magnesium deficiency negatively affects tyrosine kinase activity, increases TNF-α levels which is associated with post-receptor insulin resistance, decreases insulin secretion by pancreas, affects normal cell growth and regulation of apoptosis as magnesium is essential for DNA synthesis and repair. [123,124]

It also governs the activity of sodium-potassium ATPase pump on the endothelial membrane thereby regulating the concentration gradient of these cations and further affecting the glucose transport.[123] Glucose disposal in cells depends on tissue sensitivity to insulin. Hypomagnesemia disrupts this dependence leading to a linear relationship between serum magnesium levels and cellular glucose disposal independent of insulin secretion.[125]

Chronic hyperglycaemia is responsible for development and progression of diabetic retinopathy and causes gastrointestinal and renal disturbances which result in poor absorption of magnesium leading to hypomagnesemia. Hypomagnesemia in turn increases insulin resistance leading to hyperglycaemia.[126] Osmotic action of glycosuria and hyperglycaemia also reduces tubular absorption of magnesium.[125] Hence, hyperglycaemia and hypomagnesemia form a vicious cycle.[126]

Insulin secretion, insulin receptor interaction, post receptor events and normal carbohydrate utilization acquire magnesium in the form of magnesium dependant enzymes. Hypo magnesium in serum induces or worsens existing type 2 diabetes by alteration of cellular glucose transport, reducing pancreatic insulin secretion, defective post receptor insulin signalling and altered receptor interactions.[127] Magnesium does not alter binding of insulin to receptor but ability of insulin bound to receptor to activate tyrosine kinase is reduced with

low magnesium levels.[128] Low serum levels of magnesium correlate with development and progression of diabetic retinopathy.

Association of serum magnesium levels and HbA1c levels:

Reduced serum magnesium levels will cause impaired glucose control resulting in increase of HbA1c levels. Magnesium plays an important role in carbohydrate metabolism. It controls release of hormones controlling blood glucose levels.

AQUEOUS HUMOR

Aqueous humor is a clear fluid. It fills the anterior and posterior chamber of the eye. It is a blood surrogate of eye providing transparent and colourless medium between cornea and lens. Its funcyion is to provide nutrition, transport neurotransmitters, eliminate excretory products, regulate homeostasis and stabilize ocular structures.[129]

It provides a transparent and colorless medium between the cornea and the lens and constitutes an important component of the eye's optical system.[130]

Aqueous humor dynamics:[130-135]

The secretion of aqueous humor and regulation of its outflow are important processes required for normal functioning of eye.

Aqueous humor dynamics mainly depends on two structures:

- Site of production of aqueous humor i.e. the ciliary body
- Site for outflow of aqueous humor i.e. the limbal region, which includes trabecular meshwork

Aqueous Humor Production:[130-135]

Aqueous humor is mainly produced in the anterior pars plicata and ciliary processes.

Aqueous humor is formed from plasma in the network of capillaries of ciliary processes. The formed aqueous humor circulates around the lens and enters anterior chamber through the pupil following its initial entry into the posterior chamber. To reach the posterior chamber, it traverses through the capillary wall, stroma, pigmented and non-pigmented epithelium; all of which are a component of the ciliary process. The non-pigmented epithelium consists of multiple junctions through which the substances traverse by-

- Diffusion: transports the lipid soluble substances
- Ultrafiltration: transports the smaller sized (water and water-soluble) components down the osmotic gradient
- Secretion: larger components are transported actively via the cell membrane. This requires specific protein transporters and adenosine triphosphate (ATP) hydrolysis.it contributes to 80-90% of aqueous humor formation

Basic Physiologic Processes

The following processes occur in production of aqueous humor:

1. Accumulation of Plasma Reservoir:

The components of plasma pass from the ciliary process capillaries, through the stroma and pigmented epithelial layer to get collected posterior to the tight junctions of the non-pigmented epithelium. This process primarily involves diffusion and ultrafiltration.

2. Transport via Blood-Aqueous Barrier:

Active transport of various ions and molecules occurs by transcellular movement across the blood aqueous barrier. This barrier is composed of tight junctions in non-pigmented epithelium. Aqueous humor is secreted by movement of sodium chloride (NaCl) from the

stroma of ciliary process to the posterior chamber. This movement of NaCl is accompanied by passive movement of water.

Thus the secretion of aqueous humor is mediated by 3 main steps including:

- Movement of NaCl from the stoma by electroneutral transporter to the pigmented epithelium by Na+/H+ and Cl−/HCO3− antiports and Na+-K+-2Cl- cotransporter.
- Passage of NaCl from pigmented epithelial cells through the gap junctions to the non-pigmented epithelial cells
- Release of sodium and chloride ions via the Na+,K+-activated ATPase and Cl- channels, respectively. This is enhanced by agonists of A3 adenosine receptors (A3ARs).

Carbonic anhydrase facilitates the transport of bicarbonate through the ciliary epithelium. It causes hydration of CO2 and dehydration of H2CO3:

$CO_2 + H_2O \rightarrow (CA)\ H_2CO_3 \rightarrow HCO_3^- + H^+$

The formation of bicarbonate further facilitates fluid transport by affecting the Na+ and regulating the pH for optimal transport of Na+, actively.

3. Osmotic Flow

An osmotic gradient gets formed across the ciliary epithelium due to the active transport as described. This gradient facilitates the movement of other substances through a process of ultrafiltration and diffusion. Na+ is believed to be the main cation inducing the movement of water from the stroma of ciliary process to the posterior chamber. Aquaporin-1 and aquaporin-4 are the water transporting channels present in the nonpigmented ciliary epithelium that also facilitates the movement of water.

The rate of aqueous humor formation in a normal human eye is ~2.5 μL × min−1 [130,131].

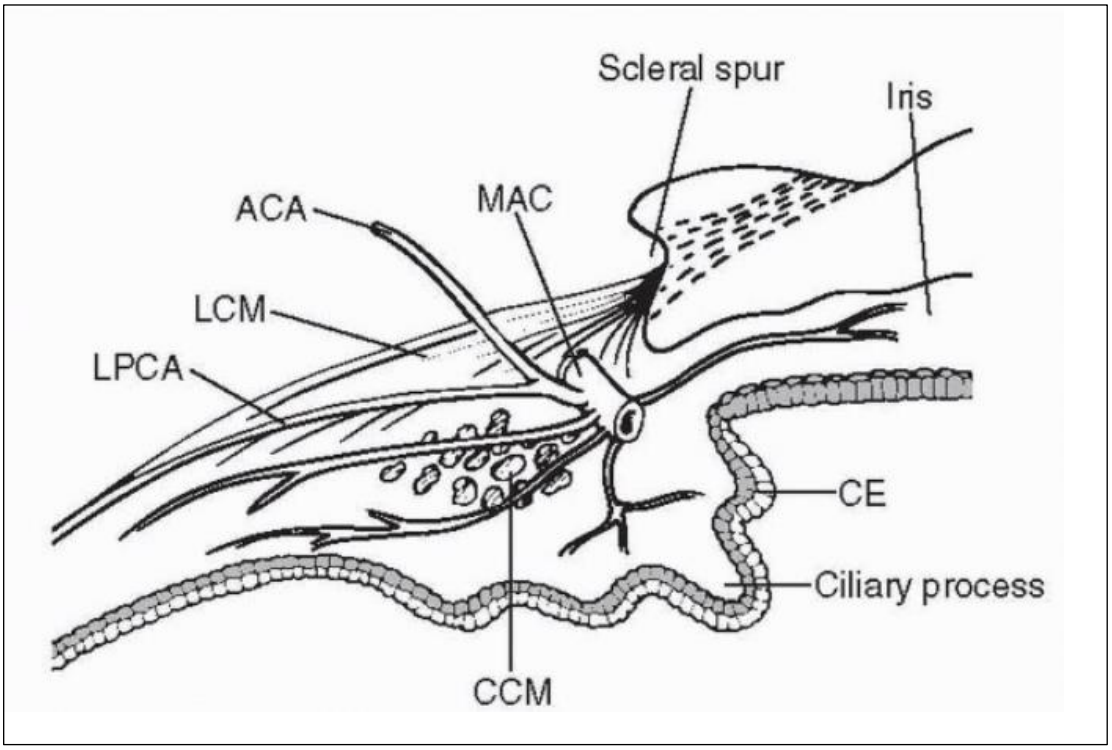


Figure 13: The three main components of the ciliary body:

1. Ciliary muscle: longitudinal (LCM), radial and circular fibres (CCM)
2. Vascular system: anterior ciliary arteries (ACA), long posterior ciliary arteries (LPCA) which form the major arterial circle (MAC)
3. Ciliary epithelium (CE): composed of outer pigmented and inner non pigmented layer

Source: Shield's Textbook of Glaucoma; Chapter: Cellular and Molecular Biology of Aqueous Humor Dynamics

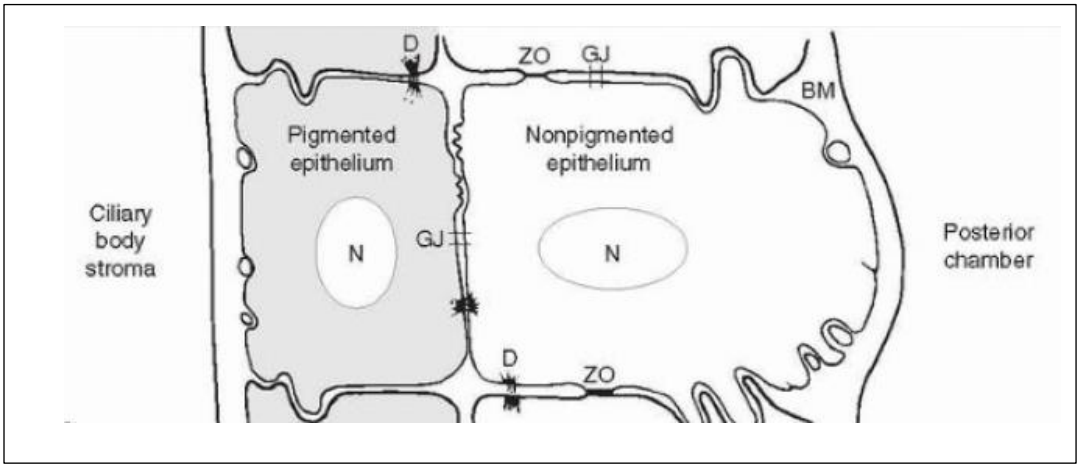


Figure 14: Composition of ciliary epithelium

Ciliary epithelium is composed of 2 layers containing nuclei with an outer pigmented layer (facing the stroma of ciliary process) and an inner non-pigmented layer (facing and lining the posterior chamber). The basement membrane (BM) lines the bilayer. It constitutes the inner limiting membrane on the inner surface.

The non-pigmented layer comprises: mitochondria, zonula occludens (ZO) and lateral surface interdigitations.

Pigmented epithelium comprises: melanin granules

[Intercellular junctions: desmosomes (D), gap junctions (G)]

Source: Shield's Textbook of Glaucoma; Chapter: Cellular and Molecular Biology of Aqueous Humor Dynamics

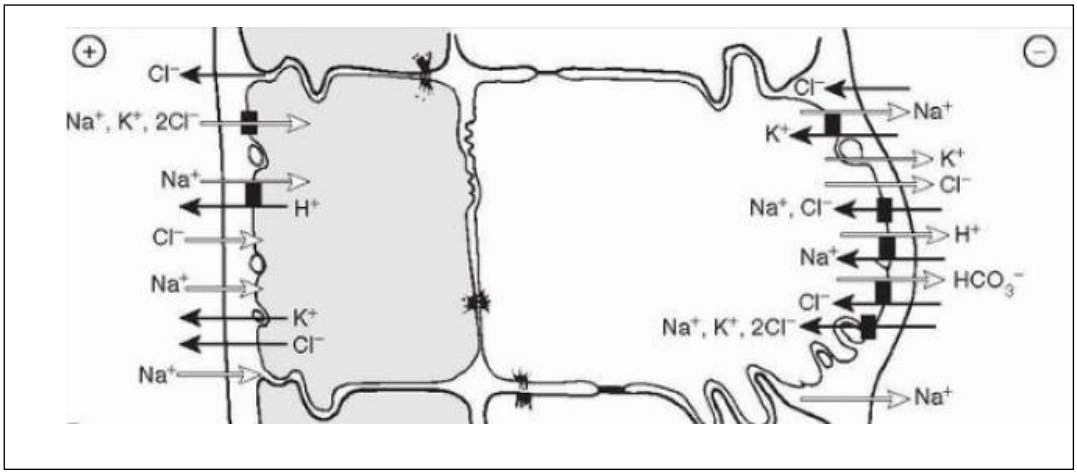


Figure 15: Physiology of ion transport mechanism across ciliary epithelium

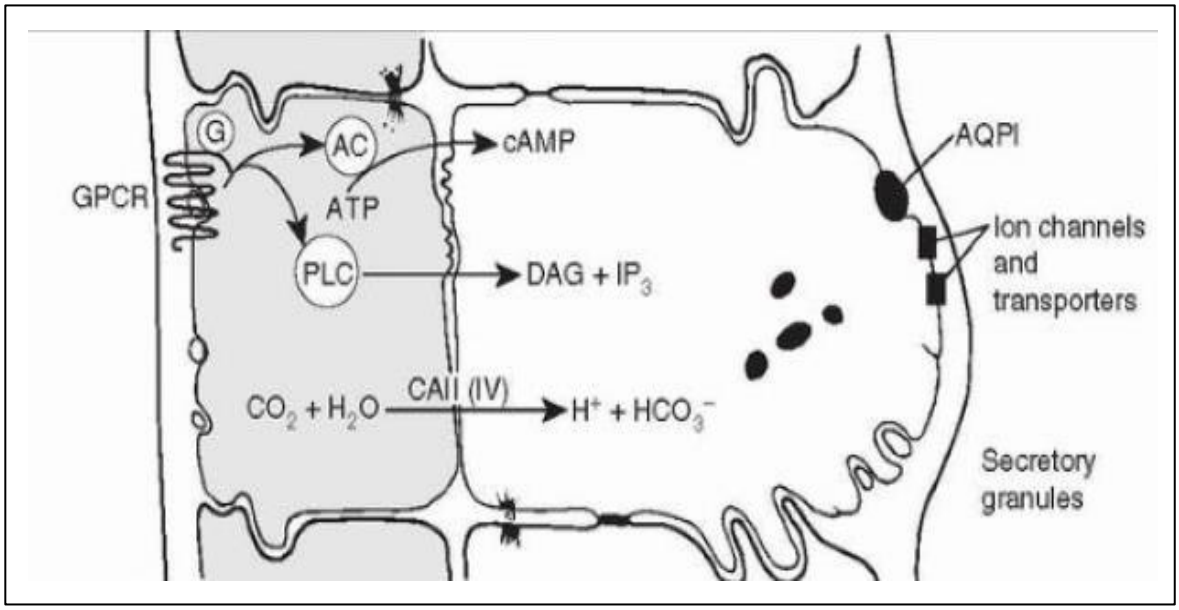


Figure 16: Transmembrane signalling and enzymatic pathways

(AC- adenylate cyclase, PLC: phospholipase C, GPCR: G-protein coupled receptor, ATP: adenosine triphosphate, cAMP: cyclic adenosine monophosphate, DAG: diacyl glycerol, IP3: inositol triphosphate, CAII/IV: carbonic hydrase type II/IV, AQP1: aquaporin type 1 channel)

Source: Shield's Textbook of Glaucoma; Chapter: Cellular and Molecular Biology of Aqueous Humor Dynamics

