ORIGINAL RESEARCH PAPER

Ophthalmology

"STUDY OF ASSOCIATION BETWEEN SERUM MAGNESIUM LEVEL WITH STAGES OF DIABETIC RETINOPATHY"

KEY WORDS: Stage of diabetic retinopathy, Serum level, magnesium concentration

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INTRODUCTION

Diabetes mellitus is defined as "Endocrinal disorder characterized by hyperglycemia and glycosuria due to reduce secretion/absence or resistance to the insulin with decreased glucose utilization and increased glucose production". **(kasper et al. 2005)**[1]

It is emerging as one of the most common disease and leading cause of blindness in the world. **(Shah et al. 2017)**[2]

The global number of cases with diabetes has risen from 108 million in 1980 to 422 million in 2014 and 500 million cases in 2018. **(Kaiser et al. 2018)**[3]

The global prevalence of diabetes among adult over 18 year of age has risen from 4.7% in 1980 to 8.5% in 2014. **(Kaiser et al. 2018)**[3]

The prevalence of diabetes among adult in India has reached 20% in urban population and approximately 10% in rural population in 2018. **(WHO India 2018)**[4]

The rising prevalence of diabetes in developing countries is closely associated with industrialization and socioeconomic development. **(Park 2005)**[5]

The metabolic dysregulation associated with diabetes mellitus causes secondary pathophysiological changes in multiple organ system and responsible for majority of morbidity and mortality associated with diabetes mellitus. **(kasper et al. 2005)**[1]

Macrovasular complication (known as macroangiography) associated with diabetes mellitus are coronary artery disease, stroke and peripheral vascular disease. **(kasper et al. 2005)**[1]

Microvasular complication (known as microangiography) associated with diabetes mellitus are nephropathy and retinopathy. **(kasper et al. 2005)**[1]

Overall prevalence as well as severity of Diabetic retinopathy is higher in type- 1 (47.26%) than in type- 2 (26.49%). **(Pedro et al. 2017)**[6]

The gravity of this problem is heightened by the finding that individuals with diabetic mellitus are 25 times more likely to become blind than that of the individuals without diabetic mellitus. **(kasperet al. 2005)**[1]

Blindness due to diabetic retinopathy is generally caused by non-resolving vitrous hemorrhage, traction retinal detachment or diabetic macular edema. **(Kanski JJ 2003)**[7]

Diabetic retinopathy is estimated to be most frequent cause of new cases of blindness in the 20-74 years age group in developed countries. **(Kanski JJ 2003)**[7]

Diabetic retinopathy is classified according to modified Airlie House System **(DRS report 1981 and ETDRS report 1991)**[8,9]
1.No Diabetic Retinopathy- had no clinical observable diabetic retinopathy.
2.Background diabetic retinopathy (BDR) - Lesions occurring in retina in this stage of retinopathy are microaneurysms, haemorrhages, hard exudates of yellow waxy appearance with distinct margin.
3.Pre-proliferative diabetic retinopahy (Pre-PDR) - changes in this stage consisted of background retinopathy lesions plus two or more of the following: venous bleading, intra-retinal microvascular abnormalities, deep intra retinal haemorrhages and multiple cotton wool spots.
4.Proliferative diabetic retinopathy (PDR) - affects about 5-10% of diabetic opulation. Neovascularization is the hallmark of PDR. It can be present at the disc (NVD) and along the course of major temporal vascular arcades (NVE).

Disturbance in lipid metabolism are said to be as essential part of deranged metabolism in diabetic mellitus and are probably responsible for high percentage of vascular complication. **(Chew et al. 1996)**[10]

Disturbance in lipid metabolism have been implicated in the pathogenesis of diabetic retinopathy. **(Chew et al. 1996)**[10]

There is however a further factor that seems to be important in determining the progression of retinopathy, might be modificable may be "cigarette smoking". **(Ackerman et al. 1992)**[11]

The paetkau and her colleagues found that in non-smokers there was no association between the duration of diabetes and proliferative retinopathy, but in smokers the number with proliferative retinopathy rise with duration of diabetes. **(PAETKAU et al. 1977)**[12]

Magnesium is the second most important intracellular cation in the body next only to potassium. **(Reinhart RA et al., 1988)**[13]

The **National Institute Of Health Published** in its website, "Magnesium is needed for the correct metabolic function of more than 300 enzymes in the human body.

Dietary magnesium deficiency is more prevalent than generally expected and remains to be one of the most common nutritional problems in the industrialized as well as the developing world. This is due to the result of current change in the dietary trends, agricultural practices and the food preparation techniques. World health organization and

other health agencies say that more than 75-80% of the people do not consume the recommended daily intake of magnesium. **(Seeling M. et al. 1995)**[14]

Magnesium depletion is described as the most under-diagnosed electrolyte abnormality in current medical practice. Magnesium is gaining greater importance, as its deficiency is found to be a common problem and the prevalence of magnesium deficiency in hospital settings has been found to be 7–11%. Its deficiency also coexists with other electrolyte abnormalities in 40% of the patients. **(Syed K. Ahsan et al.)**[15]

Due to the change in the dietary habits, an average human's diet is deficient in magnesium. This is more common in the alcoholics, young people, and people who receive certain medications. In otherwise normal people the chronic low magnesium diets are atherogenic and thrombogenic and disrupts the arterial and cardiac integrity and so, it is associated with a number of common chronic diseases like hypertension, diabetes, coronary, heart disease, stroke, osteoporosis, etc.[16]

It also helps in proper normal functioning of nerves and the muscles, maintains a strong immunity against infections, keeps the heart work in regular rhythm and supports the bone integrity. It regulates the blood sugar values, keeps the blood pressure under control, and also has a role in protein synthesis and energy metabolism. There's a developing interest in the magnesium role in the prevention and treatment of diseases like diabetes, hypertension, cardiac and cerebral disorders." It is thus magnesium deficiency is considered to be an epidemic deficiency. **(Hiroyasu Iso et al. 1999)**[17]

The atherogenic property of smoking directed our attention towards the possible importance of "hypomagnesaemia". **(PAETKAU et al. 1977)**[12]

A low serum level of magnesium (hypomagnesaemia) leading to atherosclerosis of vessels of heart land brain gave idea to study the role of magnesium as one of the risk factor in producing diabetic retinopathy, by its effect on retinal blood vessels. **(P. Mcnair et al. 1978)**[18]

Firstly, P. Mc Nair et al. (1978) found that there was a change in magnesium level of serum in diabetes patients with diabetic retinopathy and this change was found to be related to severity of diabetic retinopathy. **(P. Mcnair et al. 1978)**[18]

Magnesium deficiency inhibits the acute phase of insulin release in response to glucose challenge and also deficiency is associated with insulin resistance; conversely insulin resistance is associated with low serum magnesium. **(Paolisso G et al. 1989)**[19]

Hypomagnesemia in patients with diabetes results from poor oral intake, poor gastrointestinal absorption and enhanced renal magnesium excretion. **(Durlach J et al. 1983)**[20]

Microalbuminuria and overt proteinuria in patients with diabetes contribute to renal magnesium wasting due to protein bound magnesium loss. There exists an inverse correlation between serum magnesium level and diabetic patients with proteinuria. More the proteinuria, more the magnesium loss, lesser the serum magnesium level. **(Kroll MH et al. 1985)**[21]

Elimination of magnesium occurs through the kidney and is about 100 mg/day. The threshold for urinary excretion is the upper limit of normal range. When the serum magnesium level raise above the upper limit, excretion also increases to maintain the constant serum level. The main site for reabsorption of magnesium in the kidney is the thick ascending limb of loop of henle and in conditions of magnesium depletion, kidney has a strong capacity to reabsorb magnesium. **(Lee DB et al. 1993)**[22]

Factors which impair renal reabsorption are volume expansion, hypercalcemia and administration of diuretics such as thiazide, osmotic or loop diuretics. **(Berman M et al. 1966)**[23]

AIMS & OBJECTIVES

1.To establish association between serum magnesium and diabetic retinopathy.

2.To sort out the relationship between serum magnesium and stages of diabetic retinopathy.

REVIEW OF LITERATURE

Diabetic Retinopathy

Diabetes is a chronic metabolic disease characterised by hyperglycaemia that results from defects in insulin secretion, absolute or relative and/or insulin action. **(American Diabetes Association 2014)**[24]

The range of pathogenic processes involved include the autoimmune destruction of pancreatic β cells with insulin deficiency and abnormalities in carbohydrate, fat and protein metabolism, resulting in resistance to insulin action. **(Wild et al. 2004)**[25]

The majority of diabetes falls into two broad categories **(American Diabetes Association 2013)**[26] :-

a)**Type I diabetes** which is caused by an absolute deficiency of insulin secretion, and

b)**Type 2 diabetes** caused by a combination of resistance to insulin action and an inadequate compensatory insulin secretion response. The majority of persons with diabetes (~90%) will have type 2 diabetes.

c)There are also other types of diabetes which are either secondary to diseases of the exocrine pancreas, and drug or chemically induced or to other causes such as either to genetic defects in β-cell function and insulin action and gestational diabetes, which is diagnosed during pregnancy and is not clearly diabetes.