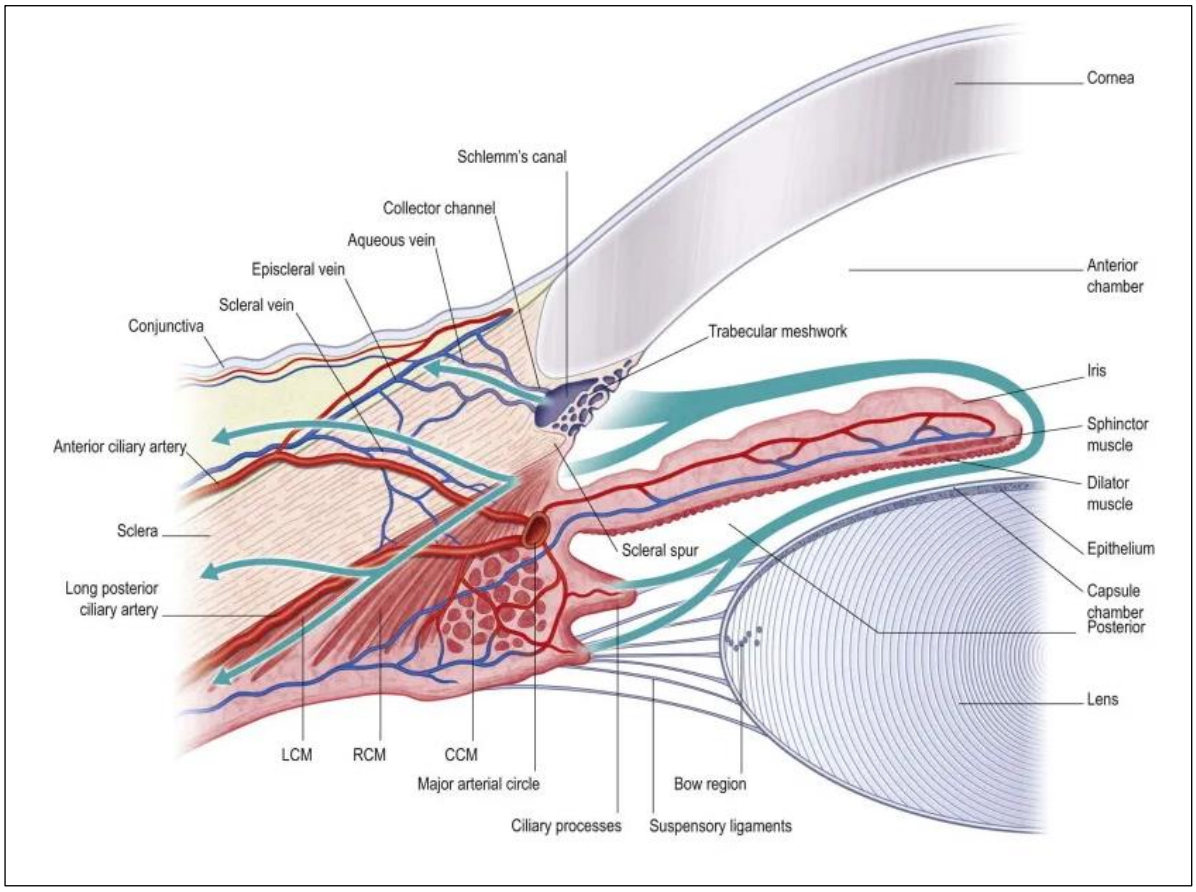


Figure 17: Normal aqueous flow

(Arrows indicate aqueous humor outflow)

Source: Adler's Physiology of the Eye; Chapter: Production and Flow of Aqueous Humor

Aqueous humor outflow: [130.131]

Aqueous leaves the eye mainly by two passive mechanisms:

(1) Trabecular or conventional route: aqueous humor passes to the Trabecular Meshwork → inner wall of Schlemm's canal and it's lumen → collector channels → aqueous veins → episcleral venous circulation

(2) Uveoscleral, posterior, or unconventional route: here, the aqueous humor passes through the iris root → uveal meshwork → anterior face of the ciliary muscle →connective tissue between the muscle bundles → suprachoroidal space → out through the sclera.

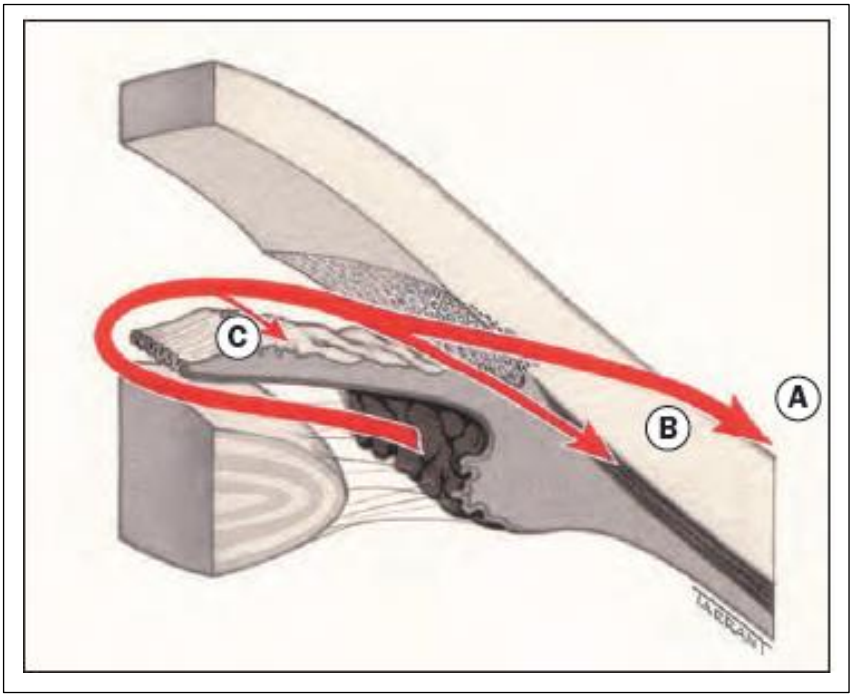


Figure 18: Routes of aqueous outflow- A: trabecular; B: uveoscleral; C: iris

Source: Kanski's Clinical Ophthalmology, A systemic approach; Chapter: Glaucoma

Major components of aqueous humor are organic and inorganic ions, carbohydrates, glutathione, urea, amino acids, proteins, oxygen, carbon-di-oxide and water.[136] Relative concentrations of substances in aqueous humor help in estimation of composition of aqueous humor which not only depends on its production but also on metabolic interchanges occurring in various tissues of its intraocular route.[137]

Inflammatory cytokines and angiogenesis factors are associated with development of diabetic retinopathy.[138] The concentrations of various cytokines increase with increase in severity of diabetic retinopathy.

Biochemistry of aqueous humor: The chemical composition of aqueous humor is derived from plasma by passive diffusion and from ciliary epithelium by active secretion. Some substances also enter from surrounding corneal endothelium, lens and vitreous by diffusion and secretion. Aqueous humor is an intraocular fluid with unique composition of electrolytes, proteins, biologically active substances, organic solutes and ascorbate. The composition of aqueous humor is maintained and controlled by many factors including pathological

conditions in anterior segment. Aqueous humor comprises of three groups of substances depending on physical state of molecules in solution: [139,140]

- Colloidal substances: The colloidal substances including proteins, fats occur in very minute acts in aqueous humor.
- Diffusible non-dissociated substances: The amino acid content is variable while urea and sugar are present in equal concentrations.
- Dissociated diffusible substances: These are unequally distributed.

Glucose and amino acids: Glucose concentration in aqueous humor is 76% of plasma in younger population while it reduced to 63% in older population. Glucose enters posterior aqueous humor from ciliary epithelium by diffusion. Steady state concentrations of glucose will be maintained when rate of inward flux from ciliary body equals its utilization by corneal endothelium and lens. Aqueous humor is the main source of glucose to corneal endothelium and lens.[139] The concentration of free amino acids varies widely in aqueous humor and is greater than plasma.

Catecholamines, hormones and prostaglandins: Norepinephrine is the most abundant catecholamine present in aqueous humor at a concentration of 1ng/ml. Steroid hormones in aqueous humor are about 10 to 25% of their concentration in plasma. Prostaglandins are synthesized by iris-ciliary body and anterior uvea in response to injury into aqueous humor.[139]

Proteins and lipids: Protein concentration in aqueous humor is very low and albumin is the main protein found in aqueous humor. Small amounts of transferrin and trace amounts of other proteins like orosomucoid, α1-acid glycoprotein, immunoglobulins IgG and IgE are also found in aqueous humor. Lipids are found in trace amounts in aqueous humor.[139]

Hyaluronic acid: The concentration of hyaluronic acid in aqueous humor is 1/100 of that in vitreous.[139]

Ions and low molecular weight solutes: Ions and low molecular weight solutes exist in steady state concentrations due to continuous entry from plasma and exit through trabeculum and uveoscleral drainage. Cations including sodium, potassium and magnesium are present in similar concentrations as in plasma but calcium ion concentration in aqueous humor is lower than that in plasma. Anions including chloride and bicarbonate ions are also present in same concentration as plasma. This combination of ions helps in maintaining electrical neutrality of aqueous humor which is essential to buffer surrounding tissues.[139]

Table 2: The following table gives the normal composition and concentration of electrolytes and solutes in aqueous humor and plasma:[139]

COMPONENT	AQUEOUS HUMOR	PLASMA
ELECTROLYTES (mM)		
Na^+	142	130-145
Cl^-	131	92-125
HCO_3^-	20	24-30
Mg^{2+}	1	0.7-1.1
K^+	4	3.5-5.0
Ca^{2+}	1.2	2.0-2..6
ORGANIC SOLUTE (Mm)		
Ascorbate	1.1	0.04-0.06
H_2O_2	0.024-0.069	0.02-0.10
Glutathione	1-10 X 10^{-3}	Most glutathione is in erythrocytes which is 1000 times higher than in plasma.
Lactate	4.5	0.5-0.8
Citrate	0.1	0.1
Glucose	2.7-3.9	5.6-6.4
Urea	4.1	3.3-6.5

DIABETES AND CATARACT

Several studies have reported that the occurrence of cataract is three to four times higher in diabetic patients, compared to non-diabetics; especially under the age of 65.[141]

Various mechanisms have been proposed for the pathogenesis of cataract formation among diabetic patients:

1. Polyol Pathway: the hyperglycaemia stage causes the flux of glucose through the polyol pathway where the aldose reductase (AR) enzyme causes the reduction of glucose into sorbitol. The production of sorbitol takes place more quickly among diabetic patients and intracellular removal of sorbitol through diffusion is slower because of its polar character. Thus, an increased accumulation of sorbitol results in an infusion of fluid creating a hyperosmotic effect. Intracellular accumulation of polyols causes liquefaction of lens fibres resulting in the formation of lens opacities.[142]

2. Osmotic and oxidative stress

The accumulation of sorbitol causes osmotic stress and fluctuation of glucose levels initiates production of reactive oxygen species. These in turn induce stress on endoplasmic reticulum and cause oxidative stress which causes damage to lens fibres.[143]

The increased glucose concentration in aqueous humor causes glycation of lens proteins and further leads to formation of advanced glycation end products.[144]

Free radical nitric oxide is elevated in lens and aqueous humor among diabetic patients. This further leads to formation of peroxynitrite that causes cell damage. Lens components of diabetic patients are more to oxidative stress as they have impaired function of antioxidant enzymes specially superoxide dismutase (SOD).

It is also seen that rapid glycaemic control can increase the lens epithelial cell damage by creating a hypoxic environment that decreased protective enzymes and increases the free oxygen radicals.

Various types of cataract are noted in diabetics, most common being senile cataract. Snowflake cataracts are noted in Type 1 Diabetics patients. Posterior subcapsular cataract, nuclear sclerosis and cortical cataracts have also been associated with increased glycated haemoglobin levels. [143]

RELEVANT STUDIES

Dipankar Kundu et al studied the correlation between serum magnesium levels, glycosylated haemoglobin and urinary total protein levels in diabetic patients with retinopathy. The study concluded that hypomagnesemia and albuminuria individually or in conjunction serve as indicators for dysglycaemia and could be used as marker for the risk of development of diabetic retinopathy.[19]

Chaudhary DP et al discussed the implications of magnesium deficiency in type 2 diabetes. The study concluded that magnesium plays a very important role in development of type 2 diabetes. Early detection and correction of magnesium deficiency will help prevent development of diabetes related complications. [145]

Pham PC et al reviewed the magnesium metabolism, hypomagnesemia incidence in patients with type 2 diabetes, risk factors, and associated complications. Hypomagnesemia ranges from 13.5 to 47.7% among patients with type 2 diabetes. They concluded that hypomagnesemia is associated with poor glycaemic control, coronary artery disease, high blood pressure, diabetic retinopathy, neuropathy, nephropathy, and foot ulcers. Increased incidence of hypomagnesemia among patients with type 2 diabetes may be multifactorial. [120]

Corsonello A et al evaluated circulating ionized magnesium concentrations in patients with non-insulin-dependent diabetes mellitus (NIDDM) and incipient or overt diabetic nephropathy. Microalbuminuria and clinical proteinuria, as well as poor glucose metabolic control and hypertriglyceridemia, are associated to relevant alterations in magnesium metabolism, and the measurement of serum ionized magnesium seems to represent a useful biochemical tool for the study of magnesium disturbances in patients with different grades of diabetic nephropathy. [146]

Ajith, AT et al discussed the role of magnesium as a possible therapeutic agent in the management of glaucoma, cataract and diabetic retinopathy. The study concluded that the ability of magnesium to attenuate epithelial oxidative stress and neuronal inflammation suggests its possible role in the management of ocular diseases.[147]

Flieger J et al studied multi-elemental composition of the aqueous humor of patients undergoing cataract surgery, suffering from coexisting diabetes, hypertension, or diabetic retinopathy. The study revealed substantial variations in elemental composition between test groups in comparison to the control group with no co-existing morbidity. [148]

Parlapally RP et al revealed a significant reduction in serum magnesium levels in diabetics cases compared to the non-diabetic controls. There was a significant correlation between serum magnesium levels and the level of control of diabetes. Uncontrolled diabetics had a low level of serum magnesium.[149]

MATERIAL & METHODS

MATERIALS AND METHODS

STUDY SITE:

This study was carried out at R.L. Jalappa. Hospital and Research Centre, attached to Sri Devaraj Urs Medical College, Kolar.

STUDY POPULATION:

Patients visiting Department of Ophthalmology, R. L. Jalappa Hospital and Research Centre, Kolar, Karnataka were included in the study. Group A included diabetic cases undergoing cataract surgery. Group B included nondiabetic controls undergoing cataract surgery

STUDY DURATION:

December 2019 to May 2021

STUDY DESIGN:

The current study is a case control study

SAMPLE SIZE:

A total of 54 subjects were included in the study:

- Group A: 27 diabetic cases undergoing cataract surgery
- Group B: 27 nondiabetic controls undergoing cataract surgery

SAMPLING PROCEDURE:

Sample size was calculated on consultation with the Institutional biostatistician and based on mean serum magnesium levels of 1.88 mg/dL and standard deviation of 0.28 mg/dL among the cases and mean magnesium level of 2.1 mg/dL and standard deviation of 0.29 mg/dL among the controls as per study done in serum magnesium levels in type 2 diabetes mellitus authored by Rajendra Prasad Parlapally et al in 2016.[149] The sample size of our study was calculated by the significant level of p value <0.03 as per the same study. A total sample size of 54 will be used.

Formula used for sample size calculation:

$$N = \frac{(u + v)^2(\sigma_1^2 + \sigma_0^2)}{(\mu_1 - \mu_0)^2}$$

N = Sample size

μ_1 , μ_0 =Difference between the means (μ_1=1.88and μ_0=2.1)

σ_1, σ_0 =Standard deviations (σ_1=0.28 and σ_0 =0.29)

U =two sided percentage point of the normal distribution corresponding to 100 % - the power = 80%, u =0.84

V =Percentage point of the normal distribution corresponding to the (two sided) significance level for significance level = 5%, *v* = 1.960

SELECTION CRITERIA:

Inclusion criteria**:**

1. Patients with senile cataract & type 2 diabetes mellitus undergoing cataract surgery.
2. Non-diabetic patients with senile cataract undergoing cataract surgery.

Exclusion criteria:

1. Patients on diuretics (loop, thiazides and osmotic); aminoglycosides.
2. Patients with gastrointestinal disorders like secretory diarrhoea, Crohn's disease, ulcerative colitis, whipple's disease, celiac disease causing hypomagnesaemia.
3. Patients who have undergone gastrointestinal surgery like gastrectomy, gastric bypass surgery.
4. Patients on proton pump inhibitors causing hypomagnesaemia.
5. Patients on any topical eye medications like brimonidine.
6. Patients who underwent any treatment of diabetic retinopathy.

ETHICAL CONSIDERATIONS:

Prior to the commencement, the study was approved by the Intuitional Ethics Committee, Sri Devraj Urs medical college, Kolar. Informed Consent was obtained from all patients included in the study. The risks and benefits involved in the study and the voluntary nature of participation were explained to the participants before obtaining informed consent. Confidentiality of the study participants was maintained. (ANNEXURE-II, ANNEXURE-III)

DATA COLLECTION TOOLS:

All the relevant parameters were documented in a structured study proforma. (ANNEXURE-I)

METHOD OF COLLECTION OF DATA:

A total 54 patients fulfilling the inclusion criteria were included in this case control study. All patients underwent similar protocol for standard cataract evaluation: such as detailed history, visual acuity by Snellen's chart, grading of cataract by slit lamp examination, intraocular pressure by Goldman applanation tonometer, keratometry and A scan for IOL power calculation, fundus evaluation by direct and indirect ophthalmoscopy. The status of severity of diabetic retinopathy was clearly documented as per Early Treatment Diabetic Retinopathy Study (ETDRS) classification. In cases where fundus could not be visualised before surgery, retinal evaluation was done and documented postoperatively. Blood samples were drawn from both the groups for calculating serum magnesium and HbA1c levels for the patients included under the study. These samples were sent to Central Diagnostic Laboratory Services (CDLS) attached to R.L. Jalappa Hospital & Research Centre for analysis. All cataract surgeries were performed by the consultant surgeon and aqueous humor sample was drawn on a priority basis before entering the globe. Later, cataract surgery was completed. The HbA1c levels, serum and aqueous humor magnesium levels of both the groups was compared

and correlated clinically with severity of diabetic retinopathy changes seen on fundus examination.

Preoperative preparation:

All patients received Xylocaine test dose, oral tab Ciprofloxacin 500mg twice daily and ciprofloxacin 0.3% eye drops 4 times per day one day before the surgery.

Before the start of surgery, the pupil was dilated with a combination of tropicamide 0.8% with phenylephrine 5% drops. Flurbiprofen 0.03% drops was used to maintain mydriasis.

Surgical technique for aspiration of aqueous humor sample:

- All surgeries were performed under local anaesthesia using peribulbar block by a single surgeon. This has ensured that there were no sampling errors.
- The eye to be operated was painted and draped as per the standard protocols.
- Lid was retracted using universal wire speculum.
- Superior rectus bridle suture was passed to fix the eye in downward gaze.
- A fornix-based conjunctival flap was prepared and hemostasis was achieved by wet field cautery.
- No. 15 blade was used to make the incision 1.5-2mm away from the superior limbus.
- A 1.5 mm side port entry was made at 9 o'clock position.
- 5 units of aqueous humor sample was aspirated in an insulin syringe, from the side port. (ANNEXURE-IV)
- Rest of the cataract surgery was carried out as per the standards which included anterior capsulorhexis, hydro dissection to separate the corticonuclear mass from the posterior capsule, nucleus prolapse and delivery, irrigation and aspiration of the remaining cortical matter, posterior chamber intraocular lens placement in the capsular bag, clearing of viscoelastic substance from the anterior chamber, stromal hydration of the side port and

subconjunctival injection of gentamycin and dexamethasone given at the end of the procedure.

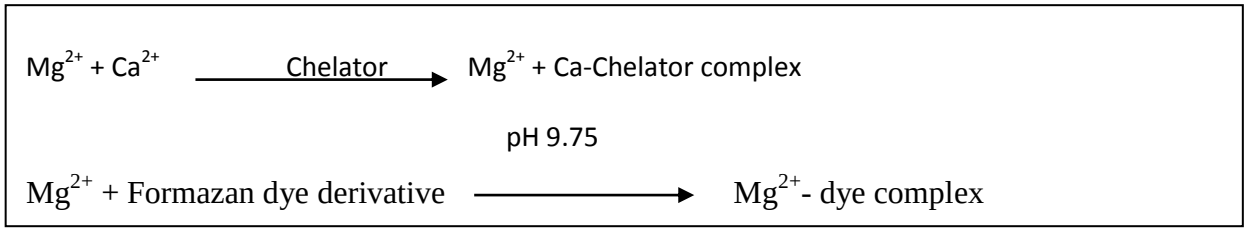
Processing of serum and aqueous humor magnesium samples (ANNEXURE-IV):

Estimation of serum and aqueous humor magnesium was done in Vitros 5.1 FS dry chemistry auto analyzer from Ortho Clinical Diagnostics, using the principle of "reflectance photometry."

Estimation of magnesium by reflectance photometry:

Instrument**:** Vitros 5.1FS dry chemistry auto analyzer based on reflectance photometry

Principle: The VITROS Mg Slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and evenly distributed by the spreading layer to the underlying layers. Magnesium (both free and protein-bound) from the sample then reacts with the formazan dye derivative in the reagent layer; the high magnesium affinity of the dye dissociates magnesium from binding proteins. The resulting magnesium-dye complex causes a shift in the dye absorption maximum. The amount of dye complex formed is proportional to the magnesium concentration present in the sample and is measured by reflection density.



Incubation time: 5 min

Temperature: 37^0 C (98.6^0 F)

Wavelength: 630 nm

Reaction sample volume: 10µL

Sample: Serum/ Aqueous humor

Reagents: Reactive Ingredients per cm^2 of slide

1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (calcium chelator) 242 µg and 1,5-bis(2-hydroxy-3,5- dichlorophenyl)-3-cyanoformazan (dye) 38 µg.

Other Ingredients: Pigment, binders, buffer, surfactants, stabilizers and cross-linking agent.

Reagent Preparation: It was importantly ensured that only after the slide cartridge reached the room temperature of 18–28 °C (64–82 °F), it was unwrapped and loaded into the slide supply.

Preparation steps:

1. The slide cartridges were removed from storage.

2. The wrapped cartridge was warmed at room temperature for 30 minutes when taken from the refrigerator or 60 minutes from the freezer.

3. The cartridge was unwrapped and loaded into the slide supply.

Note: It is important to load the cartridges within 24 hours after they reach room temperature, 18–28 °C.

Reagent Storage and Stability: VITROS Mg Slides are stable until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.

Interfering substances: Calcium, inorganic phosphorus, sodium fluoride

Detection limit: 0.2-10.0 mg/dL

Reference range: Serum: 1.2 – 2.3 mg/dL

Biological reference interval for magnesium levels in aqueous humor is not established and in this study the observed values are compared with that of non-diabetic individuals who have undergone cataract surgery.

Quality control: BIO-RAD internal and external quality assurance scheme followed strictly

Estimation of HbA1c by high performance liquid chromatography (HPLC) (ANNEXURE-IV):

Intrument: HbA1c measurements were determined using the Bio-Rad D-10® HPLC analyzer (Bio-Rad Laboratories, CA, USA)

Principle: The Bio-Rad D-10 Hemoglobin Testing System works on ionic exchange high-pressure liquid chromatography. In ion-exchange chromatography, the glycated hemoglobin components are separated according to their different electrical charge. As fractions elute, the time it takes to separate that fraction is called the retention time. The retention time for each fraction determines the identity of the component.

The auto analyzer also provides an integrated method for the separation and determination of the relative percent of specific hemoglobins (A2, F, A1c) in whole blood.

Bio-Rad D10 system uses IFCC calibration and providing derived NGSP value which is certified by the National Glycohemoglobin Standardization Program (NGSP). The technique is traceable to the Diabetes Control and Complications Trial (DCCT) reference method.

HbA1c auto analyzer is equipped with cap-piercing and onboard hemolysis systems allowing the use of a closed primary tube with whole blood.

Sample: EDTA whole blood

Interference: Hemoglobin variants but with HPLC method the interference is low

Detection limit: 3.8-18.5 %

Reference range:

- Normal: 4-6%
- Interpretation: <6.5% Good Diabetic control
- 6.5 – 7.0% Fair Diabetic control
- 7.0 – 9.0% Suboptimal Diabetic control
- 9.0% Poor Diabetic control

Quality control: BIO-RAD internal quality control is followed strictly and proficiency testing is carried out with CMC Vellore external quality assurance scheme.

STATISTICAL METHODS:

Serum magnesium and aqueous magnesium was considered as primary outcome variables. Age, gender and fundus were considered as other study relevant variables. Study group (Cases v/s Controls) was considered as primary explanatory variable.

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables. All Quantitative variables were checked for normal distribution within each category of explanatory variable by using visual inspection of histograms and normality Q-Q plots. Shapiro- wilk test was also conducted to assess normal distribution. Shapiro-wilk test p value of >0.05 was considered as normal distribution.

For normally distributed Quantitative parameters the mean values were compared between study groups using Independent sample t-test (2 groups) and ANOVA (>2 groups). For non-normally distributed Quantitative parameters the median values were compared between study groups using Mann Whitney U test. For normally distributed Quantitative parameters,

association between quantitative explanatory and outcome variables was assessed by calculating person (r) correlation coefficient. For non-normally distributed Quantitative parameters, association between quantitative explanatory and outcome variables was assessed by calculating Spearman rank (r_s) correlation coefficient.

Categorical outcomes were compared between study groups using Chi square test /Fisher's Exact test (If the overall sample size was < 20 or if the expected number in any one of the cells is < 5, Fisher's exact test was used. Data was also represented using bar charts, clustered bar charts, error bar charts, box plot and scatter plots.

p value < 0.05 was considered statistically significant. Data was analyzed by using SPSS software, Version 22.

RESULTS

RESULTS

A total of 54 participants were included in the final analysis with 27 participants among cases and 27 participants among controls.

Table 1: Comparison of age between study group by Independent Samples t-test (N=54)

Parameter	Study Group (Mean± SD)		p value
	Cases (N=27)	Controls (N=27)	
Age (in years)	67.19 ± 8.19	65.11 ± 10.02	0.409

The mean age was 67.19 ± 8.19 years among cases and 65.11 ± 10.02 years among controls. No statistically significant difference in mean age between the study group was observed (p value>0.05). (Table 1, Graph 1)

Graph 1: Pie chart showing comparison of age between study group (N=54)

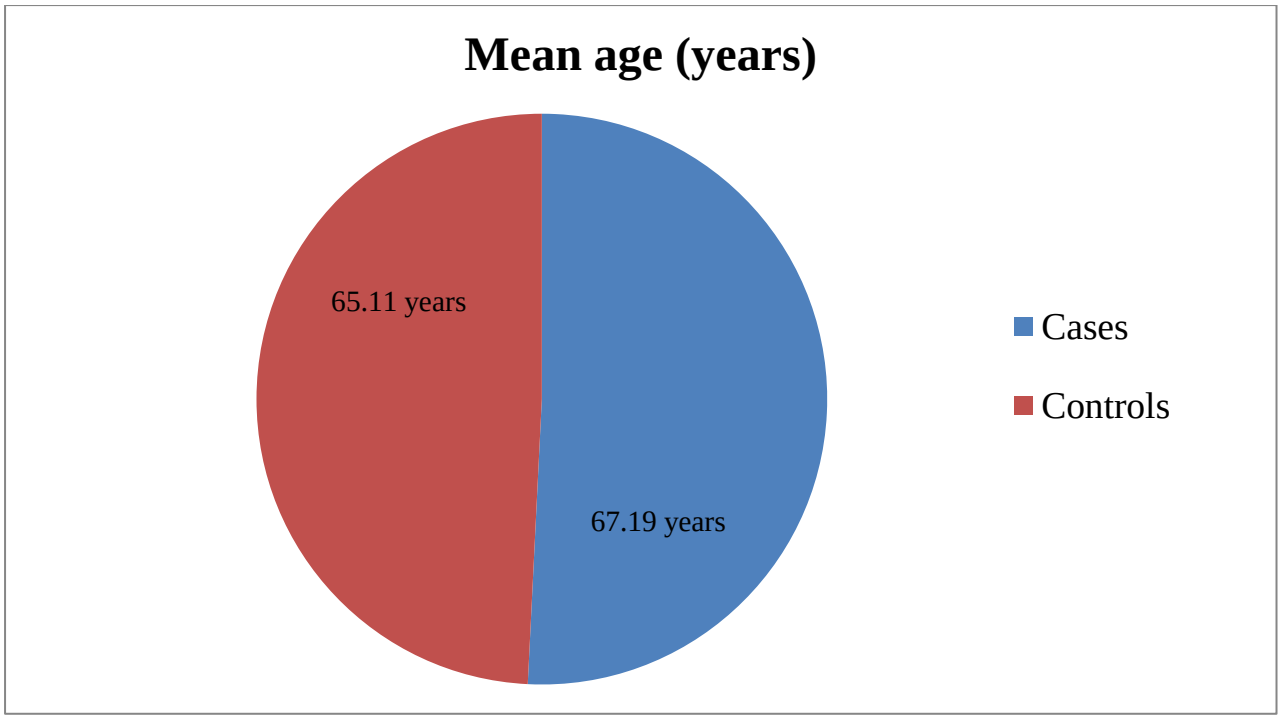


Table 2: Comparison of gender between study group by Chi-Square test (N=54)

Gender	Study Group		Chi square	p value
	Cases (N=27)	Controls (N=27)		
Female	10 (37.04%)	11 (40.74%)	0.078	0.780
Male	17 (62.96%)	16 (59.26%)		

Among the study population, there were 10 (37.04%) female participants and 17 (62.96%) male participants. Among the controls in study population, there were 11 (40.74%) female participants and 16 (59.26%) male participants. No statistically significant difference in gender between the study group was observed (p value>0.05). (Table 2, Graph 2)

Graph 2: Clustered bar chart for comparison of gender between study group

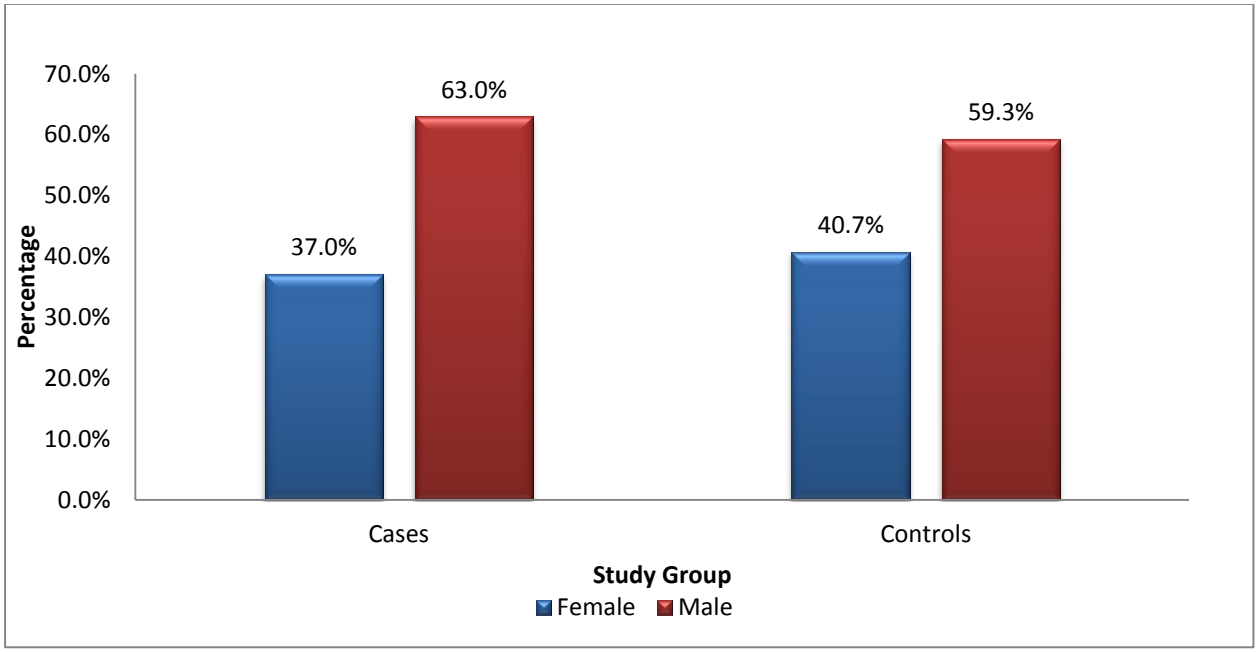


Table 3: Comparison of serum and aqueous humor magnesium between age group among cases in the study population by Independent Samples t-test (N=27)

Parameter	Age group (Mean± SD)		p value
	<60 years (N=4)	>60 years (N=23)	
Serum magnesium (mg/dL)	1.6 ± 0.18	1.74 ± 0.18	0.160
Aqueous humor magnesium (mg/dL)	1.5 ± 0.16	1.74 ± 0.2	0.035

The mean serum magnesium was 1.6 ± 0.18 mg/dL among age group <60 years and 1.74 ± 0.18 mg/dL among age group ≥60 years. The mean aqueous magnesium was 1.5 ± 0.16 mg/dL among age group <60 years and 1.74 ± 0.2 mg/dL among age group ≥60 years. A statistically significant difference in mean aqueous magnesium between the age group was observed (p value<0.05). (Table 3, Graph 3)

Graph 3: Clustered bar chart showing comparison of serum and aqueous humor (AH) magnesium between age group among cases in the study population (N=27)