Diabetes has profound effects on the structure and function of many tissues and organs in the body. **(Al-Rubeaan et al. 2010)**[27]

Complications of diabetes include,

a)**Macrovascular Disease-**

Cardiovascular disease such as stroke, myocardial infarction and peripheral vascular disease, and

b)**Microvascular Disease-**

Diabetic retinopathy (DR), diabetic neuropathy and diabetic kidney disease (diabetic nephropathy). **(Holt et al. 2010)**[28]

The prevalence of these complications is strongly related to the type and duration of diabetes and glycaemic control, other risk factors for development of these complications include hypertension, dyslipidaemia, treatment modality, the increasing global population, increasing age and predicted rise in the proportion of adults with diabetes will inevitably be accompanied by an increase in diabetic complications. **(Zhang et al. 2008)**[29]

Diabetes is a major public health problem and the incidence of blindness is 25 times greater in persons with diabetes when compared with the non-diabetic population. **(Hayward et al. 2002)**[30]

DR is the most common microvascular complication of

diabetes and was until recently regarded as the most prevalent cause of visual impairment in the working age population, DR has been overtaken by inherited retinal conditions as the leading cause of blindness in the working age group which was possibly a result of DR screening programmes and improved diabetes care. **(Liew et al. 2014)**[31]

Visual loss and blindness due to diabetes is essentially preventable in the vast majority of people, through optimal treatment of diabetes and associated hypertension and hypercholesterolemia and the implementation of screening to detect treatable DR. **(Mohamed et al. 2007)**[32]

Diabetic Retinopathy

Globally, it is estimated that there are 93 million people with DR, 17 million with proliferative DR (PDR), 21 million with macular odema and 28 million with sight threatening DR. **(Yau et al. 2012)**[33]

First Case Of Diabetic Retinopathy Is Described By Jaegar (1855)[34]

Neuro-vascular Unit :-

-The retina consists of 95% neural tissue (cone's and rod's, muller cell, astrocyte, microglia) and 5% vascular tissue. **(Antonetti et al. 2012)**[35]

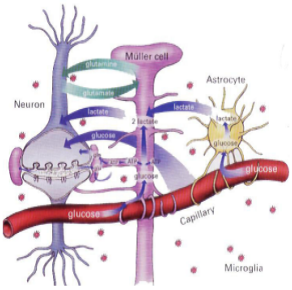


Figure 1) The Neurovascular Unit Of The Retina

Pathophysiology of DR :-

Our understanding of the pathophysiological mechanisms underlying the development of DR is constantly evolving. **(Antonetti et al. 2006)**[36]

Overall, diabetic microvascular complications are mainly caused by prolonged exposure to high glucose levels. **(Giacco F et al. 2010)**[37]

The extent of tissue damage is also determined by genetic determinants of individual susceptibility and by the presence of independent accelerating factors such as hypertension and dyslipidaemia. **(Ciulla et al. 2003)**[38]

Chronic exposure to hyperglycaemia and other causal risk factors eg. hypertension is believed to initiate a cascade of biochemical, physiological and pathological changes that ultimately lead to neuro-vascular damage and consequently retinal dysfunction. **(Cheung et al. 2010)**[39]

1) Biochemical changes in DR :-

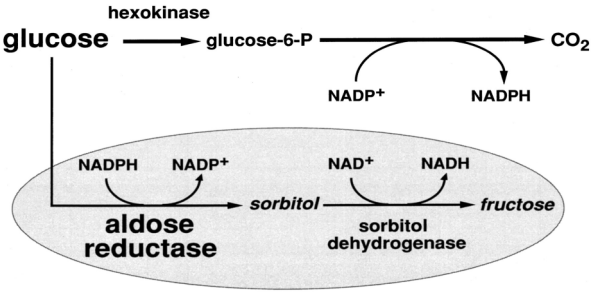
a) Advanced Glycosylation End Products (AGES) :-

Protein glycation occurs through a complex series of very slow reactions in the body, including the Amadori reaction, Schiff base formation, and the Maillard reaction. These give rise to the formation of advanced glycation end products (AGEs) which are deposited on wall of retinal capillary. **(Tooke et al. 1992)**[40]

b) Sorbital Pathway (Taylor Et Al. 1988[41]) :-

Sorbitol pathway associated with cell death/ apoptosis. **(Cheung et al. 2010)**[39]

Additional alterations include biochemical defects, such as impaired control of glutamate metabolism, which is the major neurotransmitter **(Gowda et al. 2011)**[42] as well as loss of synaptic activity and dendrites. **(Gastinger et al. 2008[43], Van Guilder et al. 2008)**[44]



2) Pathological changes in DR :-

DR is considered to be the result of changes in the retinal vasculature and the neuroretina. **(Kohner et al. 1993)**[45]

The relationship between the neural and vascular units of the retina in the pathogenesis of DR remains to be clarified. However, more recently the neurosensory retina can be observed by slit-lamp biomicroscopy and insight into the relationship between the retinal neurovascular unit could be achieved by the undertaking of a prospective assessment of diabetic patients using clinical and state-of-the-art functional and imaging technologies. **(Antonetti et al. 2012)**[35]

It is primarily concerned with the retinal capillaries and the earliest vascular changes are usually a decrease in the number of capillary pericytes and a thickening of the basement membrane these changes occur long before any clinically visible lesions develop. The effects on entire neurosensory unit occur. **(Curtis et al. 2009)**[46]

But changes in the neuro-retina may occur even before the onset of microvascular changes. **(Lieth et al. 2000)**[47]

a) Neuroretinal Changes :-

Most retinal neurons and glial cells are altered and are progressively impaired with worsening DR as Figure 2 illustrates the disruption of the neurovascular unit due to diabetes that lead to apoptosis of neurons primarily in the ganglion-cell and inner nuclear layers **(Barber et al. 1998)**[48] and activations of microglial cells that may protect the inner retina from injury but also contribute to the inflammatory response. **(Zeng et al. 2008)**[49]

There is evidence that the disruption of the neuroretina caused by diabetes leads to changes in electroretinograms (ERG) **(Lovasik et al. 1993)**[50], altered dark adaption **(Henson et al. 1979)**[51] and reduced contrast and colour sensitivity. **(Della Sala et al. 1985[52], Kurtenbach et al. 1994[53])**

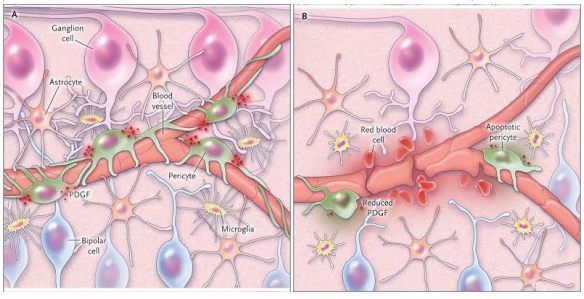


Figure 2) Illustrate The Disruption Of The Neurovascular Unit Due To Diabetes

b) Microvascular Changes :-

Pericytes are an important constituent of the wall of retinal microvasculature with contractile properties to control vessel calibre and therefore blood flow. **(Kohner et al. 1993)**[45] Figure

3, shows the location of pericytes within capillaries.

The loss of pericytes, creating ghost cells, are followed by the loss of capillary endothelial cells. **(Antonetti et al. 2006)**[36]

Apoptosis, or programmed cell death, is thought to account for the disappearance of both cell types and is caused by hyperglycaemia due to variety of mechanisms e.g. Sorbitol etc. **(Cheung et al. 2010)** [39]

The loss of pericytes means the vessels become rigid, with increased blood flow that lead to the damage of endothelium of the vessel wall by the increased shear stress. **(Kohner et al. 1993)**[45]

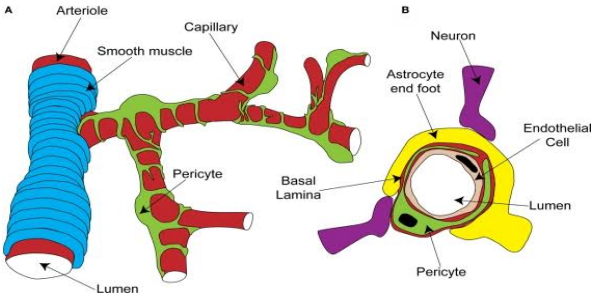


Figure 3) A) rings of smooth muscle encircle arterioles, while pericytes send processes along and around capillaries, without fully covering the vessel. B) Pericytes are located outside the endothelial cells and are separate from them and the parenchyma by a layer of basal lamina. In the parenchyma, astrocyte end-feet and neuronal terminals are closely associated with the capillary. (Hamilton et al. 2010)[54]

Destruction of the endothelial cells and increased basement membrane thickness, eventually leads to a breakdown of the blood-retina barrier, increasing the permeability of vessel walls to proteins and other substances. **(Hamilton et al. 2010)** [54]

Thickening of the basement membrane is mainly a consequence of hyperglycaemia and/or a consequence of the accumulation of advanced glycosylation end products (AGEs). **(Jennings et al. 1992[55], Roy et al. 1994[56])**

These findings suggest that DR represents a sensory neuropathy that affects the retinal parenchyma, not dissimilar to peripheral diabetic neuropathy. **(Antonetti et al. 2012)**[35]

These changes have been shown to occur well before any first clinical visible signs of DR. **(Curtis et al. 2009)**[46]

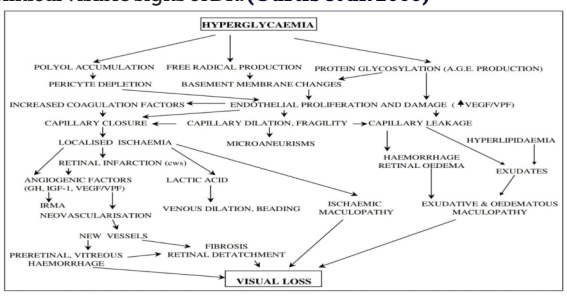


Figure 4) Patho-physiological Mechanisms In The Evolution Of DR

Clinical Feature Of DR :-

1) Microaneurysms :-

First capillary aneurysm in a case of glycosuria is described by **McKenzie and Lowenstein (1877)**[57].

These are the first feature of DR to become visible on clinical examination. fig. no 5 show a trypsin digest image of a microaneurysm and retinal image of microaneurysms respectively. However, even before these are clinically apparent, many more may be seen on fluorescein angiography (fig. no 6) accompanied by small areas of non-perfusion. **(Bernardino et al. 2006)**[58]

The response to non-perfusion in some capillaries results in dilation of others and when this is localised (as most often occurs) a microaneurysm is formed (small dot lesion), **(Kohner et al. 1993)**[45] and are lined with endothelium which has increased permeability.

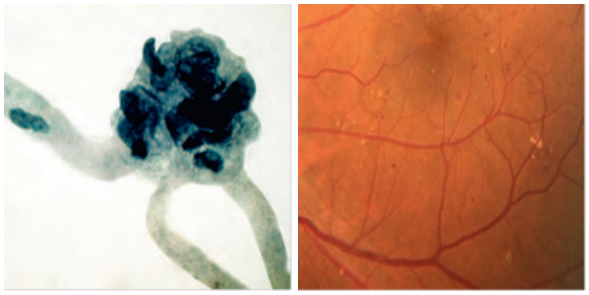


Figure 5) Retinal images showing Trypsin digest and clinical images of the formation of a microaneurysm (kanski 8th edition page no 523)

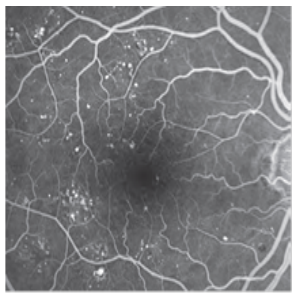


Figure6) Showing Fluorescien Angiograpgy Image Of Microaneurysm

2) Haemorrhage :-

Increasing numbers of microaneurysms are often associated with retinal haemorrhages.