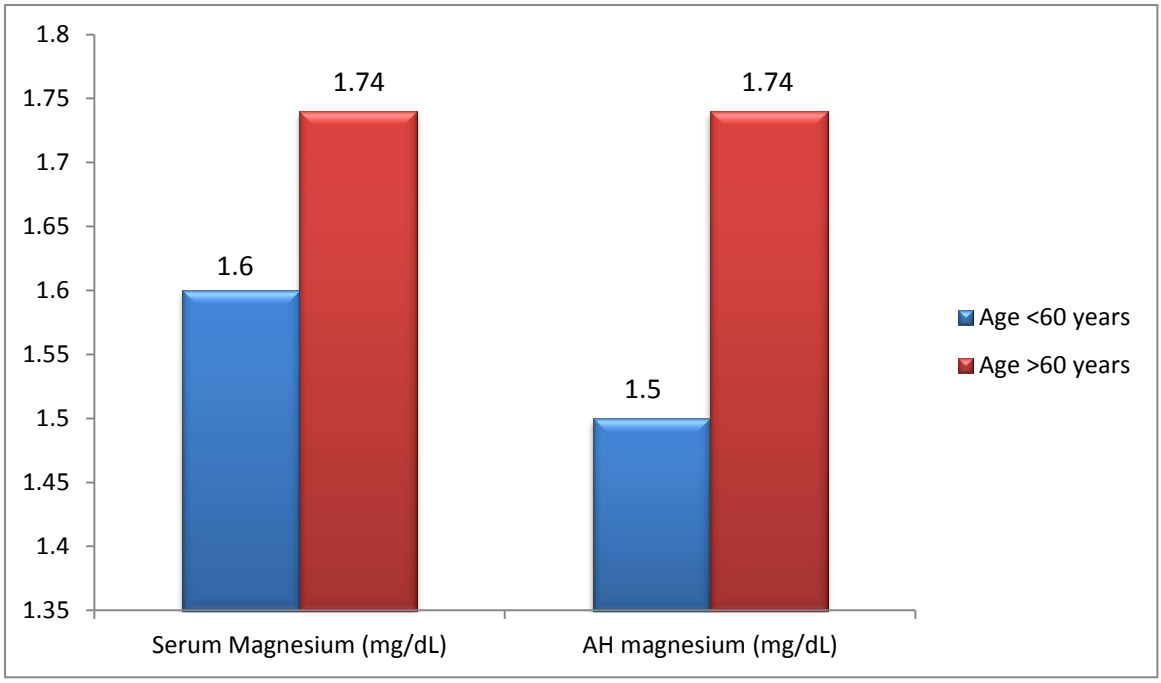


Table 4: Comparison of serum magnesium and aqueous humor (AH) magnesium between gender by Independent Samples t-test (N=27)

Parameter	Gender (Mean± SD)		p value
	Male (N=17)	Female (N=10)	
Serum magnesium(mg/dL)	1.77 ± 0.2	1.8 ± 0.23	0.963
Aqueous humor magnesium(mg/dL)	1.69 ± 0.19	1.73 ± 0.26	0.631

The mean serum magnesium was 1.77 ± 0.2 mg/dL among males in cases and 1.8 ± 0.23 mg/dL among females in cases. The mean aqueous magnesium was 1.69 ± 0.19 mg/dL among males in cases and 1.73 ± 0.26 mg/dL among females in cases. No statistically significant difference in mean aqueous magnesium between the gender (cases) was observed (p value>0.05). (Table 4, Graph 4)

Graph 4: Cluster bar chart showing comparison of serum magnesium and aqueous humor (AH) magnesium between gender (N=27)

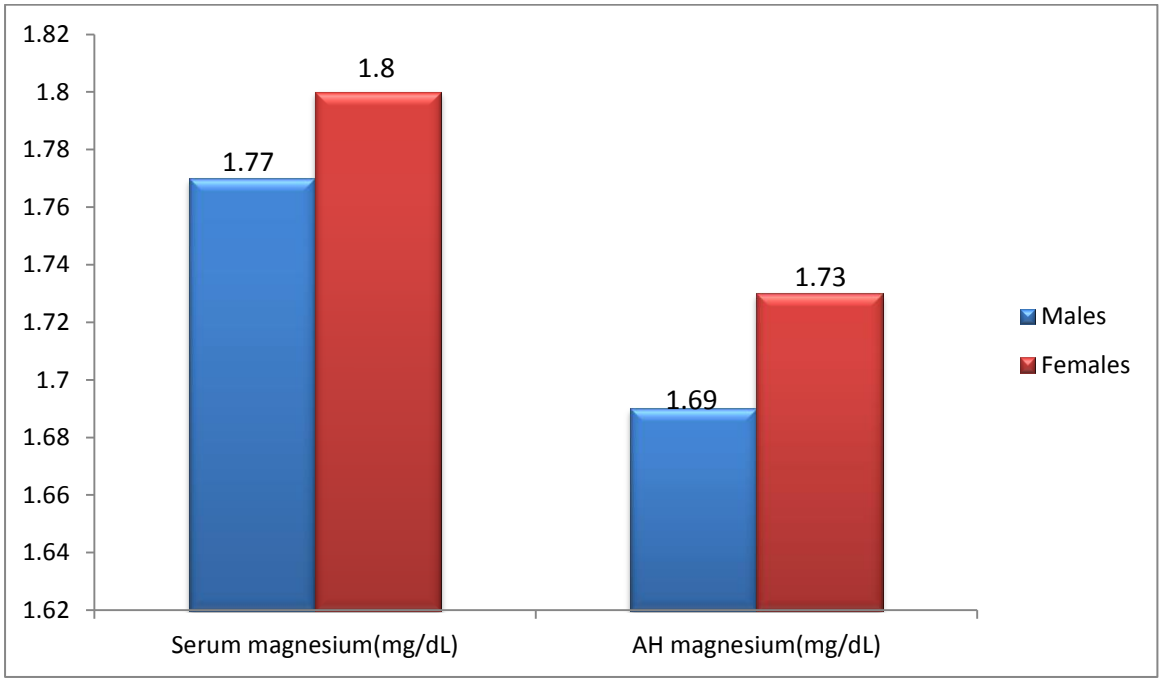


Table 5: Descriptive analysis of duration of T2DM given among cases (N=27)

Parameters	Mean ± SD	Median	Minimum	Maximum
Duration of T2DM	3.41 ± 2.8	3.0	0.0	10.0

The mean duration of T2DMamong the cases was 3.41 ± 2.8 years with maximum duration being T2DM. (Table 5)

Table 6: Correlation of duration of diabetes with serum and aqueous humor magnesium among cases in the study population (N=27)

Parameters		Serum magnesium	Aqueous humor magnesium
Duration of diabetes	Spearman Correlation Coefficient	-0.262	-0.206
	Sig. (2-tailed)	*0.188*	*0.303*

Among the study population, there was a weak negative correlation between duration of diabetes and serum magnesium (r value= -0.262) and the association was not statistically significant (p value 0.188). Among the study population, there was a weak negative correlation between duration of diabetes and aqueous magnesium (r value= -0.206) and the association was not statistically significant (p value 0.303). (Table 6)

Table 7: Distribution of medications given among cases (N=27)

Medications	Frequency	Percentage
Oral hypoglycemic agents (OHA)	26	96.30%
Medical Nutrition Therapy	1	3.70%

Majority of 26 (96.30%) participants had Oral Hypoglycemic Agents (OHA) as medications and 1 (3.70%) participant was started treatment with medical nutrition therapy which dietary control and regular exercise. (Table 7, Graph 5)

Graph 5: Bar chart for medications

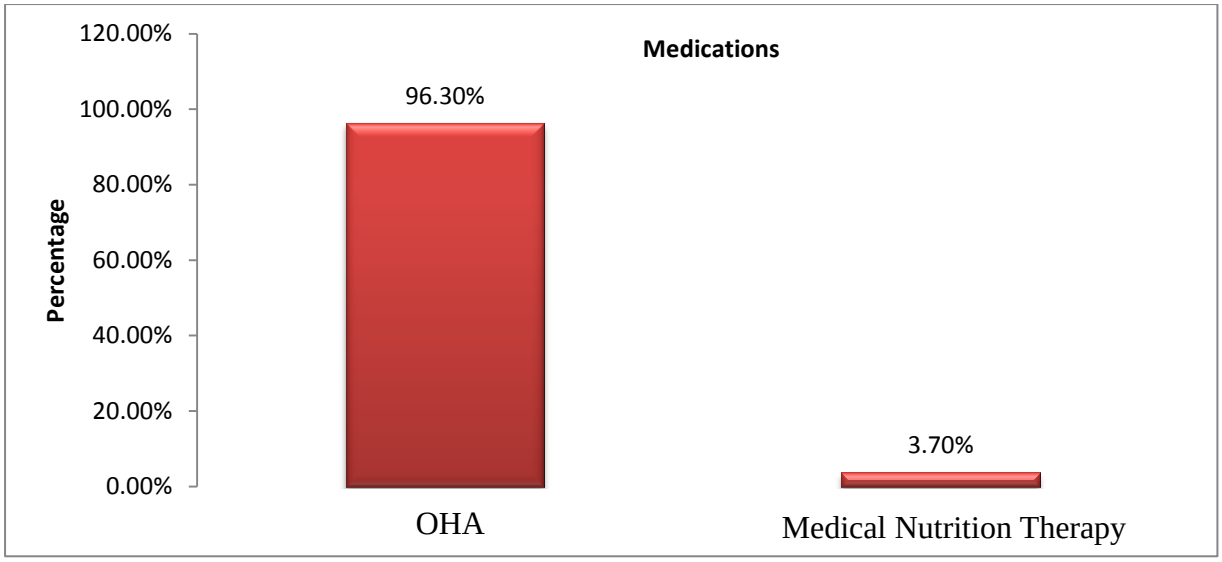
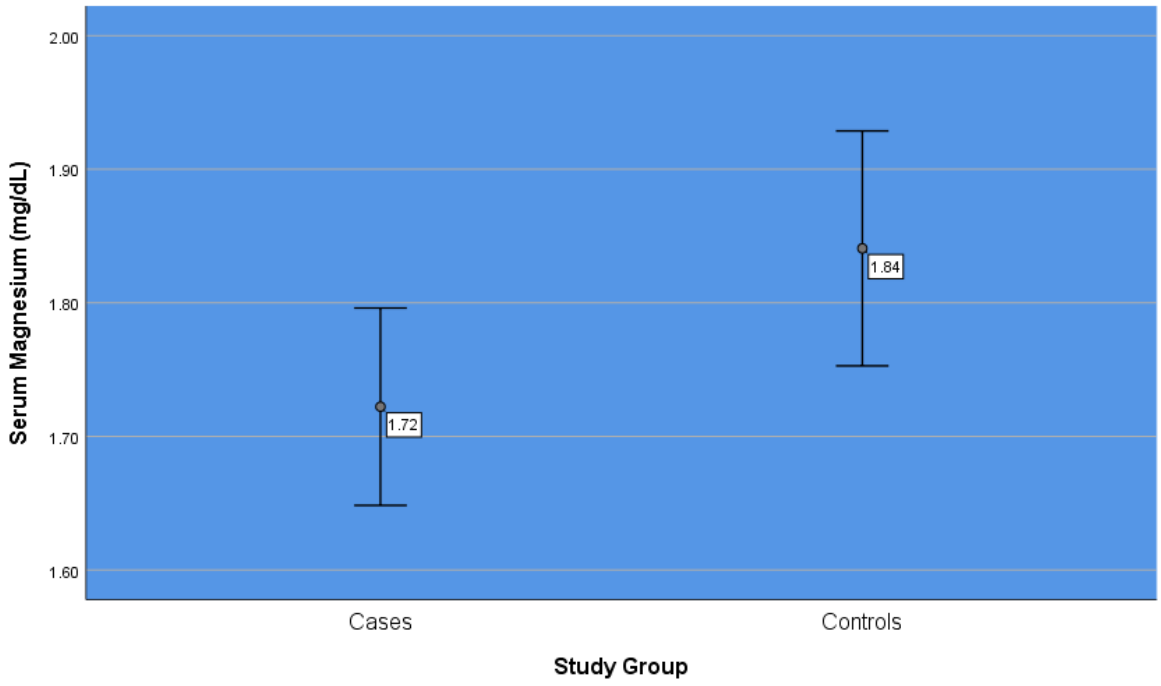


Table 8: Comparison of serum and aqueous humor magnesium between study group by Independent Samples t-test (N=54)

Parameter	Study Group [Mean±SD]		p value
	Cases (N=27)	Controls (N=27)	
Serum Magnesium (mg/dL)	1.72 ± 0.19	1.84 ± 0.22	0.039
Aqueous Humor Magnesium (mg/dL)	1.7 ± 0.21	1.83 ± 0.19	0.027

The mean serum magnesium was 1.72 ± 0.19 mg/dL among cases and 1.84 ± 0.22 mg/dL among controls. The mean aqueous magnesium was 1.7 ± 0.21 mg/dL among cases and 1.83 ± 0.19) mg/dL among controls. A statistically significant difference in mean serum magnesium and aqueous magnesium between the study group was observed (p value<0.05). (Table 8, Graph 6, Graph 7)

Graph 6: Error bar chart for comparison of Serum Magnesium between study group



Graph 7: Error bar chart for comparison of Aqueous Humor Magnesium between study group

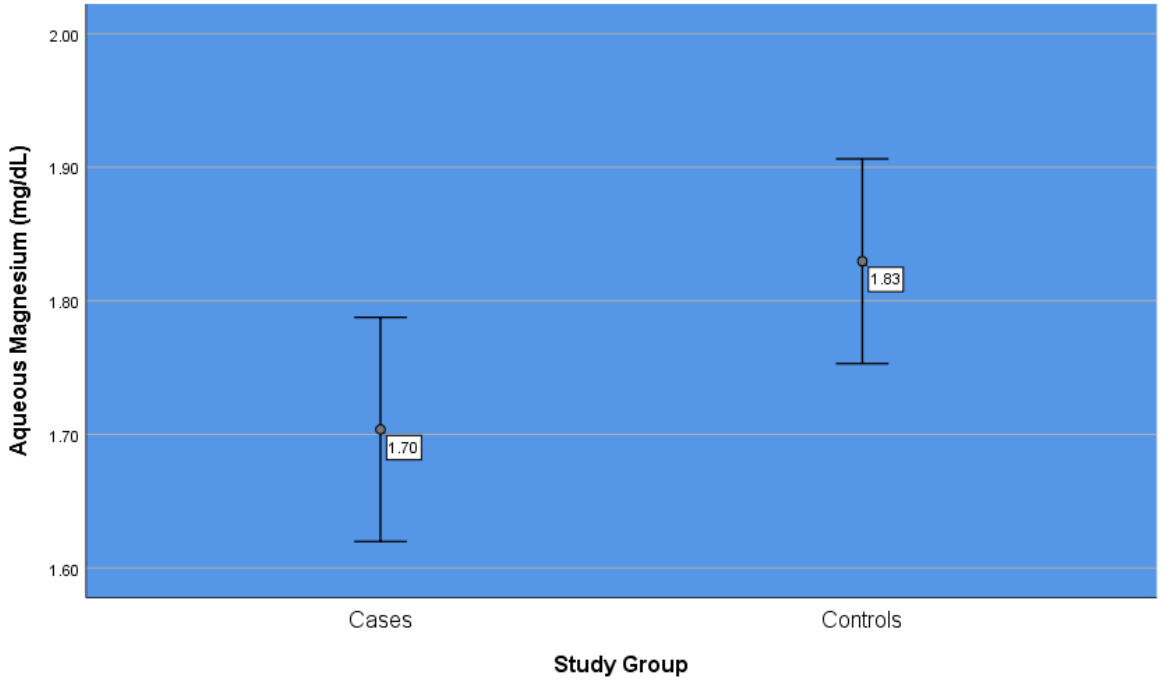


Table 9: Comparison of HbA1c levels between study group by Mann Whitney U test (N=54)

Parameter	Study Group [Median(IQR)]		p value
	Cases (N=27)	Controls (N=27)	
HbA1c (%)	7.50 (7.20 to 7.90)	5.70 (5.50 to 5.90)	<0.001

The median HbA1c was 7.50% (7.20 to 7.90) among cases and 5.70% (5.50 to 5.90) among controls. A statistically significant difference in median HbA1c concentration between the study group was observed (p value <0.05). (Table 9, Graph 8)

Graph 8: Box and whisker plot chart for comparison of HbA1c between study group

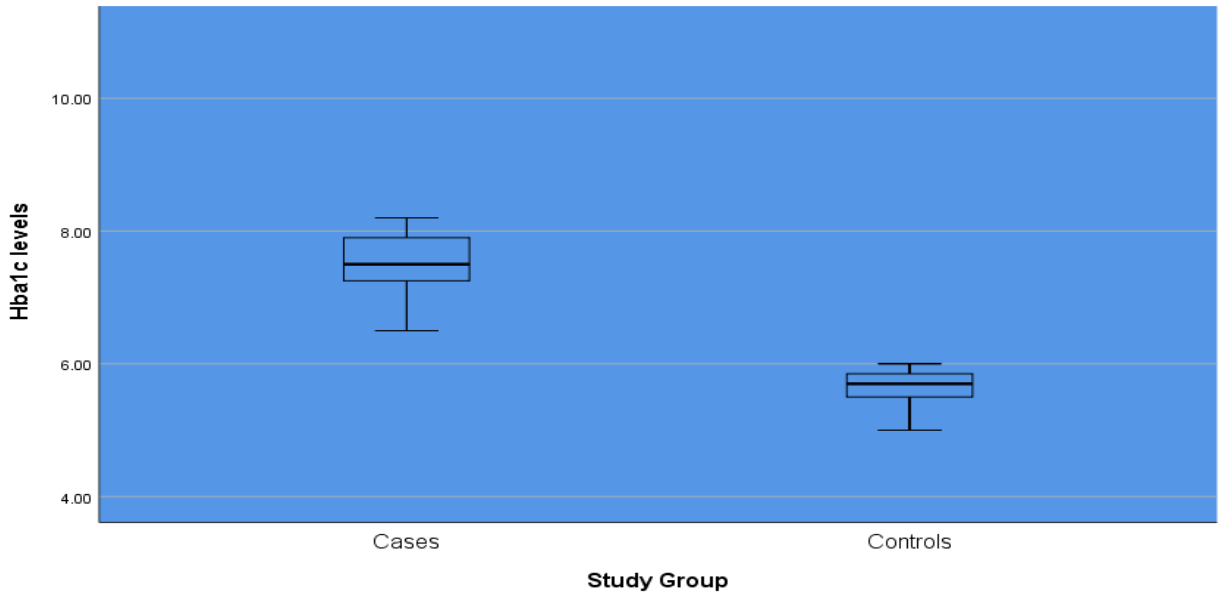
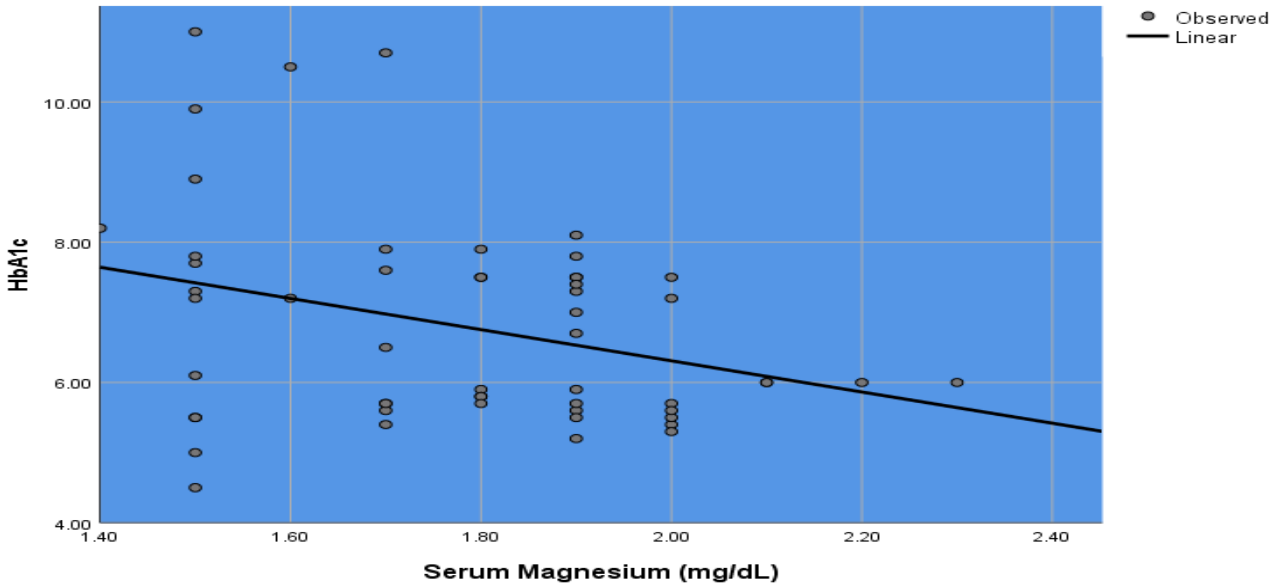


Table 10: Correlation of HbA1c with serum and aqueous humor magnesium in the study population (N=54)

Parameters		**Serum magnesium**	**Aqueous magnesium**
HbA1c levels	Pearson Correlation Coefficient	-0.153	-0.132
	Sig. (2-tailed)	*0.270*	*0.341*

Among the study population, there was a very weak negative correlation between HbA1c levels and serum magnesium (r value= -0.153) and the association was not statistically significant (p value 0.270). Among the study population, there was a very weak negative correlation between HbA1c levels and aqueous magnesium (r value= -0.132) and the association was not statistically significant (p value 0.341). (Table 10, Graph 9, Graph 10)

Graph 9: Scatter plot for correlation of HbA1c with serum magnesium in the study population



Graph 10: Scatter plot for correlation of HbA1c with aqueous humor magnesium in the study population

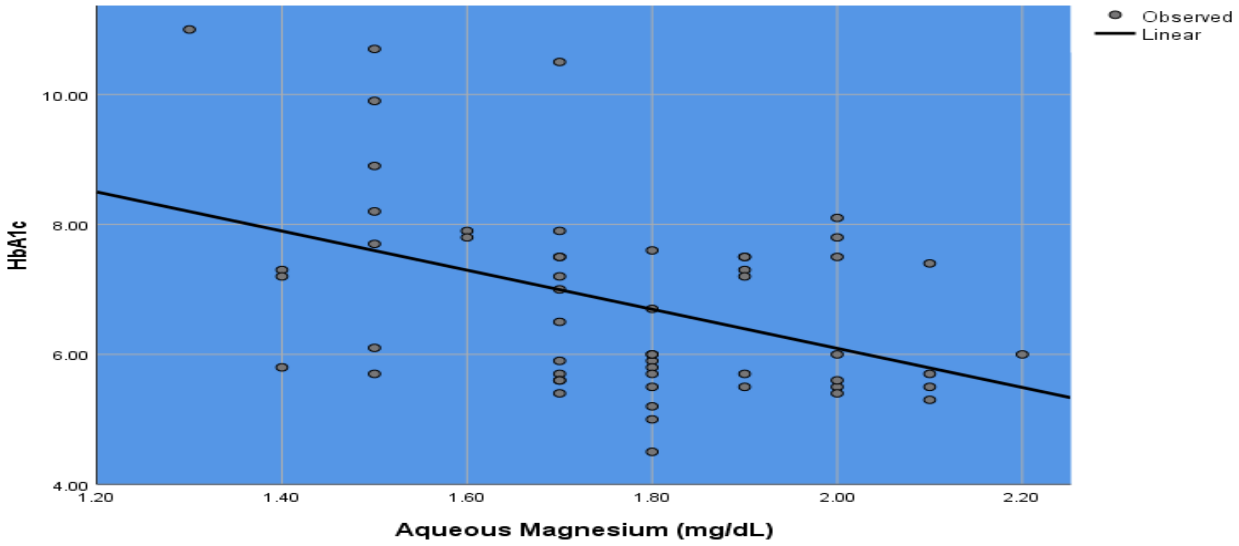


Table 11: Distribution of serum magnesium between HbA1c levels among cases (N=27)

Serum Magnesium (in mg/dL)	HbA1c levels %	
	<7 (N=3)	≥7 (N=24)
<1.5	0 (0%)	1 (4.17%)
1.5-1.7	2 (66.67%)	11 (45.83%)
>1.7	1 (33.33%)	12 (50%)

Among the cases in study population, the serum magnesium was <1.5 mg/dL for no participants with HbA1c <7% and 1 (4.17%) participants with HbA1c ≥7%. The serum magnesium was 1.5-1.7 mg/dL for 2 (66.67%) participants with HbA1c <7% and 11 (45.83%) participants with HbA1c ≥7%. The serum magnesium was >1.7 mg/dL for 1 (33.33%) participants with HbA1c <7% and 12 (50%) participants with HbA1c ≥7%. (Table 11, Graph 11)

Graph 11: Bar chart showing distribution of serum magnesium (Mg^{2+}) between HbA1c levels among cases

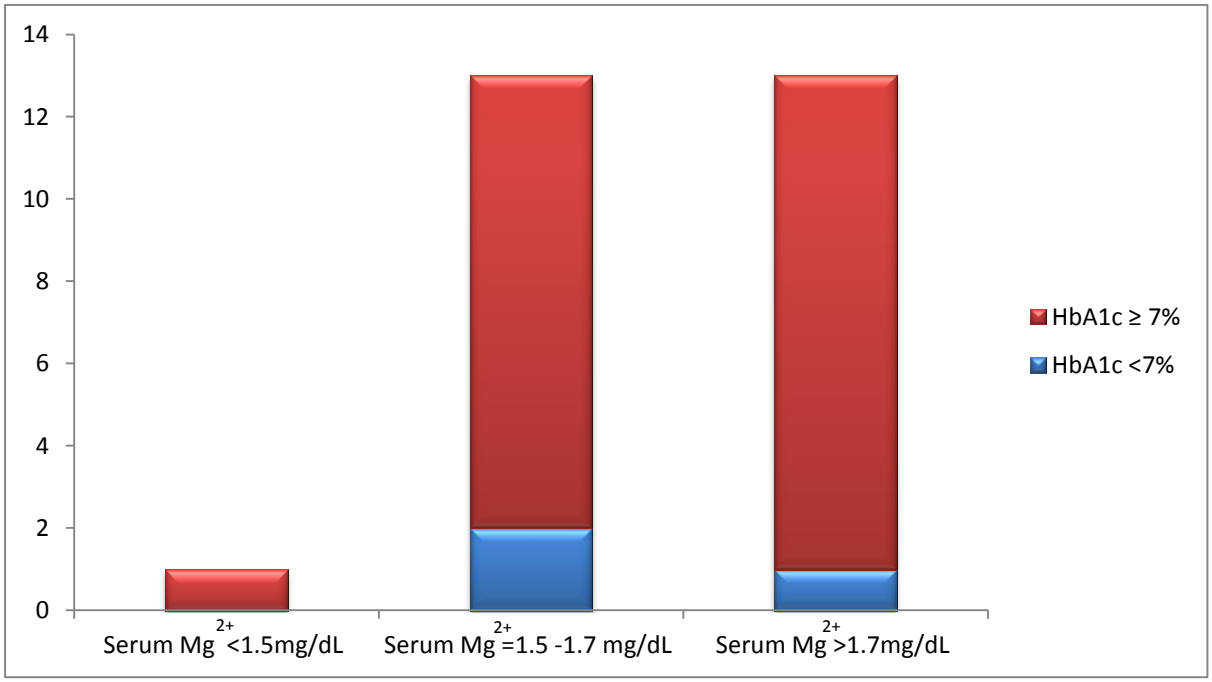


Table 12: Comparison of serum magnesium between HbA1c levels among cases (N=27)

Serum Magnesium (in mg/dL)	HbA1c levels%		Fisher exact p value
	<7 (N=3)	≥7 (N=24)	
<1.7	1 (33.33%)	9 (37.5%)	1.000
≥1.7	2 (66.67%)	15 (62.5%)	

Among the cases in study population, the serum magnesium was <1.7 mg/dL for 1 (33.33%) participant with HbA1c <7% and 9 (37.50%) participants with HbA1c ≥7%. The serum magnesium was ≥1.7 mg/dL for 2 (66.67%) participants with HbA1c <7% and 15 (62.5%) participants with HbA1c ≥7%. No statistically significant difference in serum magnesium between the HbA1c levels was observed (p value>0.05). (Table 12)

Table 13: Distribution of aqueous humor magnesium between HbA1c levels among cases (N=27)

Aqueous humor magnesium (in mg/dL)	HbA1c levels %	
	<7 (N=3)	≥7 (N=24)
<1.5	0 (0%)	3 (12.5%)
1.5-1.7	2 (66.67%)	12 (50%)
>1.7	1 (33.33%)	9 (37.5%)

Among the cases in study population, the aqueous magnesium was <1.5 mg/dL for no participants with HbA1c <7% and 3 (12.5%) participants with HbA1c ≥7%. The aqueous magnesium was 1.5-1.7 mg/dL for 2 (66.67%) participants with HbA1c <7% and 12 (50%) participants with HbA1c ≥7%. The aqueous magnesium was >1.7 mg/dL for 1 (33.33%) participants with HbA1c <7% and 9 (37.50%) participants with HbA1c ≥7%. (Table 13, Graph 12)

Graph 12: Bar chart showing distribution of aqueous humor (AH) magnesium between HbA1c levels among cases (N=27)

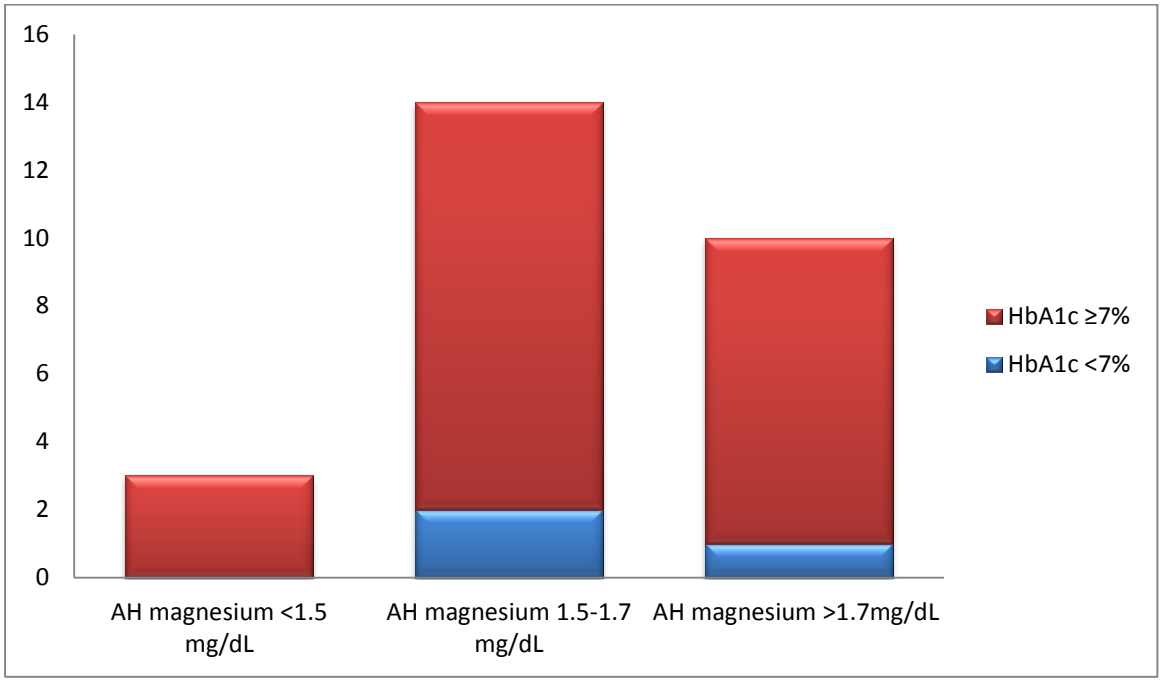


Table 14: Comparison of aqueous humor magnesium between HbA1c levels among cases (N=27)

Aqueous humor magnesium (in mg/dL)	HbA1c levels (%)		Fisher exact p value
	<7 (N=3)	≥7 (N=24)	
<1.7	1 (33.33%)	9 (37.5%)	1.000
≥1.7	2 (66.67%)	15 (62.5%)	

Among the cases in study population, the aqueous magnesium was <1.7 mg/dL for 1 (33.33%) participant with HbA1c <7% and 9 (37.50%) participants with HbA1c ≥7%. The aqueous magnesium was ≥1.7 mg/dL for 2 (66.67%) participants with HbA1c <7% and 15 (62.5%) participants with HbA1c ≥7%. No statistically significant difference in aqueous magnesium between the HbA1c levels was observed (p value>0.05). (Table 14)

Table 15: Correlation of HbA1c with serum and aqueous humor magnesium among cases in the study population (N=27)

Parameters		Serum magnesium	Aqueous humor magnesium
HbA1c levels	Pearson Correlation Coefficient	-0.301	-0.311
	Sig. (2-tailed)	*0.127*	*0.114*

Among the cases in study population, there was a weak negative correlation between HbA1c levels and serum magnesium (r value= -0.301) and the association was not statistically significant (p value 0.127). Among the study population, there was a weak negative correlation between HbA1c levels and aqueous magnesium (r value= -0.311) and the association was not statistically significant (p value 0.114). (Table 15)

Table 16: Distribution of fundus findings between study group (N=54)

Fundus	Study Group	
	Cases (N=27)	**Controls (N=27)**
Normal	16 (59.26%)	27 (100%)
CSME	1 (3.7%)	0 (0%)
Mild non-proliferative diabetic retinopathy	6 (22.22%)	0 (0%)
Mild non-proliferative diabetic retinopathy + CSME	1 (3.7%)	0 (0%)
Moderate non-proliferative diabetic retinopathy	1 (3.7%)	0 (0%)
Moderate non-proliferative diabetic retinopathy + CSME	1 (3.7%)	0 (0%)
ARMD	1 (3.7%)	0 (0%)