Types Of Hemorrhages-

a) **Superficial:-** these are flame shaped and form in RNFL.

b) **Deep:-** rounded lesions ('Dot') and associated with small hard exudates, which are an accumulation of plasma lipoprotein. **(Cunha-Vaz et al. 2010)** [59], large blot haemorrhages tend to form at the interface of the perfused and ischaemic areas of the retina.

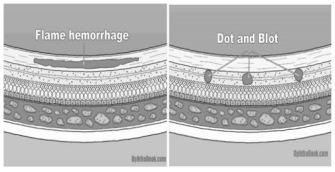


Figure 7) Showing location of the different types of retinal haemorrhages within the layers of the retina (kanski 8th edition page no 524)

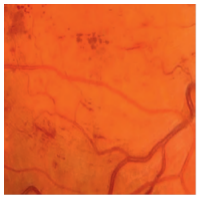


Figure 8) Showing Clinical Photograph Of The Retinal Haemorrhage

3) Exudates :-
Are composed of lipid material develop and appear as yellow deposits often larger than the microaneurysms. They have a distinctive yellow shiny appearance. Their origin is thought to be leaky capillary blood vessels and microaneurysms in the retina and they may be transient and reabsorbed by the retina, though they tend to increase in total number over time. **(Kohner et al. 1991)**[60]

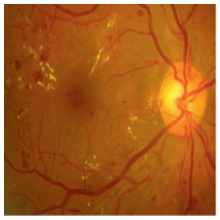


Figure 9) Showing Retinal Photographs Of Exudates

4) Cotton Wool Spots (CWS) :-
Evidence of focal ischaemia in the form of Cotton Wool Spots (CWS) may appear which are infarctions of the retinal nerve fibre layer.

CWS and haemorrhages may be present in ischaemic retinal conditions other than DR, but in the presence of microaneurysms are considered to be part of the process of DR. **(Early Treatment Diabetic Retinopathy Study Research Group 1991c)**[61]

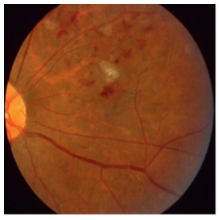


Figure 10) Retinal Images Showing CWS

5) Intra-Retinal Microvascular Abnormalities (IRMAs) :-
Dilation of the retinal capillary bed, can form in what appear to be avascular areas of the retina, these IRMAs may represent the earliest form of neovascularisation. **(Early Treatment Diabetic Retinopathy Study Research Group 1991c)**[61]

Abnormalities may also be observed in the larger blood vessels eg. the venules may develop looping, segments which are dilated alternating with segments that are constricted resembling beading, duplication of veins may also develop so that there appear to be parallel veins in some areas (reduplication). **(Early Treatment Diabetic Retinopathy Study Research Group 1991c)**[61]

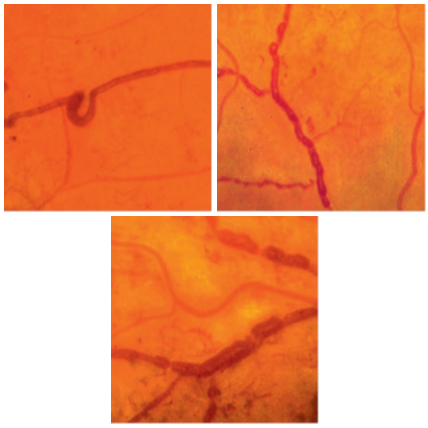


Figure 11) Retinal image depicting a)Venous loop/reduplication, b)Venous beading, and c) Segmentation (kanski 8th edition page no 528)

6) Neovascularization :-
New vessels usually develop from the veins in the retinal periphery (new vessels elsewhere, NVE) or on the optic disc (new vessels on the disc, NVD). The location of the new vessels determine the prognosis in terms of visual outcome. **(Kohner et al. 1991)**[60] When they occur at the disc, more than 50% of affected eyes may become blind within 5 years, compared with less than one third when new vessels are located away from the disc.

They arise generally, although not exclusively when there are large areas of avascular retina in the vicinity **(Davis et al. 1992)**[62] & later develop more characteristic frond-like patterns.

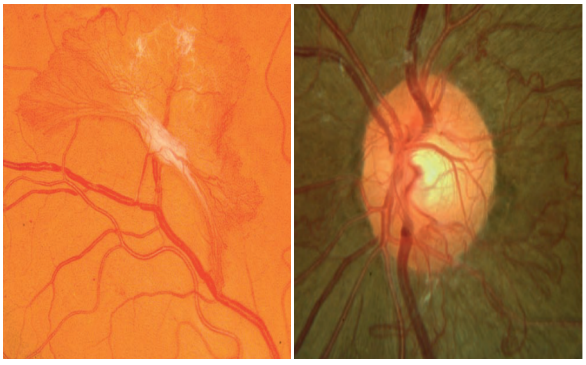


Figure 12) Showing a) NVE, and b) NVD (kanski 8th edition page no 530)

Certain features of new vessels and vitreous and pre-retinal **haemorrhage** conferred a greater likelihood of severe visual loss if untreated. **(Diabetic Retinopathy Study Research Group 1981a)**[63]

- These features were termed **'high risk characteristics' (HRCs) (Diabetic Retinopathy Study 1981)**[8]:-
a)New vessels on the disc (NVD) greater than about 1/3 disc area.
b)Any NVD with vitreous haemorrhage.
c)NVE greater than 1/2 disc area with vitreous haemorrhage.

7) Vitreous haemorrhage :-
The new vessels usually develop a fibrous sheath covering and break through the internal limiting membrane when they become attached to the posterior surface of the vitreous, which they use as a scaffold on which they continue to grow. The retracting vitreous often pulls on the vessels causing haemorrhage. **(Bunce et al. 2008)**[64]

The development and progression of DR may be asymptomatic up to this stage and symptoms only occur if these new vessels bleed causing vision to become obscured as new vessels themselves do not cause any visual symptoms, it is the bleeding from the new vessels that are responsible for the visual loss. **(Chantelau et al. 1997)**[65]

Bleeding at this stage may initially produce 'boat shaped' haemorrhages if limited to the pre-retinal space (fig no 13) with only transient visual loss, but may be an indication of impending major haemorrhage. vitreous haemorrhage with sudden visual loss if more generalized. **(Cook et al. 2013)**[66]

Small haemorrhages may clear within a few weeks, but large ones may never clear, or do so very slowly. **(Davis et al. 1992)**[62]

Without treatment, it has been demonstrated that approximately one third of patients will be blind in both eyes within one year of their first vitreous haemorrhage, and only 14% of patients will have good vision within five years of developing new vessels. **(Caird et al. 1968)**[67]

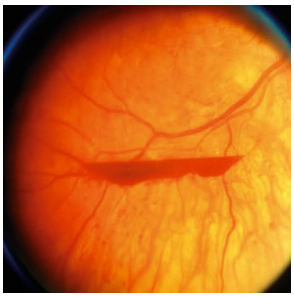


Figure 13) Image Showing Pre-retinal Haemorrhage

8) Fibrovascular Traction :-
Fibrous tissue accompanying new vessels can exert traction on the retina, either due to its own contraction, or secondary to posterior vitreous detachment (fig. no 14). **(Davis et al. 1992)** [62]

Traction or retinal detachment normally involves the posterior pole and hence the macular area and will cause a decrease or distortion in vision. **(Cunha et al. 2010)** [59]

It is uncommon for spontaneous regression to occur at this stage and visual loss related to these fibro-vascular abnormalities is usually sudden and unexpected. **(Fong. et al. 2001)** [68]

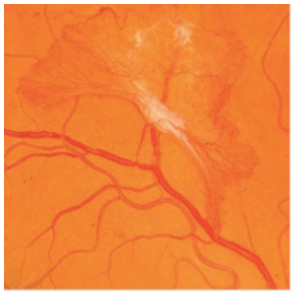


Figure 14) Image Showing Fibrous Tissue With Traction

9) Rubeosis Iridis :-
When ischaemia is a major feature, new vessels and fibrosis may also occur on the iris and in the angle of the anterior chamber. The resulting obstruction to normal drainage of aqueous fluid gives rise to painful glaucoma, which, if untreated, rapidly leads to blindness. **(Kohner et al. 1991)** [60]

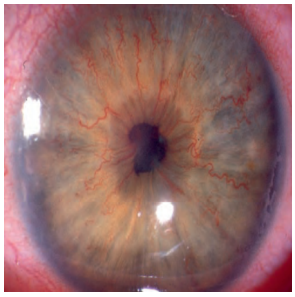


Figure 15) Image Showing Rubeosis Iridis

10) Maculopathy :-
Diabetic maculopathy is defined as DR within one disc diameter (DD) of the centre of the fovea. **(Chowdhury et al. 2002)** [69]

There are two aspects of maculopathy:-
a) **Macular Oedema** where lipoproteins accumulate within the retina.

b)**Macular Ischaemia** where there is a closure of perifoveal capillaries occur.

Diabetic maculopathy may be central involving or non-central involving as well as tractional due to vitreo-retinal pathology or non-tractional (intraretinal). **(the Royal College Of Ophthalmologists, 2012)** [70]

- Macular oedema may be focal or diffuse
a)**focal Macular Oedema**, is characterised by an increase in retinal thickening due to leakage from microaneurysms which are frequently associated with hard exudates. **(Cunha-Vaz 2010)** [59]

b)**Diffuse Macular Oedema**, is caused by leakage from abnormally dilated capillaries, arterioles and venules in the macular area, and sometimes associated with cystic lesions, but with less visible focal vascular damage and fewer hard exudates. **(Chowdhury et al. 2002)** [69]

Table 1) Describes The Principle Features Of Each Type Of Maculopathy

Oedematous	Focal	Localised areas of retinal thickening associated with focal leakage of individual microaneurysms or clusters of microaneurysms or dilated capillaries (Bhagat et al. 2009)
	Diffuse/cystoid	More generalised and chronic form of oedema, with widespread macular leakage and pooling of dye in cystic spaces (Bhagat et al. 2009)
Ischaemic	Ischaemic	Microaneurysms and haemorrhages with a small amount of capillary nonperfusion evident with fluorescein angiography. Varying oedema is present ranging from mild to cystoid in appearance.