Among the cases in study population, the fundus was normal for 16 (59.26%) participants, CSME for 1 (3.7%) participants, mild non-proliferative diabetic retinopathy for 6 (22.22%) participants, mild non-proliferative diabetic retinopathy + CSME for 1 (3.7%) participants,

moderate non-proliferative diabetic retinopathy for 1 (3.7%) participants, moderate non-proliferative diabetic retinopathy + CSME for 1 (3.7%) participants ARMD and for 1 (3.7%) participants. Among the controls in study population, the fundus was normal for all 27 (100%) participants. (Table 16, Graph 13)

Graph 13: Pie chart showing distribution of fundus findings among diabetic cases (N=27)

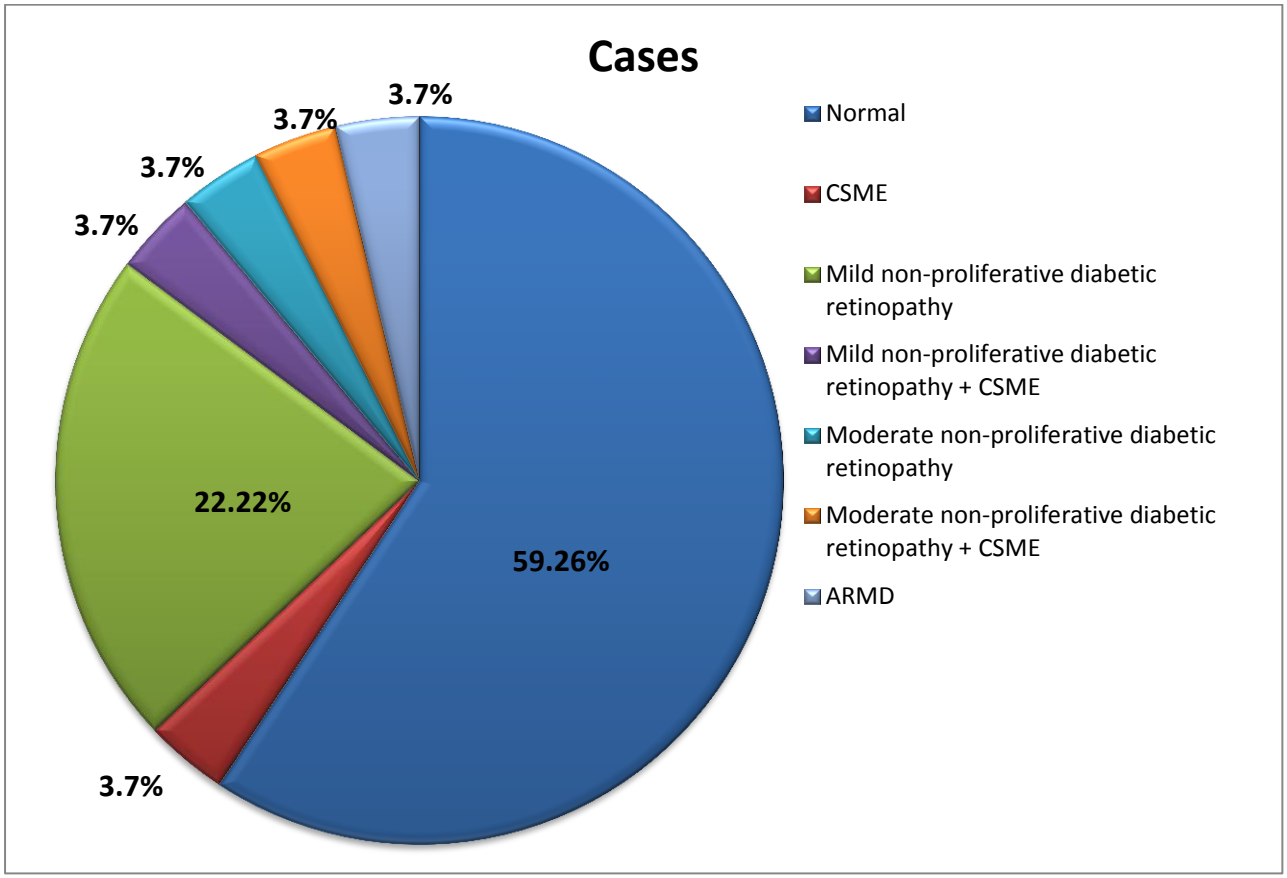


Table 17: Comparison of serum and aqueous magnesium in diabetic retinopathy cases by One-Way ANOVA test (N=27)

Parameters	Diabetic retinopathy				p value
	CSME	Mild NPDR	Moderate NPDR	Normal	
Serum Magnesium (mg/dL) (Mean ± SD)	1.6 ± 0.14	1.7 ± 0.19	1.7 ± 0.28	1.77 ± 0.17	0.595
Aqueous humor Magnesium (mg/dL) (Mean ± SD)	1.65 ± 0.21	1.67 ± 0.23	1.7 ± 0.28	1.74 ± 0.22	0.891

The mean serum magnesium was 1.6 ± 0.14 mg/dL in CSME, 1.7 ± 0.19 mg/dL in mild NPDR, 1.7 ± 0.28 mg/dL in moderate NPDR and 1.77 ± 0.17 mg/dL in normal diabetic retinopathy. The mean aqueous magnesium was 1.65 ± 0.21 mg/dL in CSME, 1.67 ± 0.23 mg/dL in mild NPDR, 1.7 ± 0.28 mg/dL in moderate NPDR and 1.74 ± 0.22 mg/dL in normal diabetic retinopathy. No statistically significant difference in mean serum magnesium and aqueous magnesium between the diabetic retinopathy was observed (p value>0.05). (Table 17, Graph 14)

Graph 14: Bar chart showing comparison of serum and aqueous humor magnesium in diabetic retinopathy (N=27)

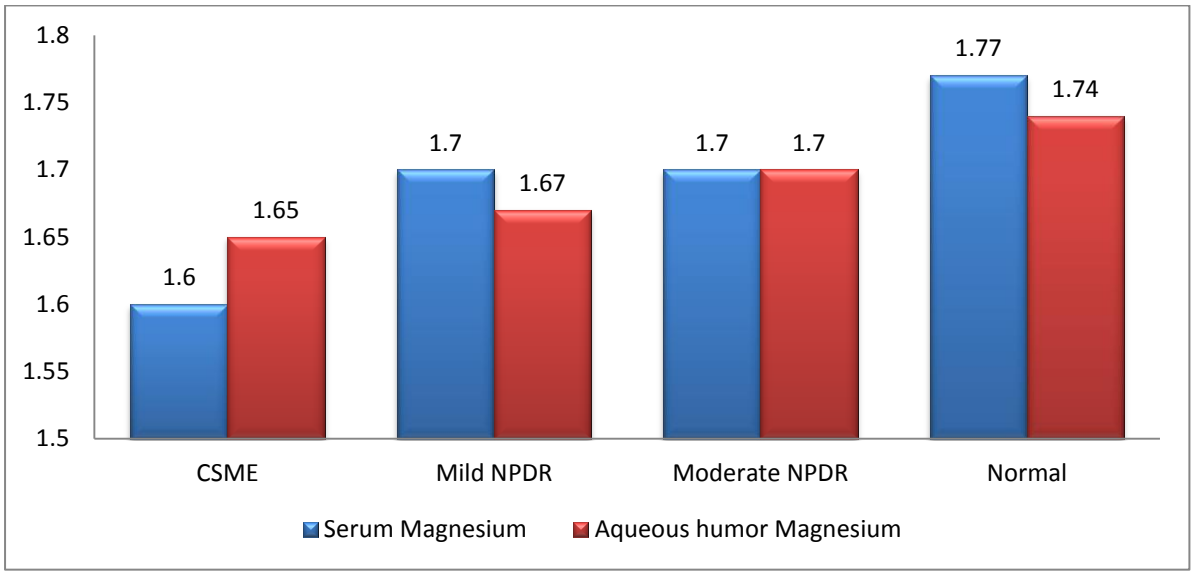
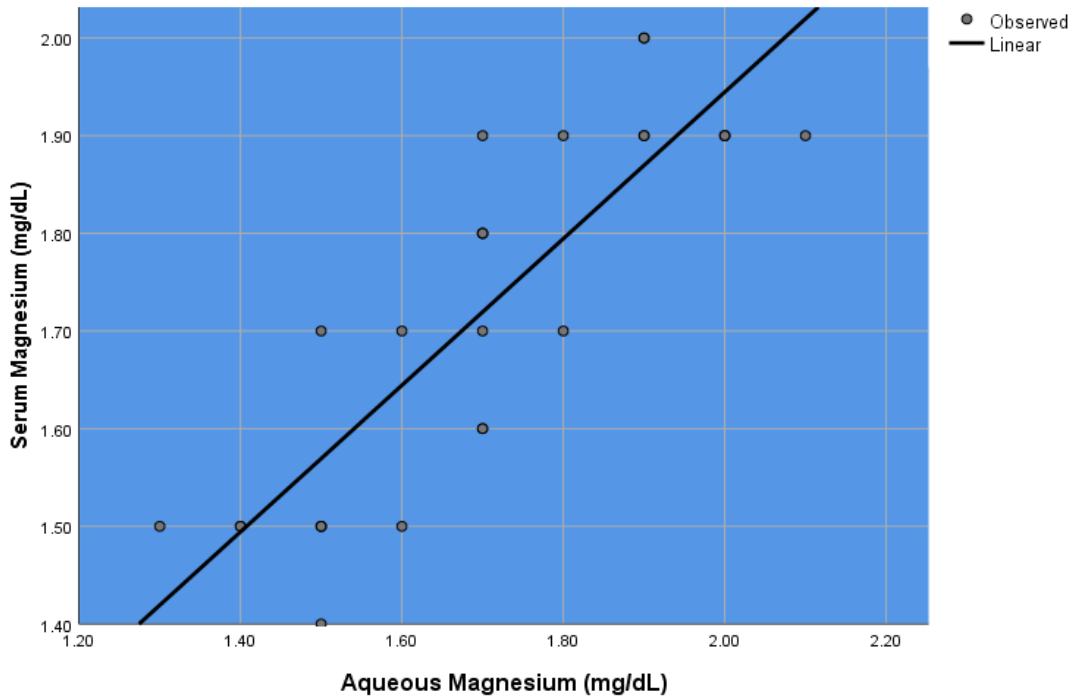


Table 18: Correlation of serum and aqueous magnesium among cases in the study population (N=27)

Parameters		**Aqueous magnesium**
Serum magnesium	Pearson Correlation Coefficient	0.852
	Sig. (2-tailed)	*<0.001*

Among the cases in the study population, there was a very strong positive correlation between serum magnesium and aqueous magnesium (r value= 0.852) and the association was statistically significant (p value <0.001). (Table 18, Graph 15)

Graph 15: Scatter plot for correlation of serum magnesium with aqueous magnesium in the study population (N=27)



DISCUSSION

DISCUSSION

The present study comprises of 54 patients divided into 2 groups based on their glycaemic status, each group comprising 27 patients each.

Group A included the cases i.e. senile cataracts diagnosed with Type 2 diabetes mellitus

Group B included the controls i.e. senile cataracts without diabetes mellitus

Studies done previously, suggest that an inverse correlation exists between serum magnesium levels and diabetic retinopathy changes. However, based on our literature review, correlation of aqueous humor magnesium levels with diabetic retinopathy status has not been established yet. Therefore, this study aims to do so.

Demography:

In our study, the mean age of subjects among Group A (cases) was 67.19 years and in Group B (controls) was 65.11 years. There was no significant difference in age distribution between two groups. Group A consisted of 10 females (37.04%) and 17 males (62.96%). Group B consisted of 11 females (40.74%) and 16 males (59.26%). There was no significant difference in gender distribution between two groups.

Correlation of age and sex with serum magnesium levels among diabetics remains ambiguous. While studies done by Zhang Y et al and Sudha S et al have demonstrated that, there exists a statistically significant difference between serum magnesium and age; a study by Arpaci D et al reported statistically insignificant relationship between the two. [150-152] Sudha S et al demonstrated 100% hypomagnesemia among diabetic patients above 70 years of age.[151] However, both Zhang Y et al and Sudha S et al demonstrated a statistically insignificant relationship between serum magnesium and sex. [150, 151] Daruka KM reported a statistically insignificant relationship of serum hypomagnesemia with both age and sex. [153]

In our study, we found that there was a statistically insignificant relationship of serum magnesium with age and gender among the diabetics. However, a statistically significant relationship of aqueous humor magnesium and age among the diabetics was observed. There was an insignificant relationship between aqueous humor magnesium and gender.

From our study, it was observed that aqueous humor magnesium levels are affected more by age. Patients <60 years had mean aqueous magnesium of 1.5 ± 0.16 mg/dL and 1.74 ± 0.2 mg/dL among age group ≥60 year. However, the significance of this should be further determined on a larger sample population.

Serum and aqueous humor magnesium with duration of diabetes:

Murthy and Palvai, Shashidhar G et al have reported a significant association between the duration of diabetes and serum magnesium levels, concluding that more the duration of diabetes, lower will be the serum magnesium levels. They concluded that hypomagnesemia starts increasing after 5 years of duration of diabetes, with maximum within 11-16 years of duration. [154, 155]

Arpaci D and Daruka et al demonstrated a statistically insignificant relationship of low serum magnesium with respect to the duration of diabetes. The average duration of diabetes in both the studies was around 6 years. [152, 153]

In our study, we found that there was a weak negative correlation of duration of diabetes with both serum and aqueous humor magnesium; however, this relationship was statistically insignificant. This can be substantiated by the fact that among the 27 cases in our study, the average duration of diabetes was 3.41 ± 2.8 years, and significant hypomagnesemia is seen after 5 years of diabetes duration as reported above. [152, 155]

M Rema et al have reported that diabetic retinopathy risk increases by 1.89 times for every five years increase in diabetes duration.[126]

HbA1c with serum and aqueous humor magnesium:

HbA1c level estimation is the most commonly used diagnostic test for diabetes providing a standardized criteria for accurate glycaemic measurements. The serum values reflect average plasma glucose levels over the previous eight to 12 weeks. The test does not require any special fasting conditions and can be performed at any time of the day. Thus, it is being used as a diagnostic as well as screening gold standard test. [156, 157] HbA1c has a distinct affinity towards oxygen. Therefore, increased HbA1c causes tissue anoxia and also affects the causality of diabetes related micro and macroangiopathy. [125]

Various studies done previously have compared the relationship between HbA1c levels and serum magnesium and established an inverse correlation between the two as reported by Bankir et al. [158]

Lu J et al, studied 3100 diabetic patients. They reported average HbA1c values to be 9.3% and serum hypomagnesemia of 0.87mmol/L which was statistically significant. [159] Sudha S et al revealed that 100% diabetics in 9-10% HbA1c range had hypomagnesemia, 94.11% patients with 7-8% HbA1c had hypomagnesemia. Only 34.48% of normal HbA1c patients reported hypomagnesemic. These values were statistically significant. [151] Shashidhar G et al reported that Serum magnesium levels were low (<1.7 mg/dL) when HbA1c was >9.80 and HbA1c was <7.20 then serum magnesium level was >1.7 mg/dL.[155]

Similarly, in our study, the median HbA1c values among diabetic cases and non- diabetic controls were 7.50% and 5.70% respectively. This value was statistically significant.

Among the diabetic cases, 24 patients had HbA1c value >7%, 45.83% and 50% of which had median serum and aqueous humor magnesium in the range of 1.5-1.7 mg/dL, respectively. No statistically significant difference was seen among the comparison groups. Even though, a weak negative correlation was observed between serum and aqueous humor magnesium with HbA1c levels, it was not statistically significant. This can be substantiated by the fact that

only controlled diabetic cases (mean HbA1c = 7.50%) were included in our study owing to the fact that cataract surgery cannot be performed in uncontrolled diabetics and studies by Kumar et al and Duruka KM et al reported that mean serum magnesium value was lowest in patients who had HbA1c values >8%. [18, 153]

In a study by Arpaci D et al who studied 673 diabetics, reported 502 patients to have HbA1c levels above or equal to 7% and established a statistically insignificant relationship of HbA1c with hypomagenesemic and normomagnesemic groups was found. [152]

Serum and aqueous humor magnesium with diabetic retinopathy and maculopathy:

Retinopathy in hypomagnesaemia results from vascular injuries in eye due to increased intracellular formation of advanced glycation end products, activation of protein kinase-C isoforms and stimulation of hexosamine pathway. [123]

Studies done by various authors have reported a statistically significant relationship between hypomagnesemia and diabetic retinopathy. They have demonstrated retinopathy changes at serum magnesium level <1.7 mg/dL. [19, 126, 123, 124, 151, 153-155]

In a study by Phadnis P et al, hypomagnesemia was noted in 22.64% patients with diabetic retinopathy, of which majority cases had nonproliferative diabetic retinopathy (NPDR), followed by proliferative diabetic retinopathy (PDR), and clinically significant macular edema (CSME). All the values were statistically significant. Hypomagnesemia showed positive correlation with the severity of diabetic retinopathy.[160] A cross sectional study by Kumar P et al demonstrated that maximum patients (62.7%) with serum magnesium <1.7 mg/dL, had NPDR and 21.8% had PDR, whereas among normomagnesemic patients, only 14.3% had NPDR and 8.6% had PDR. There was significant difference among these two groups ($P < 0.001$). [18]

Kareem I et al reported that the average concentration of serum magnesium in diabetic patients with retinopathy was 1.2meq/1. These values were lower compared to diabetic with no retinopathy and control groups. The values were statistically significant. [125]

Haddad NS and Zuhair S found that serum magnesium remained statistically significant among the groups of patients with different stages of retinopathy (p value <0.05). Such differences correlated negatively with advancing stages of retinopathy i.e. more advanced the stage of retinopathy, lower was the serum magnesium concentration. They also concluded that lowest level of serum magnesium was observed in patients with advanced retinopathy and maculopathy. [124]

Lu J et al demonstrated that associated retinopathy decreased by approximately 20% with every 0.1mmol/l increase in serum magnesium values. [159]

Arpaci D et al conducted a study on 673 diabetic patients to study the association between serum magnesium level, glycaemic regulation, and microvascular complications. They divided the study population based on serum magnesium values into low and normal magnesium levels. 8.2% patients in the study population had diabetic retinopathy. No difference for retinopathy was noted between the two groups. (p= 0.597).[152]

Fewer studies have compared a relationship between serum magnesium and diabetic maculopathy. A study by Haddad NS and Zuhair S showed that patients with maculopathy had the lowest value for the serum magnesium level (1.35 mg/dL), which was statistically significant. [124] It has been postulated that the maculopathy is due to increased microaneurysm leak because of damage to the endothelial cells. Serum hypomagnesemia promotes this endothelial cell damage. This further causes aggravated platelet aggregation and vascular calcifications and thus, thrombogenesis ensues. Decreased serum magnesium levels further stimulate proinflammatory and pro-fibrogenic reaction and decrease the production of protective enzymes involved in oxidative stress. [18, 124]

In our study, normal fundus study was seen in 16 (59.26%) of the 27 diabetic cases. Diabetic retinopathy changes were reported in 10 patients among whom, majority i.e. 7 (25.92%) patients had mild non-proliferative diabetic retinopathy (mild NPDR) followed by CSME in 11.11% patients and moderate non-proliferative diabetic retinopathy (moderate NPDR) in 7.40 % patients. The mean serum magnesium among mild and moderate NPDR cases was 1.7 mg/dL. These values were comparable to mean aqueous humor magnesium (1.67 mg/dL in mild NPDR and 1.7 mg/dL in moderate NPDR). Mean serum and aqueous humor magnesium among CSME patients was 1.60 mg/dL and 1.65 mg/dL respectively. Statistical insignificance among the groups could be attributed to a small sample size of the study population.

Serum and aqueous humor magnesium:

The chemical components of aqueous humor are derived principally from plasma by passive diffusion and the ciliary epithelium by active secretion. Cations such as Na^{+}, K^{+} and Mg^{2+} are present at similar concentrations in plasma and aqueous, Ca^{2+} is considerably lower in the aqueous than in plasma. The two principal anions in aqueous humor, CI^{-} and $HC03^{-}$, are also present at nearly the same concentrations as in plasma.[139]

In our study the median serum magnesium was 1.70 mg/dL among cases and 1.90 mg/dL among controls. The median aqueous magnesium was 1.70 mg/dL among cases and 1.80 mg/dL among controls. A statistically significant difference in median serum magnesium and aqueous magnesium between the study group was observed (p value<0.05).

A strong positive correlation was observed between serum and aqueous humor magnesium among the diabetic cases. This correlation was statistically significant. Thus, we propose that aqueous humor magnesium can be used as an effective biomarker in diabetes.

Limitations of study:

Our study does not study the therapeutic effect of magnesium supplementation on metabolic control and the associated enhancement or waning of diabetic retinopathy changes.

Serum magnesium is a relatively insensitive measurement of magnesium status of the body because the major bulk of magnesium is found within the cell. Thus, intra erythrocyte magnesium would give a better analysis of magnesium concentration.

A more detailed evidence of aqueous humor hypomagnesemia and its range can be obtained by solely performing an aqueous tap on a larger diabetic sample population. Since our study included aqueous humor aspiration as well as cataract surgery, we included only controlled diabetics because previous literature has shown progression of diabetic retinopathy changes after cataract surgery. [161-164]

CONCLUSION

CONCLUSION

A vicious cycle exists between serum hypomagnesemia and type 2 diabetes mellitus. Serum hypomagnesemia causes increased diabetic retinopathy changes as well. Since, the biological reference interval for aqueous humor has not been established yet; our study provides valuable information of normative data from the non-diabetic population in such aspects. In our study, the median serum and aqueous humor magnesium level among diabetic cases was lower than non-diabetic controls. The average HbA1c among the diabetics was 7.5%. Majority of the diabetic cases with HbA1c >7% had serum and aqueous humor magnesium in the range of 1.5-1.7 mg/dL. The most common retinopathy changes observed were mild nonproliferative diabetic retinopathy. The mean serum and aqueous humor magnesium among these cases was 1.7 mg/dL and 1.67 mg/dL. A strong positive correlation was observed between serum and aqueous humor magnesium, thus giving insight into its use as a potential biomarker in future and a more accurate estimation and prediction of diabetes retinopathy changes. However, since it is an invasive procedure, further studies on a bigger sample population should be conducted to establish its complete role.

SUMMARY

SUMMARY

This case control study was conducted on 54 patients fulfilling the inclusion criteria, undergoing cataract surgery and admitted under the Department of Ophthalmology of R. L. Jalappa Hospital and Research Center, Kolar, from December 2019 to May 2021, after obtaining ethical clearance from Institutional Ethical Committee of Sri Devaraj Urs Medical College and written informed consent from the subjects.

The study population consisted of 2 groups, Group A included patients with senile cataract & type 2 diabetes mellitus and Group B included non-diabetics with senile cataract.

All patients underwent standard cataract evaluation and fundus findings were documented. Blood samples were drawn for calculating serum magnesium and HbA1c levels for the patients included. 5 units of aqueous humor sample was drawn in an insulin syringe intraoperatively while performing cataract surgery before entering the globe. All samples were analysed in Central Diagnostic Laboratory Services (CDLS) attached to R.L. Jalappa Hospital.

The median serum magnesium level among cases and controls was 1.70 mg/dL and 1.90 mg/dL respectively. The median aqueous humor magnesium levels among cases and controls were 1.70 mg/dL and 1.80 mg/dL respectively. The average HbA1c among the diabetics and non-diabetics controls was 7.5% and 5.70% respectively. All the above values were statistically significant ($P < 0.05$). Majority of the diabetic cases with HbA1c >7% had serum and aqueous humor magnesium in the range of 1.5-1.7 mg/dL.

Diabetic retinopathy was observed among 10 of 27 diabetic cases, majority of which had mild non proliferative diabetic retinopathy (NPDR), followed by moderate non proliferative diabetic retinopathy and clinically significant macular edema (CSME). The mean serum and

aqueous humor magnesium among these mild NPDR cases was 1.7 mg/dL and 1.67 mg/dL. Moderate NPDR cases showed mean of 1.7 mg/dL serum and aqueous humor magnesium and 1.6 mg/dL and 1.65 mg/dL, respectively among CSME cases.

A strong positive correlation was noted among serum and aqueous humor magnesium for the diabetic cases indicating its effective use as a biomarker in diabetic retinopathy evaluation.

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ANNEXURE

ANNEXURE- I

CASE PROFORMA

Group:	Case no:
Name:	Date:
Age:	IP no:
Sex:	DOA:
Occupation:	DOS:
Address:	

Chief complaints:

History of Presenting illness:

Past history:

DM/HTN/BA/Epilepsy

Family history:

Personal history:

Appetite – Sleep – Bowel –

Diet – Habits – Bladder –

GPE:

Pallor / Edema /Icterus / Cyanosis / Clubbing / Lymphadenopathy

Vital signs:

a. Pulse – c) RR –

b. BP – d) Temp –

Systemic examination:

a. CVS – c. RS –

b. PA – d. CNS –

OCULAR EXAMINATION				
	RE		**LE**	
1. Head posture 2. Ocular posture 3. Facial symmetry				
4. Ocular movements				
5. Visual Acuity a) Distant b) Near				
6. Anterior Segment				
7. Fundus (IDO & Slit Lamp +90D)				
8. B Scan				
9. Keratometry K1 K2				
10. Axial Length				
11. Intraocular Lens Power				
12. Intraocular Pressure				
13. Lab Investigations a. RBS b. Glycosylated haemoglobin c. Serum Magnesium d. Aqueous humor magnesium e. Serum creatinine f. Serum urea				
14. Intraoperative Complications				
15. Postoperative Visual Acuity		1 day	1 week	I month
Distant vision				
Near Vision				

ANNEXURE- II

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,TAMAKA, KOLAR - 563101.

PATIENT INFORMATION SHEET

TITLE: "ROLE OF SERUM AND AQUEOUS HUMOR MAGNESIUM LEVELS AS A MARKER IN ASSESSMENT OF DIABETIC RETINOPATHY IN TYPE 2 DIABETIC AND NON DIABETIC PATIENTS."

The selected patient is invited to take part voluntarily in this research study, it is important that you read and understand the purpose, procedure, benefits and discomforts of the study.

1. Purpose of this study is to To evaluate the interelation between diabetic retinopathy and serum and aqueous humor magnesium levels.

2. The various investigations being used are detailed history, visual acuity by Snellens chart, grading of cataract by slit lamp examination, intraocular pressure by applanation tonometer, keratometry and A scan for IOL power calculation, fundus evaluation by direct and indirect ophthalmoscopy. Blood samples for HbA1c levels, and serum magnesium levels and Aqueous humor sample for estimaton of magnesium levels. The blood and aqueous humor invesigations cost, it will be borne by the researcher. These tests are not associated with any risk.

3. Your Participation in this research study may not change the final outcome of your eye condition. However, patients in the future may benefit as a result of knowledge gained from this study. Your taking part in this study is entirely voluntary. You may refuse to take part in the study or you may stop your participation in the study at any time, without a penalty or loss of any benefits to which you were otherwise entitled.

CONFIDENTIALITY

Your medical information will be kept confidential by the study doctor and staff and will not be made publicly available. Your original records may be reviewed by your doctor or ethics review board. For further information/ clarification please contact.

Dr. Aastha Garg

Junior Resident

Department of ophthalmology

SRI DEVARAJ URS MEDICAL ACADEMY

TAMAKA, KOLAR

Contact no:9535399137

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR - 563101.

ರೋಗಿಯ ಮಾಹಿತಿ ಪತ್ರ

ಈ ಮಾಹಿತಿಯು ಅಧ್ಯಯನದ ಉದ್ದೇಶವನ್ನು ಅರ್ಥಮಾಡಿಕೊಳ್ಳಲು ನಿಮಗೆ ಸಹಾಯ ಮಾಡುತ್ತದೆ "ಟೈಪ್ 2 ಮಧುಮೇಹದ ರೆಟಿನೋಪತಿಯ ಮತ್ತು ಮಧುಮೇಹವಿಲ್ಲದ ರೋಗಿಯ ಮೌಲ್ಯಮಾಪನದಲ್ಲಿ ಸೀರಮ್ ಮೆಗ್ನೀಸಿಯಮ್ ಮತ್ತು ಅಕ್ಯೂಸ್ ಹ್ಯೂಮರ್ ಮ್ಯಾಗ್ನೆಸಿಯಮ್ ಮಾರ್ಕರ್‌ಗಳು ಪಾತ್ರ"

ಡಯಾಬಿಟಿಕ್ ಮತ್ತು ನಾನ್ ಡಯಾಬಿಟಿಕ್ ರೋಗಿಗಳು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮನ್ನು ಆಹ್ವಾನಿಸಲಾಗಿದೆ. , ಅಧ್ಯಯನದ ಉದ್ದೇಶ, ಕಾರ್ಯವಿಧಾನ, ಪ್ರಯೋಜನಗಳು ಮತ್ತು ಅಸ್ವಸ್ಥತೆಗಳನ್ನು ನೀವು ಓದುವುದು ಮತ್ತು ಅರ್ಥಮಾಡಿಕೊಳ್ಳುವುದು ಬಹಳ ಮುಖ್ಯ.

1. ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶವೆಂದರೆ ಮಧುಮೇಹ ರೆಟಿನೋಪತಿ ಮತ್ತು ಸೀರಮ್ ಮೆಗ್ನೀಸಿಯಮ್ ಮಟ್ಟಗಳು ಮತ್ತು ಅಕ್ಯೂಸ್ ಹ್ಯೂಮರ್ ಮೆಗ್ನೀಸಿಯಮ್ ಮಟ್ಟಗಳ ನಡುವಿನ ಪರಸ್ಪರ ಸಂಬಂಧವನ್ನು ಮೌಲ್ಯಮಾಪನ ಮಾಡುವುದು.
2. ಬಳಸಲಾಗುವ ವಿವಿಧ ತನಿಖೆಗಳು ವಿವರವಾದ ಇತಿಹಾಸ, ಸ್ನೆಲೆನ್ಸ್ ಚಾರ್ಟ್ ದೃಷ್ಟಿ ತೀಕ್ಷ್ಣತೆ, ಸ್ಲಿಟ್ ಲ್ಯಾಂಪ್ ಪರೀಕ್ಷೆಯಿಂದ ಕಣ್ಣಿನ ಪೊರೆಯ ಶ್ರೇಣೀಕರಣ, ಅಪ್ಲ್ಯಾನೇಷನ್ ಟೋನೊಮೀಟರ್ ಮೂಲಕ ಕಣ್ಣಿನ ಒತ್ತಡ, ಕೆರಾಟೊಮೆಟ್ರಿ ಮತ್ತು ಐಒಎಲ್ ವಿದ್ಯುತ್ ಲೆಕ್ಕಾಚಾರಕ್ಕಾಗಿ ಸ್ಕ್ಯಾನ್, ನೇರ ಮತ್ತು ಪರೋಕ್ಷ ನೇತ್ರವಿಜ್ಞಾನದ ಫಂಡಸ್ ಮೌಲ್ಯಮಾಪನ. ಎಚ್‌ಬಿಎ 1 ಸಿ ಮಟ್ಟಗಳಿಗೆ ರಕ್ತದ ಮಾದರಿಗಳು, ಮತ್ತು ಸೀರಮ್ ಮೆಗ್ನೀಸಿಯಮ್ ಮಟ್ಟಗಳು. ಮೆಗ್ನೀಸಿಯಮ್ ಮಟ್ಟವನ್ನು ಅಂದಾಜು ಮಾಡಲು ಅಕ್ಯೂಸ್ ಹ್ಯೂಮರ್ ಮಾದರಿ. ಈ ಪರೀಕ್ಷೆಗಳು ಯಾವುದೇ ಅಪಾಯದೊಂದಿಗೆ ಸಂಬಂಧ ಹೊಂದಿಲ್ಲ. ಸೀರಮ್ ಮೆಗ್ನೀಸಿಯಮ್ ಮಟ್ಟಗಳು ಮತ್ತು ಅಕ್ಯೂಸ್ ಹ್ಯೂಮರ್ ಮೆಗ್ನೀಸಿಯಮ್ ಮಟ್ಟಗಳ ನಡುವಿನ ಪರಸ್ಪರ ಸಂಬಂಧವನ್ನು ಆವಿಷ್ಕಾರಗಳಿಗೆ, ಅದನ್ನು ಸಂಶೋಧಕರು ಭರಿಸುತ್ತಾರೆ.
3. ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆಯು ನಿಮ್ಮ ಕಣ್ಣಿನ ಸ್ಥಿತಿಯ ಅಂತಿಮ ಫಲಿತಾಂಶವನ್ನು ಬದಲಾಯಿಸುವುದಿಲ್ಲ. ಆದಾಗ್ಯೂ, ಈ ಅಧ್ಯಯನದಿಂದ ಪಡೆದ ಜ್ಞಾನದ ಪರಿಣಾಮವಾಗಿ ಭವಿಷ್ಯದಲ್ಲಿ ರೋಗಿಗಳು ಪ್ರಯೋಜನ ಪಡೆಯಬಹುದು.. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನೀವು ಭಾಗವಹಿಸುವುದು ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿರಾಕರಿಸಬಹುದು ಅಥವಾ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವ ಮೊದಲು ನಿಮಗೆ ಅರ್ಹತೆ ದೊರೆತ ಯಾವುದೇ ಪ್ರಯೋಜನಗಳ ದಂಡ ಅಥವಾ ನಷ್ಟವಿಲ್ಲದೆ ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವುದನ್ನು ನಿಲ್ಲಿಸಬಹುದು.