Clinically Significant Macular Edema (CSME)
Maculopathy and decreased visual acuity associated with oedema known as clinically significant macular edema. **(Hirai et al. 2008)** [71]

- Retinal thickening within 500µm of the centre of the fovea
- Hard exudates within 500µm of the centre of the fovea with adjacent retinal thickening
- Retinal thickening 1DD or larger in size located within 1 DD of the fovea.

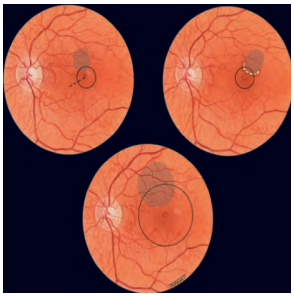


Figure 16) Image Showing Clinical Photograph Of CSME

Maculopathy can occur in the presence of microaneurysms, haemorrhages, CWS, IRMA, and venous changes. **(Klein et al. 2003)**[72], and associated visual loss is progressive.

Without treatment about one third of affected eyes may be expected to become blind within five to seven years. **(British Multicentre Study Group 1983, Early Treatment Diabetic Retinopathy Research Study Group 1985)**[73]

Blood Sugar Control[4] **:-**
Blood sugar level defined as-
a) **Good Control –** Fasting Blood Sugar Below 130 mg %
b) **Fair Control –** Between 130-150 mg %
c) **Poor Control –** Above 150 mg %

Classification of DR :-
The need for a classification of DR, to allow the evaluation of treatment and natural progression, early classifications were disadvantaged by the need for detailed and time consuming fundal drawings to complete a full evaluation. **(Lee et al. 1966)**[74]

1) Hammersmith Classification :-
- It was one of the first to use. **(Oakley et al. 1967)**[75]

Method- Five types of lesion were recognized (Microaneurysm, haemorrhage, exudate, new vessels, venous irregularities and proliferation) then these lesions were graded on a scale from 1 to 5 depending on severity.

- As Macular involvement was not identified separately and CWS were ignored, this initial classification was limited, but the use of standard photographs and lesion grading was firmly established.

2) Airlie House Classification :-
- The first internationally recognised classification of DR which was established in 1968. **(Davis et al. 1969)**[76]

Method- Fifteen clinical features and two assessments one based on fluorescein angiography and one including seven standard colour photographs were each graded as 'absent' (0), 'mild to moderate'
(1), and 'moderate to severe' (2).

- Most subsequent classifications of DR have been adapted from the Airlie House classification. **(DRS 1981)**[8]

3) ETDRS classification (1991)[9] **:-**
Early Treatment Diabetic Retinopathy Study (ETDRS) more emphasis being placed on early changes and lesions at and around the macula, particularly macular oedema.

ETDRS allowed a more detailed assessment of the severity of DR and risk of progression to PDR to be made.

Category	Description
a) Non-proliferative diabetic retinopathy (NPDR)	
1) No DR	
2) Very mild NPDR	Microaneurysm only
3) 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.
4) Moderate NPDR	Severe retinal haemorrhages (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
5) 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
5) Very severe NPDR	Two or more of the criteria for severe NPDR
b) Proliferative diabetic retinopathy (PDR)	
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 • NVD about 1/3 disc area • Any NVD with vitreous haemorrhage • NVE greater than 1/2 disc area with vitreous haemorrhage

Magnesium
Magnesium is the fourth most abundant the body besides sodium, potassium and calcium, and the second most abundant intracellular cation next only to potassium. **(Reinhart RA et al. 1988)**[13]

It has got atomic number 12 and its atomic weight is 24. **(Chemical element.com 2019)**[77]

Normal adult human body contains 21 to 28 grams of magnesium which is approximately equal to 2000meq. 53% of total body magnesium is located in the bone and 27% is located in the muscle, 19% in soft tissue and 1% is located in the extracellular fluid. **(Shils ME et al. 1998)**[78] Most of the intracellular magnesium occurs in bound form and only 0.25 to 1 mmol occurs as free magnesium. **(Grubbs RD et al. 2002)**[79]

Serum concentration of magnesium ranges from 0.7 to 1 mmol/l or 1.6 to 2.1 mEq/litre. The plasma concentration in healthy adults remain constant. **(Rude RK et al. 1998)**[80]

Magnesium acts as a cofactor in more than 300 enzymatic reaction involving protein and nucleic acid synthesis and energy metabolism. The active form of the element is the free ionized magnesium. **(Mazur A et al. 2007)**[81]

REGULATION OF MAGNESIUM :-
Absorption :-
Magnesium is absorbed from the gastrointestinal tract. The average daily intake of magnesium is about 140 to 360 mg/day (25meq/day). Around 40% of dietary magnesium is

absorbed in the small intestine particularly in the ileum. absorption occurs through paracellular and transcellular pathway. **(David B.N et al. 1991)**[82]

The fractional absorption of ingested magnesium depends on the amount of magnesium in the food and the presence of enhancing or inhibiting substances in the food eg. Calcium and phosphate influences the amount of magnesium absorbed apparently by competition for a common absorptive pathway and the magnesium hemostasis. **(Fordtran JS et al. 1991)**[83]

Excretion :-

Magnesium is mainly excreted by the kidneys, but a smaller amount is also excreted through the gastrointestinal secretion and a negligible amount through sweat and menstrual losses. **(Lee DB et al. 1993)**[22]

The important sites of magnesium reabsorption are distal small intestine and colon. magnesium is re-excreted into the intestine by bile ,pancreatic and other intestinal excretion and cell sloughing **(Berman M et al. 1966)**[23]

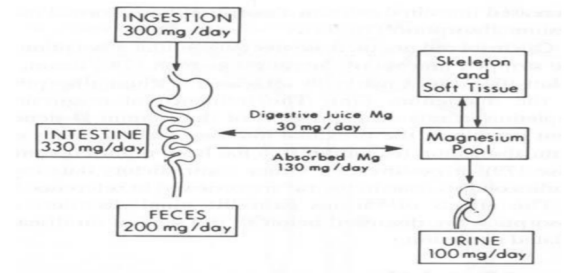


Figure:17) Flow Chart Showing The Renal Handling Of Magnesium

Renal Excretion :-

The kidney is the main organ that regulates magnesium homeostasis, around 80% of the magnesium is filtered through the glomerulus. The important sites of magnesium reabsorption are the thick ascending limb of loop of henle (70-80%), the proximal tubule (5-15%), and the distal convoluted tubule (5-10%). 3% of filtered magnesium appears in the urine. **(Quamme GA et al. 1997)**[84]

The mechanism by which magnesium gets reabsorbed is mainly by paracellular pathway in the loop of henle and transcellular pathway in the proximal and distal tubules and studies have shown that magnesium reabsorption increases in magnesium deficiency. **(Schwarz S et al. 2006)**[85]

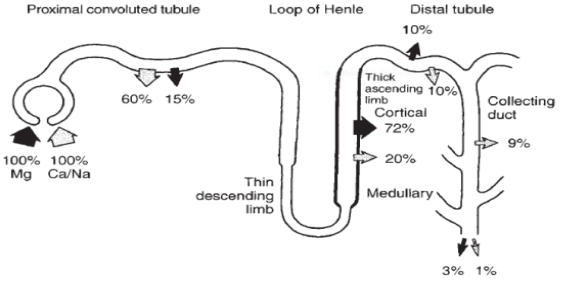


Figure:18) Schematic representation showing the renal handling of Mg

Role Of Hormones In Magnesium Homeostasis :-

Hormones which increase serum magnesium level are **(Haenni A et al. 2001)**[86] :-

- Parathyroid hormone
- Glucagon
- 1,25 dihydroxy cholecalciferol

Hormones which decreases serum magnesium level are **(Haenni A et al. 2001)**[86] :-

- Aldosterone
- Vasopressin
- Calcitonin
- Thyroxine

FOOD SOURCES OF MAGNESIUM (USDA, 2003)[87]

(Magnesium content in mg/100gm)

Legumes

- Split beans- 50
- Soyabean – 86

Nuts

- Peanuts -175
- Almonds -315
- Cashews -260

Dairy Products

- Milk -24
- Butter -20
- Yoghurt -12

Fruits

- Dates -35
- Banana -30
- Oranges -10
- Apple -5

Cereals

- Shredded wheat – 110
- Rice -40

Meat And Fish

- Pork -22
- Chicken -21
- Beef - 18
- Fish -22

Recommended Dietary Allowances, Of Magnesium As Developed By Food And Drug Administration[88]

AGE	MALE	FEMALE	PREGNANCY	LACTATION
1-3	80	80	NA	NA
4-8	130	130	NA	NA
9-13	240	240	NA	NA
14-18	410	360	400	360
19-30	400	310	350	310
31+	420	320	360	320

(magnesium values in mg/day)

BIOCHEMICAL IMPORTANCE OF MAGNESIUM

Magnesium is an activator of enzyme system which are particularly involved in cellular metabolism. Important enzymes are one which hydrolyze and transfer phosphate groups, especially the reaction involving adenosine phosphate as adenosine tri-phosphate is required for fat, protein, glucose utilization, nucleic acid and coenzyme synthesis, muscle contraction and other reactions, by inference magnesium has an active role in the above reactions. **(Hernik et al. 2018)**[89]

Magnesium is required as a cofactor in oxidative phosphorylation, which occurs in the mitochondria. **(Kotaro Oka et al. 2016)**[90]

The highly ordered organization of DNA, RNA and ribosome is stabilized by the presence of magnesium. **(Lehman et al. 1960)**[91]

Magnesium is involved in protein synthesis. it helps in binding of the messenger RNA to the 70s ribosome. **(Vernon WB et al. 1988)**[92]

Pharmacological properties magnesium ion as a potential anticonvulsant and anaesthetic agent. **(Sang-Hwan Do et al. 2013)**[93]

The second messenger cyclic adenosine monophosphate is involved in many reaction including secretion of hormones such as parathyroid hormone. Cyclic adenosine monophosphate is formed from magnesium adenosine triphosphate and the enzyme adenylate cyclase, which is activated by magnesium by its two binding sites. **(Maguire ME et al. 1984)**[94]

Magnesium is also involved in membrane stabilization, ion transport and calcium channel activity. **(Bellorin-Font E et al. 1998)**[95]

Effect Of Magnesium On Blood Coagulation :-

Hughes et al. (1965) [96] observed that magnesium prolongs the clotting time of whole blood and delays the peak thrombin time in vivo and vitro.