ಗೌಪ್ಯತೆ

ನಿಮ್ಮ ವೈದ್ಯಕೀಯ ಮಾಹಿತಿಯನ್ನು ಅಧ್ಯಯನ ವೈದ್ಯರು ಮತ್ತು ಸಿಬ್ಬಂದಿ ಗೌಪ್ಯವಾಗಿಡುತ್ತಾರೆ ಮತ್ತು ಸಾರ್ವಜನಿಕವಾಗಿ ಲಭ್ಯವಾಗುವುದಿಲ್ಲ. ನಿಮ್ಮ ಮೂಲ ದಾಖಲೆಗಳನ್ನು ನಿಮ್ಮ ವೈದ್ಯರು ಅಥವಾ ನೈತಿಕ ಪರಿಶೀಲನಾ ಮಂಡಳಿಯು ಪರಿಶೀಲಿಸಬಹುದು. ಹೆಚ್ಚಿನ ಮಾಹಿತಿಗಾಗಿ / ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ದಯವಿಟ್ಟು ಸಂಪರ್ಕಿಸಿ

ಡಾ ಆಸ್ತಾ ಗರ್ಗ್
ನೇತ್ರಶಾಸ್ತ್ರ ವಿಭಾಗ
ಶ್ರೀ ದೇವರಾಜ್ ಯುಆರ್ಎಸ್ ಮೆಡಿಕಲ್ ಅಕಾಡೆಮಿ
ತಮಾಕಾ ಕೋಲಾರ
ಸಂಪರ್ಕ ಸಂಖ್ಯೆ: 9535399137

ANNEXURE- III

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR - 563101.

INFORMED CONSENT FORM

Group:

Case no:

IP no:

TITLE: **ROLE OF SERUM AND AQUEOUS HUMOR MAGNESIUM LEVELS AS A MARKER IN ASSESSMENT OF DIABETIC RETINOPATHY IN TYPE 2 DIABETIC AND NON DIABETIC PATIENTS."**

I, the patient, agree to participate in this study and authorize the collection and disclosure of my personal information as outlined in this consent form.

I understand the purpose of this study, the risks and benefits of the technique and the confidential nature of the information that will be collected and disclosed during the study.

The information collected will be used only for research.

I have had the opportunity to ask questions regarding the various aspects of this study and my questions have been answered to my satisfaction.

I understand that I remain free to withdraw from this study at any time and this will not change my future care.

Participation in this research project does not involve any financial burden to me.

Name	Signature	Date	Time
Patient:			
Witness1:			
Witness 2:			
Primary Investigator/ Doctor:			

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR - 563101.

ರೋಗಿ **ಮಾಹಿತಿಯ ಬರೆಯಲಾಗಿದ ಒಪ್ಪಿಗೆ**

"ಟೈಪ್ 2 ಮಧುಮೇಹದ ರೆಟಿನೋಪತಿಯ ಮತ್ತು ಮಧುಮೇಹವಿಲ್ಲದ ರೋಗಿಯ ಮೌಲ್ಯಮಾಪನದಲ್ಲಿ ಸೀರಮ್ ಮೆಗ್ನೀಸಿಯಮ್ ಮತ್ತು ಅಕ್ಯೂಸ್ ಹ್ಯೂಮರ್ ಮ್ಯಾಗ್ನೆಸಿಯಮ್ ಮಾರ್ಕರ್‌ಗಳು ಪಾತ್ರ"

ಈ ಸಂಶೋಧನೆಗೆ ರೋಗಿಯ ಗುರುತಿನ ಸಂಖ್ಯೆ:

ಐಪಿ ಸಂಖ್ಯೆ:

ನಾನು, ರೋಗಿ, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪುತ್ತೇನೆ ಮತ್ತು ಈ ಒಪ್ಪಿಗೆಯ ರೂಪದಲ್ಲಿ ವಿವರಿಸಿರುವಂತೆ ನನ್ನ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯ ಸಂಗ್ರಹ ಮತ್ತು ಬಹಿರಂಗಪಡಿಸುವಿಕೆಯನ್ನು ಅಧಿಕೃತಗೊಳಿಸುತ್ತೇನೆ.

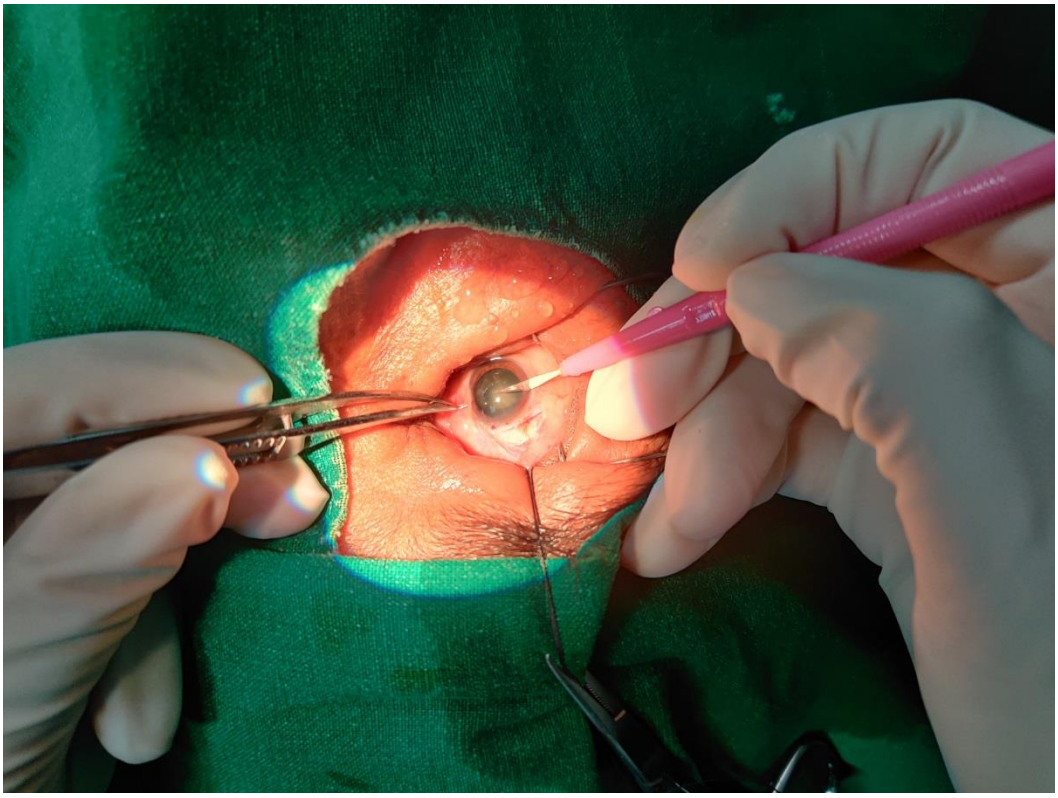
ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶ, ತಂತ್ರದ ಅಪಾಯಗಳು ಮತ್ತು ಪ್ರಯೋಜನಗಳು ಮತ್ತು ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಸಂಗ್ರಹಿಸಿ ಬಹಿರಂಗಪಡಿಸುವ ಮಾಹಿತಿಯ ಗೌಪ್ಯ ಸ್ವರೂಪವನ್ನು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.
ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಸಂಶೋಧನೆಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ.

ಈ ಅಧ್ಯಯನದ ವಿವಿಧ ಅಂಶಗಳಿಗೆ ಸಂಬಂಧಿಸಿದಂತೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುವ ಅವಕಾಶ ನನಗೆ ಸಿಕ್ಕಿದೆ ಮತ್ತು ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನ್ನ ತೃಪ್ತಿಗೆ ಉತ್ತರಿಸಲಾಗಿದೆ.

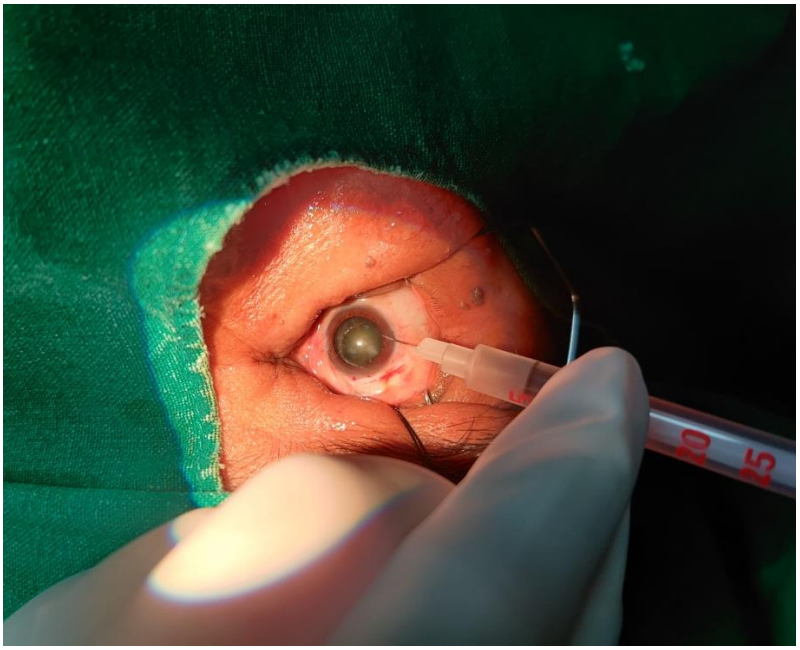
ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಈ ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಲು ಮುಕ್ತನಾಗಿರುತ್ತೇನೆ ಮತ್ತು ಇದು ನನ್ನ ಭವಿಷ್ಯದ ಆರೈಕೆಯನ್ನು ಬದಲಾಯಿಸುವುದಿಲ್ಲ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.
ಈ ಸಂಶೋಧನಾ ಯೋಜನೆಯಲ್ಲಿ ಭಾಗವಹಿಸುವುದರಿಂದ ನನಗೆ ಯಾವುದೇ ಆರ್ಥಿಕ ಹೊರೆ ಬರುವುದಿಲ್ಲ.

ಹೆಸರು	ಸಹಿ	ದಿನಾಂಕ	ಸಮಯ
ರೋಗಿಯ:			
ಸಾಕ್ಷಿ 1:			
ಸಾಕ್ಷಿ 2:			
ಪ್ರಾಥಮಿಕ ತನಿಖೆದಾರ / ಡಾಕ್ಟರ್:			

ANNEXURE– IV



Photograph 1- RE: Lance tip blade being used to make side port entry 9 o'clock position



Photograph 2- RE:5 Units of Aqueous Humor sample being aspirated in insulin syringe

Photograph 3- Vitros 5.1FS dry chemistry auto analyser machine

Photograph 4- Processing of subject's aqueous humor sample

Photograph 5- Processing of subject's serum magnesium sample

MASTER CHART

ANNEXURE V

KEY TO MASTER CHART

T2DM –Type 2 Diabetes Mellitus

S. Mg (mg/dL) –Serum Magnesium concentration in mg/dL

AH Mg (mg/dL) – Aqueous humor Magnesium concentration in mg/dL

OHA –Oral Hypoglycaemic Agents

Mild NPDR – Mild Nonproliferative Diabetic Retinopathy

Moderate NPDR – Moderate Nonproliferative Diabetic Retinopathy

CSME – Clinically Significant Macular Edema

MASTER CHART FOR CASES									
UHID NO	**AGE**	**SEX**	**T2DM**	**DURATION**	**MEDICATION**	**S. Mg (mg/dL)**	**AH Mg (mg/dL)**	**HbA1c**	**FUNDUS**
899837	71	F	YES	NEWLY DIAGNOSED	OHA	1.8	1.7	7.5	NORMAL
901795	75	M	YES	2 YEARS	OHA	1.8	1.7	7.9	NORMAL
909698	52	M	YES	6 YEARS	OHA	1.9	1.9	7.5	MILD NPDR
908520	68	M	YES	10 YEARS	OHA	1.7	1.5	10.7	MODERATE NPDR
908598	55	M	YES	5 YEARS	OHA	1.9	2	7.8	NORMAL
933375	77	M	YES	2 YEARS	OHA	1.9	2	7.5	NORMAL
933381	67	F	YES	4 YEARS	OHA	2	1.9	7.5	MILD NPDR
933379	70	M	YES	3 YEARS	OHA	1.5	1.5	7.7	NORMAL
933377	73	M	YES	2 YEARS	OHA	1.6	1.7	7.2	NORMAL
933373	68	F	YES	NEWLY DIAGNOSED	OHA	1.9	1.9	7.3	NORMAL
933385	86	F	YES	2 YEARS	OHA	1.7	1.8	7.6	NORMAL
933376	60	M	YES	1 YEAR	OHA	1.9	2.1	7.4	CSME
934942	70	F	YES	5 YEARS	OHA	1.5	1.4	7.3	NORMAL
938602	65	F	YES	4 YEARS	OHA	1.4	1.5	8.2	MILD NPDR
938641	50	M	YES	6 YEARS	OHA	1.9	2	8.1	ARMD
941809	73	F	YES	NEWLY DIAGNOSED	OHA	2	1.9	7.2	NORMAL
941227	60	M	YES	NEWLY DIAGNOSED	OHA	1.5	1.3	11	NORMAL
943750	52	M	YES	2 YEARS	MEDICAL NUTRITION THERAPY	1.6	1.7	10.5	NORMAL
943766	69	M	YES	2 YEARS	OHA	1.5	1.5	9.9	MILD NPDR
946717	70	F	YES	8 YEARS	OHA	1.7	1.7	6.5	MILD NPDR + CSME
946716	65	M	YES	10 YEARS	OHA	1.9	1.8	6.7	NORMAL
948399	66	M	YES	3 YEARS	OHA	1.5	1.4	7.2	MILD NPDR
948405	75	F	YES	3 YEARS	OHA	1.7	1.6	7.9	MILD NPDR
948411	70	F	YES	1 YEAR	OHA	1.5	1.5	6.1	NORMAL
948427	65	M	YES	6 YEARS	OHA	1.5	1.6	7.8	MODERATE NPDR+ CSME
948417	72	M	YES	4 YEARS	OHA	1.9	1.7	7	NORMAL
951028	70	M	YES	1 YEAR	OHA	1.5	1.5	8.9	NORMAL

MASTER CHART FOR CONTROLS							
UHID NO	**AGE**	**SEX**	**T2DM**	**S. Mg (mg/dL)**	**AH Mg (mg/dL)**	**HbA1c**	**FUNDUS**
838465	75	F	NO	2.1	1.9	5.6	Normal
838457	75	M	NO	1.8	1.8	5.9	Normal
838462	70	F	NO	2	1.9	5.7	Normal
871918	60	M	NO	1.7	1.7	5.7	Normal
871921	65	F	NO	1.8	1.8	5.8	Normal
871922	72	F	NO	1.7	1.7	5.6	Normal
873210	72	M	NO	1.7	1.7	5.4	Normal
873216	70	M	NO	1.9	1.7	5.9	Normal
875616	65	M	NO	1.5	1.8	5.5	Normal
875624	51	F	NO	1.5	1.8	5	Normal
874476	58	M	NO	1.5	1.8	4.5	Normal
863743	60	M	NO	1.5	2	5.5	Normal
899829	52	M	NO	1.9	1.7	5.6	Normal
899842	55	F	NO	1.8	1.4	5.8	Normal
933391	74	M	NO	2	2	5.4	Normal
933396	50	F	NO	2.2	1.8	6	Normal
933392	81	M	NO	2	1.9	5.5	Normal
933389	70	F	NO	2.3	1.8	6	Normal
908518	65	M	NO	1.9	2.1	5.5	Normal
908515	46	F	NO	1.9	1.8	5.2	Normal
908253	50	F	NO	1.8	1.8	5.7	Normal
946717	60	F	NO	2	2	5.6	Normal
946812	60	M	NO	2.1	2	6	Normal
943892	75	M	NO	2.3	2.4	5.7	Normal
938598	75	M	NO	2.1	2.2	6	Normal
938591	75	M	NO	1.7	2.1	5.7	Normal
938589	80	M	NO	2	2.1	5.3	Normal
941832	65	F	NO	1.6	1.7	5.4	Normal
943771	72	M	NO	1.9	1.5	5.7	Normal