Anstall et al. (1959) [97] and **Huntsman et al. (1960)** [98] have observed that small amount of magnesium added to fresh unclotted human plasma prolonged the clotting time considerably. This action was explained by a cation antagonism between magnesium ions and calcium ions, both competing for combination with clotting factors.

Hypomagnesemia has been described in acute renal failure and chronic nephritis. Abnormal bleeding is common in uremic patients and has been described to failure in the activation of platelet factors 3 (PF 3). Addition of magnesium to fresh platelet rich plasama (PRM) with anticoagulants, prolongs platelet clumping time and antagonizes the clumping activity of ADP **(Hughes et al. 1968)**[96]

Helle et al. (1960)[99] observed that a heat stable extract of red cells factor "R" later on identified as A.D.P. could induce platelet clumping in vitro **(Havig et al. 1963)**[100] has shown the release of ADP from damaged platelets. ADP is said to require the presence of ionized calcium before it causes platelet aggregation, and helle and oween have Proposed that ADP is bound to the platelet surface and to a second molecule of ADP by calcium. Thus linking the platelets into an aggregate or clump. If the action of magnesium is to reduce or prevent this activity at the surface of platelet, it is possible that its action might result from cation antagonism between magnesium and calcium ions.

Hughes and Tonks (1965)[96]observed that rabbits with intravascular coagulation (induced by minerals corticoids and monobasic sodium phosphate) have 40 % reduction of plasma magnesium. It suggested to them that the subnormal levels of plasma magnesium in their patients of myocardial infarction might be related to their enhanced platelet aggregation.

The antagonism of magnesium with calcium for clotting factors **(Greville et al. 1944)**[101] , even at concentration produced after oral administration **(Huntsman et al. 1960)**[98] has given an explanation of its being an anticoagulant in his study.

Anstall et al. (1959)[97] and **Graville et al. (1944)**[101] showed that a single oral dose of magnesium has produced a delay in thrombin time lasting for 4-6 hours in patients with myocardial infarction. Magnesium influences thrombosis by interfering with intravascular coagulation through its competition with calcium and by stabilizing fibrinogen and platelets by promtiong fibrinolysis and by causing vasodilatation **(Stevenson et al. 1972 ; Szelenyi et al. 1967)**[102,103]

As magnesium takes part in many cellular activities ,it plays an important role in control of neuronal activity, cardiac excitability, muscle contraction, neuromuscular transmission, vasomotor tone, blood pressure and peripheral blood flow. **(Altura BM et al. 1996)**[104]

Causes Of Hypomagnesemia[105]**:-**

1) Impaired Intestinal Absorption
- Primary infantile hypomagnesemia
- Malabsorption syndrome
- Vitamin D deficiency

2) Increased Intestinal Loss
- Intestinal drainage, fistula
- Protracted vomiting, diarrhea

3) Impaired Renal Tubular Absorption
- Genetic magnesium wasting syndrome
- Bartter syndrome
- Gitelman syndrome
- Na K ATP ase g-subunit mutation
- Acquired renal disease
- Tubulointerstitial disease
- Renal transplantation
- Post obstruction/Acute tubular necrosis (diuretic phase)

4) Drugs
- Ethanol
- Diuretics(osmotic, loop and thiazide)
- Cisplatin, Cyclosporin
- Aminoglycosides, Amphotericin B

5) Metabolic Causes
- Diabetes mellitus
- Metabolic acidosis
- Hyperaldosteronisim
- Syndrome of inappropriate ADH secretion
- Hypercalcemia
- Hyperthyroidism

6) Others
- Pancreatitis
- Excessive sweating
- Osteoblastic metastasis

Clinical Features Of Hypomagnesemia (Saris NE et al. 2000)[106]

Symptom's :-
- Patient may present with non specific symptoms.
- Symptoms include weakness, muscle cramps, vertigo, ataxia, depression, seizure and altered mental status.
- Symptoms and signs occur only when serum magnesium concentration is less than 1.2mg/dl.

Sign's:-
- Muscle cramps.
- Hyperactive deep tendon reflexes.
- Trousseau and chovstek sign.
- Dysphagia due to esophageal dysmotility.
- Irritability /disorientation.
- Ataxia, nystagmus and seizure.
- Tachycardia.

ECG changes in hypomagnesemia (Purvis JR et al. 1992)[107]**:-**

ECG changes in hypomagnesemia are non specific. Modest level of magnesium deficiency causes widening of the QRS complex and peaking of T wave. Severe magnesium deficiency causes prolongation of PR interval, progressive widening of QRS complex, flattening/inversion of T wave and U wave[64].

MAGNESIUM AND DIABETES :-

Magnesium plays an important role in carbohydrate metabolism. **(Mooren FC, 2015)**[108]

Diabetes mellitus is one of the most common metabolic disorder associated with magnesium deficiency. **(Jackson et al. 1968)**[109]

The prevalence of magnesium deficiency in diabetes is around 25-39% **(Rude RK et al. 1995)** [80]

This diagnosis was listed in 20 % of the patients with serum magnesium level below 1.48 mEq/litre, in 14 % of those with magnesium levels between 1.48 and 1.52, but in only 3.8% of those in the "normal" 1.55 to 1.99 range. One half of the diabetic patients in the law magnesium groups (less than 1.53 mEq/litre) had admitting blood sugar elevations of this magnitude. A review of records of all diabetics in the study has also found in those patients with poorly controlled disease. However, ketonuria was not encountered in any of the 16 diabetics in the less than 1.48 mEq/litre magnesium category. **(Jackson et al. 1968)**[109]

In early studies of serum magnesium levels, diabetic acidosis was shown to be associated with hypomagnesemia. **(atchley et al. 1933)** [110] In **1942, Haury and Cantarow** [111] reported low levels of magnesium in seven of 56 diabetics studied. **Stutzman et al. (1953)**[112] revealed that in diabetes mellitus and portal cirrhosis a significantly low serum magnesium and portal cirrhosis a significantly low serum magnesium may be found. They suggested that the low serum magnesium levels in diabetes were related in some way to the fact that magnesium is cofactor in carbohydrate metabolism and also an increased urinary excretion of magnesium has been reported after ingestion of glucose.

Large amount of magnesium are excreted during acidosis after experimental withdrawl of insulin from patient with diabetes, a loss of 0.9 mEq/Kg over a period of three and half day of insulin deprivation has been measured. As much as 40 mEq/of magnesium is retained during the 1st week of restored function. **(Wacker et al. 1968)**[113]

In a study of 100 patients with controlled diabetes the serum magnesium rise significantly with age form normal levels to a mean high of 2.33 mEq/ liter in these over 60 years of age. No such systemic fluctuation was observed in non-diabetic controls. **(Butler et al. 1950)**[114]

The mean serum magnesium concentration of patients with controlled diabetes receiving insulin is said to be low' however the difference of the reported mean value form that of normal person is not significant. **(stutzman et al. 1953)**[112]

P. Mcnair et al. (1978) [18] has tried to find out a correlation between serum magnesium and severity of diabetic retinopathy. he observe that there was a definite lowering of serum magnesium in long term, insulin treated diabetic out patients having fairly normal renal function (P<0.001). The division of his patients according to severity of their retinopathy yielded groups that were strictly comparable in all respects i.e. potassium, calcium, creatinine, triglycerides, cholesterol, c-peptide, blood glucose and urine glucose, except serum magnesium. Consequently, he suggested hypomagnesemia as a possible risk factor in the development and progress of diabetic retinopathy. According to him the cause of diabetic hypomagnesemia was unknown but an increased urinary loss of magnesium may contribute to it.

So it was very clear from above that although much work was done on diabetic retinopathy but only **P. Mc nair et al. (1978)** [18] was first to find out a correlation between the diabetic retinopathy and magnesium level which was supposed to be another risk factor in diabetic retinopathy.

A. CERIELLO et al. (1981)[115], suggested that diabetes patients have lower level of serum magnesium than healthy people, regardless of therapy.

A link between hypomagnesaemia and the complication of Diabetes mellitus and suggesting that patients who have documented hypomagnesaemia and DM should receive magnesium supplementation. **(John R. White Jr., R. Keith Campbell, 1993)**[116]

Phuong-Chi T. Pham et al. (2007)[117] linked hypomagnesaemia to poor glycemic control, coronary artery diseases, hypertension, diabetic retinopathy, nephropathy, neuropathy, and foot ulcerations and described that hypomagnesaemia among patients with type 2 diabetes presumably is multifactorial.

Dipankar Kundu, Manish Osta, Divyendu Gautam (2013)[118] suggest hypomagnesaemia and albuminuria individually or in conjunction serve as indicator for dysglycemia and could be used as marker for the risk of development of diabetic retinopathy.

Shahbah D et al. Medicine (Baltimore) (2017)[119] suggest oral magnesium supplementation improves glycemic control and lipid profile in children with type 1 diabetes and hypomagnesaemia.

Aetiology of hypomagnesemia in diabetes (Durlach J et al. 1983)[20]

- Diet tends to be low in magnesium.
- Reduced intestinal absorption of magnesium.
- Inceased renal magnesium loss due to glycosuria.
- Insulin effect causes redistribution of magnesium from plasma to red blood cells.
- Insulin insensitivity affects magnesium transport and glucose metabolism.
- Loop and thiazide diuretic use promotes magnesium loss.

A specific tubular defect has been postulated for magnesium deficiency in diabetes. The site of the defect is not yet defined. The proposed site of defect is the thick ascending limb of loop of henle or more distally. Reduced tubular magnesium absorption results in hypermagnesuria. Treatment with insulin will correct renal magnesium loss. **(Anwana AB J et al. 1990)**[120]

Role Of Magnesium In Glucose Homeostasis And Insulin Sensitivity :-

In patients with diabetes magnesium deficiency have shown a negative impact on glucose homeostasis and insulin sensitivity. **(Rosolova H J et al. 1997)**[121]

In diabetics uptake of magnesium in erythrocytes in response to insulin is reduced. This change was associated with an increase in erythrocyte membrane microviscosity. Changes in the physical state of plasma membrane and insulin resistance were responsible for the lower erythrocyte magnesium level. Plasma membrane changes impair the interaction of insulin with its receptors and reduces glucose tolerance.

The reduced insulin sensitivity is due to defective tyrosine kinase activity of the insulin receptor. Several enzymes are involved in glucose metabolism, which requires high energy phosphate bonds. Magnesium acts as a cofactor in these enzymatic reactions. Intracellular magnesium deficiency leads to worsening of insulin action and insulin resistance.

Thus low magnesium level contributes to insulin resistance ,which in turn reduces magnesium uptake in insulin sensitive tissues. **(Paolisso G et al. 1989)**[19]

MATERIALS AND METHODS

To estimate the **"ASSOCIATION OF SERUM MAGNESIUM**

LEVEL WITH STAGES OF DIABETIC RETINOPATHY" study conducted on patients taken from wards and attending the outpatient department of ophthalmology, **GOVT. MEDICAL COLLEGE AND ASSOCIATED GROUP OF HOSPITALS, KOTA.**

SOURCE OF DATA

Study Place :Ward and Out-patient department, Department of Ophthalmology, Govt. Medical College and associated group of hospitals, Kota.

Study Subjects : Classified in 4 groups

1. Non-diabetic patients (Control group)	30 Cases
2. Diabetic patients without retinopathy	30Cases
3. Diabetic patients with NPDR	30 Cases
4. Diabetic patients with PDR	30 Cases

Study Design : Cross sectional study
Sample Size : 120

DIAGNOSTIC CRITERIA:

The criteria chosen for the diagnosis of diabetes are those proposed by WHO study.

Glucose conc. in mmol/L (mg/dl)

Impaired Glucose Tolerance	Venous Plasma
Fasting Value	< 7.0 mmol/L (126 mg/dl)
2 hrs after 75 g glucose load	≥ 7.8 mg/dl and < 11.1 mmol/L (140mg/dl to 200 mg/dl)

Glucose conc. in mmol/L (mg/dl)

Diabetes Mellitus	Venous Plasma
Fasting Value	≥ 7.0 mmol/L (126 mg/dl)
2 hrs after 75 g glucose load	≥ 11.1mmol/L (200mg/dl)

Blood Sugar Control[4] :-

Blood sugar level defined as-
d)**Good Control** – Fasting Blood Sugar Below 130 mg %
e)**Fair Control** – Between 130-150 mg %
f)**Poor Control** – Above 150 mg %

Inclusion Criteria:

1)Known cases of diabetes mellitus.
2)Mentally and physically fit up to a level to participate in study.
3)Patients who gave voluntary informed consent.

Exclusion Criteria:

1)Patients with primary malignant hypertension.
2)Chronic alcoholic patients
3)Pregnant and lactating women
4)Glaucomatous patients
5)Patients with primary renal disorders
6)Patients with gastro-intestinal disorder
7)Patient taking diuretics and antacid

METHOD

Venous blood is drawn into 2 ml disposable syringe after all aseptic precautions and then emptied in a vial containing heparin as anticoagulant. Blood collected in tubes containing EDTA is not used in the study. Fresh, unhemolyzed serum is used. Hemolyzed serum, Grossly icteric or lipemic specimens not used in study.

PRINCIPLE

Atomic Absorption Spectrometry (AAS)-This method for magnesium detection, first proposed by J.B. DAWSON and F.W. HEATON in 1960.[122]

Serum magnesium ions react with Xylidyl Blue (magnon) in an alkaline solution to produce a red complex that is measured spectrophotometrically. The intensity of color produced is directly proportional to magnesium concentration.

The color produced is measured at 550 nm.

$$Mg^{2+} + xylidyl\ blue \xrightarrow{pH\ 11.4} Purple\ Complex$$

This coloured complex so obtained will be measured by spectrophotometer. Spectrophotometery is based on Lambert Beers law which states that when light passes through a coloured medium it is absorbed in direct proportion to the concentration of coloured substance.

So the light transmitted through a coloured solution which is observed in a spectrophotometer is inversely proportional to the concentration of the solution.

The concentration of coloured substance is directly proportional to the Absorbance.

Absorbance is defined as the logarithm of ratio of the incident to transmitted light.
C1/C2=A1/A2
Where, C1 and C2 are the concentration and A1 and A2 as their Absorbance

$$\text{Conc. Of unknown} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of standard}} \times \text{Conc of standard}$$

Calcium interference is virtually eliminated by use of EGTA and a surfactant system is included to remove protein interference.

COMPOSITION OF THE REAGENTS USED

1.Enzyme Reagents

The reagents are supplied as ready to use liquids.

Xylidylblue	0.1mM
EGTA	0.13mM
DMSO	1.4M

2.Buffer

Potassium cyanide	0.02% w/v

PROCEDURE

(I) Three test tubes will be taken and marked as blank (B) standard (S) and Test (T) and the solution were pipetted out into, the test tube as follows.

1. Pipette into 3 test tubes	**Blank mL**	**Standard (STD) Ml**	**Test Ml**
Working Reagent	1	1	1
Standard	-	.01	-
Sample	-	-	.01

(ii)Mix well Incubate for 3 minutes at 37*C

(iii)Read the A (Absorbance) of the Test and Standard against Blank at 550nM.

RESULTS

$$\text{Magnesium Conc.} = \frac{\text{Absorbance of magnesium}}{\text{Absorbance of standard}} \times 2\ (\text{mg/dl})$$

- Magnesium mg/dl converted to mEq/litre by multiply 0.83 to mg/dl value.

- Statistical analysis done with ANOVA T test.

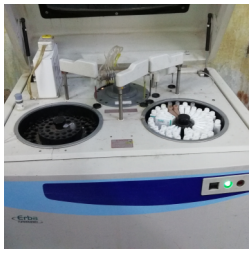


Fig. 19) Autoanalyser Using Magnesium Reagent (xylidine Blue)

OBSERVATIONS AND RESULTS

Age And Sex Distribution Of Cases :-

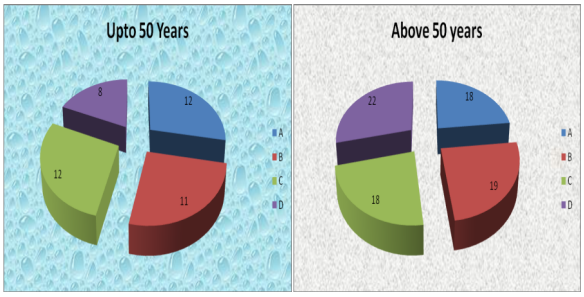
According to age, patients are divided into 2 groups, to establish the values separately for younger and older age group and also to note the relative distribution of cases in different groups with regard to age.

a. Those upto the age of 50 years.

b. Those above the age of 50 years.

Table -1 Age Distribution Of Cases Studied

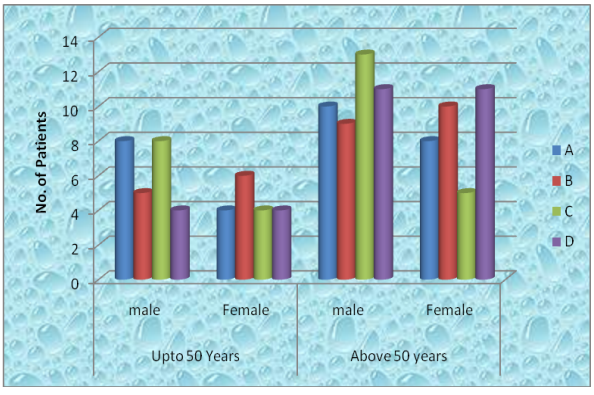
Group	Age (in years)			
	Upto 50 years		Above 50 years	
	no.	%	no.	%
A	12	40.00	18	60.00
B	11	36.66	19	63.33
C	12	40.00	18	60.00
D	8	26.66	22	73.33



Graph 1) Age Distribution Of Study Cases

Table– 2 Sex Distribution Of Cases Studied

Group	Upto 50 Years				Above 50 Year			
	Male		Female		Male		Female	
	No	%	No	%	No	%	No	%
A	8	26.66	4	13.33	10	33.33	8	26.66
B	5	16.66	6	20.00	9	30.00	10	33.33
C	8	26.66	4	13.33	13	43.33	5	16.66
D	4	13.33	4	13.33	11	36.66	11	36.66



Graph 2) Sex Distribution Of Study Cases

In Group A:- 40% (12 cases) are upto 50 years, out of these 26.66 % (8 cases) are male and 13.33 % (4 cases) are female. 60% (18 cases) are above 50 years, out of these 33.33% (10 cases) are male and 26.66 % (8 cases) are female.

In Group B:- 36.66 % (12 cases) are below 50 years, out of these 16.66 % (5 cases) are male and 20.00 % (6 cases) are female. 63.33% (19 cases) are above 50 years and out of these 30% (9 cases) are male and 33.33% (10cases) are female.

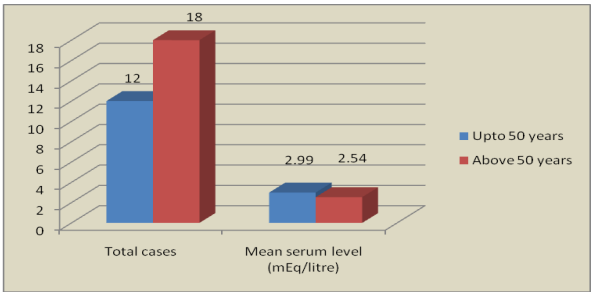
In Group C :- 40.00 % (12 cases) are below 50 years, out of these 26.66 % (8 cases) are male and 13.33 % (4 cases) are female. 60.00 % (18 cases) are above 50 years and out of these 43.33 % (13 cases) are male and 16.66 % (5 cases) are female.

In Group D :- 26.66 % (8 cases) are below 50 years, out of these 13.33 % (4 cases) are male and 13.33 % (4 cases) are female. 73.33% (22 cases) are above 50 years and out of these 36.66 % (11 cases) are male and 36.66 % (11 cases) are female.

Table –3 Mean Serum Magnesium Level According To Age In Control Group

Age	Total cases	Mean serum level (mEq/litre)	S.D.	p value
Upto 50 years	12	2.99	0.16	<0.0001
Above 50 years	18	2.54	0.19	

In control (Non-diabetic) group, mean serum magnesium level in patients upto 50 year of age is 2.99 mEq/litre with standard deviation 0.16 and in patients above 50 year of age is 2.54 mEq/litre with standard deviation 0.19 and calculated p value is <0.0001.

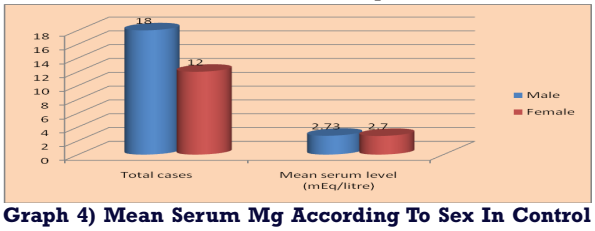


Graph 3) Mean Serum Mg According To Age In Control Group

Table –4 Mean Serum Magnesium Level According To Sex In Control Group

Sex	Total cases	Mean serum level (mEq/litre)	S.D.	p value
Male	18	2.73	0.28	0.7817
Female	12	2.70	0.30	

In control (Non-diabetic) group, mean serum magnesium level in male patients is 2.73 mEq/litre with standard deviation 0.28 and in female patients is 2.70 mEq/litre with standard deviation 0.30 and calculated p value is 0.7817.



Graph 4) Mean Serum Mg According To Sex In Control Group

Duration Of Diabetes :-

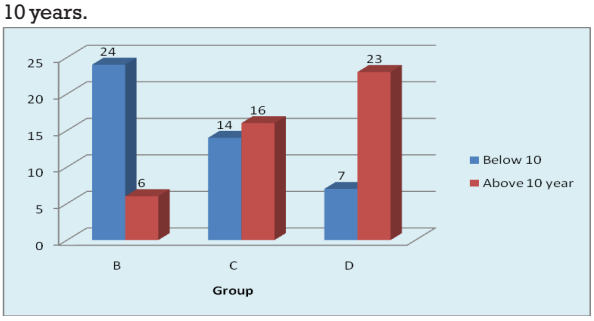
Table – 5 Duration Of Diabetes

Group	Below 10 years		10 years or above		Total %
	No	%	No	%	
B	24	80.00	6	20.00	100
C	14	46.66	16	53.33	100
D	7	23.33	23	76.66	100

In Group B, 80.00 % cases had diabetes below 10 years and 20.00 % cases are those with duration of diabetes more than 10 years.

In Group C, 46.66 % cases had diabetes below 10 years and 53.33 % cases are those with duration of diabetes more than 10 years.

In Group D, 23.33 % cases had diabetes below 10 years and 76.66 % cases are those with duration of diabetes more than 10 years.



Graph 5) Duration Of Diabetes In Study Cases

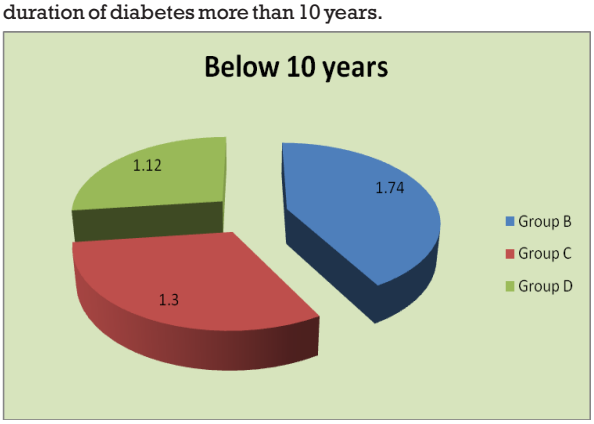
Table – 6 Mean Serum Magnesium Level In Relation To Duration Of Diabetes (According To Groups)

Duration of diabetes	Mean serum Magnesium level		
	Group B	Group C	Group D
Below 10 years	1.74 mEq/liter	1.30 mEq/liter	1.12 mEq/liter
10 years or above	1.41 mEq/liter	1.25 mEq/liter	0.80 mEq/liter

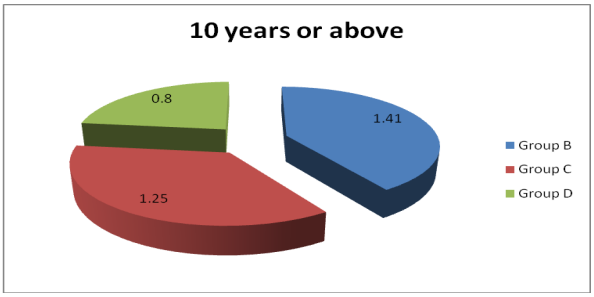
In Group B, mean serum mg level is 1.74 mEq/liter in diabetes below 10 years and 1.41 mEq/liter is in those with duration of diabetes more than 10 years.

In Group C, mean serum mg level is 1.30 mEq/liter in diabetes below 10 years and 1.25 mEq/liter is in those with duration of diabetes more than 10 years.

In Group D, mean serum mg level is 1.12 mEq/liter in diabetes below 10 years and 0.80 mEq/liter is in those with duration of diabetes more than 10 years.



Graph 6) Mean Serum Magnesium Level In Below 10 Years Of Diabetes

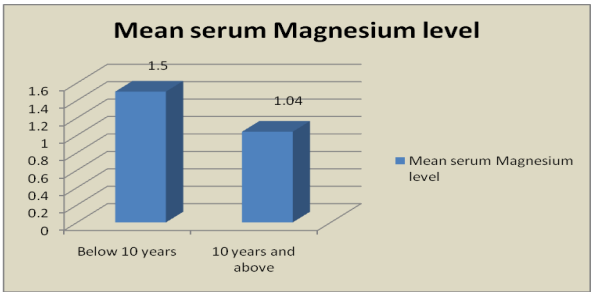


Graph 7) Mean Serum Magnesium Level In 10 Years Or Above Of Diabetes

Table – 7 Mean Serum Magnesium Level In Relation To Duration Of Diabetes (90 patients)

Duration of diabetes	Mean serum Magnesium level	S.D.	pvalue
Below 10 years	1.50 mEq/liter	0.30	<0.0001
10 years and above	1.04mEq/liter	0.28	

In patients below 10 years of diabetes, mean serum magnesium level is 1.50 mEq/liter with standard deviation 0.30 and In patients with 10 years or more with diabetes it is 1.04 mEq/litre with standard deviation 0.28 and calculated p value is <0.0001.



Graph 8) Mean Serum Mg Level In Association With Duration Of Diabetes

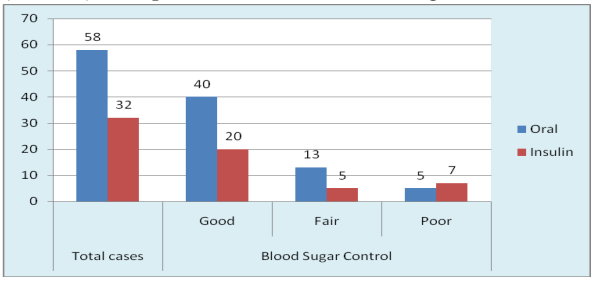
Blood Sugar Control & Type Of Treatment:-

Table–8 Type Of Therapy In Relation To Blood Sugar Control

Type of Therapy	Total cases	Blood Sugar Control		
		Good	Fair	Poor
Oral	58	40	13	5
Insulin	32	20	5	7

There are 58 patients, who treated with oral hypoglycemic agent. Out of which 40 (68.96 %) have good control, 13 (22.41 %) having fair and 5 (8.6%) have poor control of their blood sugar.

There are 32 patients, who treated with insulin. Out of which 20 (62.5%) have good control, 5 (15.62%) having fair and 7 (21.87 %) have poor control of their blood sugar.



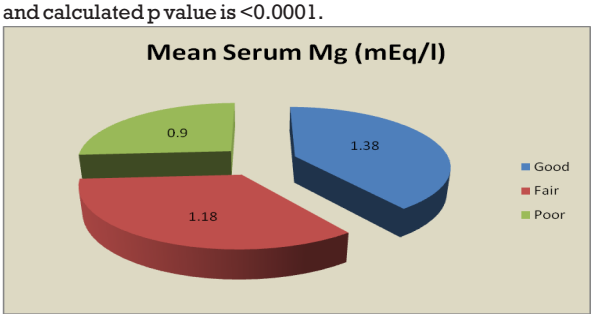
Graph 9) Blood Sugar Control In Study Cases

Table–9 Blood Sugar Control In Diabetes And Mean Serum Magensium Level

Blood Sugar Control	Mean Serum Mg (mEq/l)	S.D.	p value
Good	1.38 mEq/litre	0.368	<0.0001
Fair	1.18 mEq/litre	0.261	
Poor	0.90 mEq/litre	0.298	

The table show that in our study there is difference in mean serum magnesium level between good, fair and poor control.

In good control it is 1.38 mEq/liter with standard deviation 0.368, in fair it is 1.18 mEq/liter with standard deviation 0.261 and in poor it is 0.90 mEq/liter with standard deviation 0.298 and calculated p value is <0.0001.

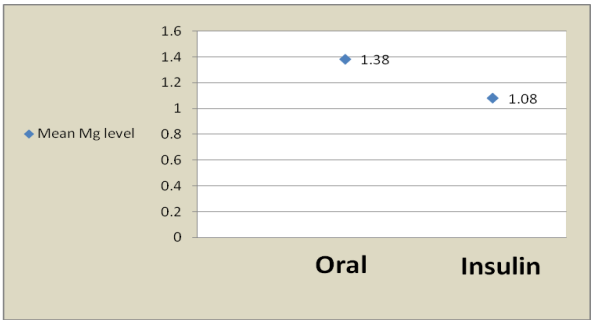


Graph 10) Mean Serum Mg & Blood Sugar Control In Study Cases

Table-10 Mean Serum Magnesium Level According To Type Of Treatment

Type of Therapy	Total Cases	Mean Serum level (mEq/l)	S.D.	p value
Oral	58	1.38	0.37	0.0002
Insulin	32	1.08	0.31	

We observed that patients treated by oral hypoglycemic agent (58 patients) the mean serum magnesium level is 1.38 mEq/litre with standard deviation 0.37 and & In patients on insulin treatment (32 patients) it is 1.08 mEq/liter with standard deviation 0.31 and which is lower than that with oral hypoglycemic drugs. On statistical analysis estimated p value is 0.0002.

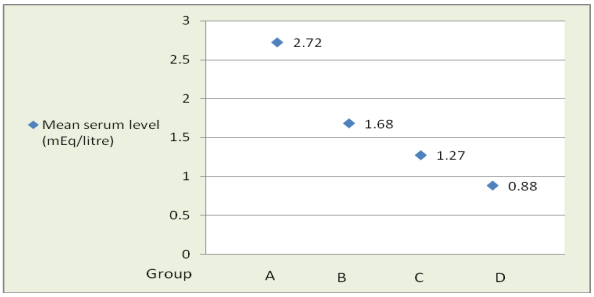


Graph 11) Mean Serum Mg In Relation To Type Of Treatment

Mean Serum Magnesium Level Comparison :

Table – 11 Mean Serum Magnesium Level In Different Group

Group	Total cases	Mean serum level (mEq/litre)
A	30	2.72
B	30	1.68
C	30	1.27
D	30	0.88



Graph 12) Mean Serum Magnesium Level In Study Group

In Group A, 30 cases were studied. In this group mean serum magnesium level (2.72 mEq/litre) which is higher than the normal (1.6 to 2.1 mEq/litre).

In Group B, 30 cases were studied. In this group mean serum magnesium level (1.68 mEq/litre) which is within the range the normal serum magnesium level.

In Group C, 30 cases were studied. In this group mean serum magnesium level (1.27 mEq/litre) which is lower than the range the normal serum magnesium.

In Group D, 30 cases were studied. In this group mean serum magnesium level (0.88 mEq/litre) which is lower than the range the normal serum magnesium.

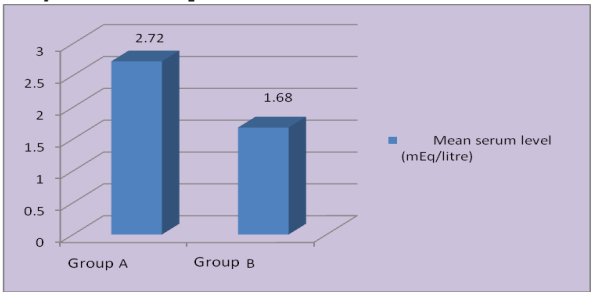
So in group C & D mainly patients are those with a low serum magnesium as compared to control.

So, Magnesium level decreased according to the appearance of diabetic retinopathy. In control group the mean serum level is 2.72 mEq/litre, In patients without retinopathy it is 1.68 mEq/litre, in patients with non-proliferative diabetic retinopathy (NPDR) the level is 1.27 mEq/litre and in patients with Proliferative diabetic retinopathy (PDR) the level is 0.88 mEq/litre.

Table –12 Comparison Between Control Group And Diabetic Without Retinopathy Group

Group	Total cases	Mean serum level (mEq/litre)	S.D.	p value
A	30	2.72	0.28	<0.0001
B	30	1.68	0.22	

A Marked difference of serum magnesium level is seen between control group and those with diabetic without retinopathy. In group A mean serum magnesium level is 2.72 mEq/litre with standard deviation 0.28 and in group B it is 1.68 mEq/litre with standard deviation 0.22 and On statistical analysis estimated p value is <0.0001.



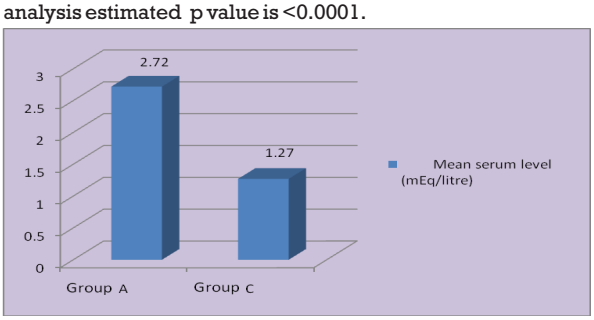
Graph 13) Comparison Between Group A And Group B

Table – 13 Comparison Between Control Group And Npdr Group

Group	Total cases	Mean serum level (mEq/litre)	S.D.	p value
A	30	2.72	0.28	<0.0001

C	30	1.27	0.059	

A difference of serum magnesium level is seen between control group and those with Non-proliferative diabetic retinopathy. In group A mean serum magnesium level is 2.72 mEq/litre with standard deviation 0.28 and in group C it is 1.27 mEq/litre with standard deviation 0.059 and On statistical analysis estimated p value is <0.0001.

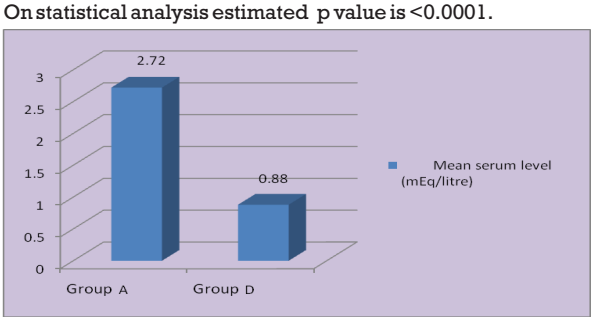


Graph 14) Comparison Between Group A And Group C

Table – 14 Comparison Between Control And Proliferative Diabetic Retinopathy Group

Group	Tatal cases	Mean serum level (mEq/litre)	S.D.	p value
A	30	2.72	0.28	<0.0001
D	30	0.88	0.21	

A difference of serum magnesium level is seen between control group and those with Proliferative diabetic retinopathy (PDR) group. In group A mean serum magnesium level is 2.72 mEq/litre with standard deviation 0.28 and in group D it is 0.88 mEq/litre with standard deviation 0.21 and On statistical analysis estimated p value is <0.0001.

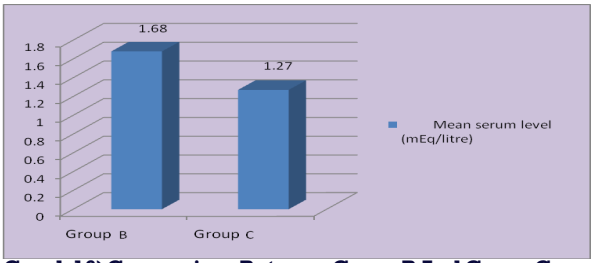


Graph 15) Comparison Between Group A And Group D

Table – 15 Comparison Between Diabetic Without Retinopathy And NPDR Group

Group	Tatal cases	Mean serum level (mEq/litre)	S.D.	p value
B	30	1.68	0.22	<0.0001
C	30	1.27	0.059	

A difference of serum magnesium level is seen between diabetic without retinopathy group and those with Non-proliferative diabetic retinopathy (NPDR) group. In group B mean serum magnesium level is 1.68 mEq/litre with standard deviation 0.22 and in group C it is 1.27 mEq/litre with standard deviation 0.059 and On statistical analysis estimated p value is <0.0001.

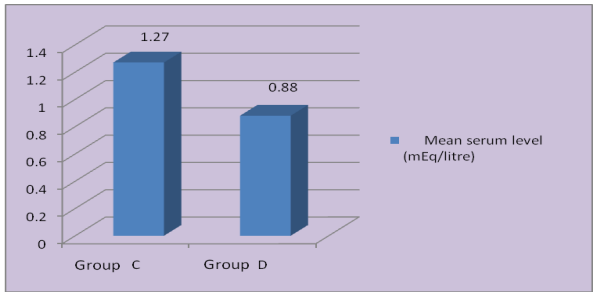


Graph 16) Comparison Between Group B And Group C

Table – 16 Comparison Between NPDR And PDR Group

Group	Total cases	Mean serum level (mEq/litre)	S.D.	P value
C	30	1.27	0.059	<0.0001
D	30	0.88	0.21	

A difference of serum magnesium level is seen between Non-proliferative diabetic retinopathy group (NPDR) and Proliferative diabetic retinopathy (PDR) group. In group C mean serum magnesium level is 1.27 mEq/litre with standard deviation 0.059 and in group D it is 0.88 mEq/litre with standard deviation 0.21 and On statistical analysis estimated p value is <0.0001.



Graph 17) Comparison Between Group C And Group D

DISCUSSION

A group of 30 patients with Proliferative diabetic retinopathy (PDR) and 30 patients with Non-proliferative diabetic retinopathy (NPDR) were subject of study for estimation of serum magnesium. The results are compared to the controls (Non-diabetic – 30 cases) and with diabetics without retinopathy (30 cases). A relationship with age and sex, duration, control of blood sugar, type of therapy, stages and severity of retinopathy observed is as under :-

1)In our study, with reference to Table no. 1, cases below 50 year age are 36.66% in diabetes without retinopathy, 40% in NPDR, 26.66% in PDR and above 50 year age are 63.33% in diabetes without retinopathy, 60% in NPDR, 73.33% in PDR respectively. This is in according to **CDC's 2017 National Diabetes Statistics Report,** stated that cases affected with diabetes with retinopathy were mainly above 45 years. This may be because of the longer duration of the disease in old persons.

2)In our study, with reference to Table no. 2, male to female ratio in diabetes without retinopathy (group B) is 7:8 and diabetics with retinopathy (group C & D) was 3:2, which is similar sex incidence given by **Ozawa et al., 2014**[124] said that female affected more in diabetes without retinopathy and male affected more in diabetes with retinopathy.

3)With reference to Table no. 3, in control (Non-diabetic) group (as there is no effect of diabetes on magnesium level), mean serum magnesium level in patients upto 50 year of age is 2.99 mEq/litre and in patients above 50 year of age is 2.54 mEq/litre. This is clinically significant as p value is <0.05. This showing age related decrease in level of serum magnesium may be due to age related decrease in renal reabsorption function.

4)With reference to Table no. 4, In control (Non-diabetic) group, mean serum magnesium level in male patients is 2.73 mEq/litre and in female patients is 2.70 mEq/litre. This is clinically Insignificant as p value is >0.05.But as the sample size is small in our study so a study with large sample size is required to evaluate this relationship.

5)In our study, with reference to Table no. 5, the patients of diabetes without retinopathy are mainly those with duration of diabetes less than 10 years (80%) while those with more

than 10 years of duration are only 20 percent. In group of diabetes with retinopathy (Group C & D) the cases with less than 10 years of duration are 35 percent and those with more than 10 years of duration are 65 percent, According to this study the diabetic retinopathy is proportional to the duration of the disease which is in agreement with other workers **(Gupta et.al.,2013)**[125].

6)With reference to Table no. 7, There is an inverse relationship between the duration of disease and serum magnesium level i.e. in patients upto 10 years mean serum mg level is 1.50 mEq/litre in contrast to patients with duration of diabetes more than 10 years in which level is 1.04 mEq/litre, so as the duration of the diabetes increased there occurred a decrease in serum magnesium level. This relation between the serum magnesium level and duration of diabetes is clinically significant as p value is <0.05. This is probably because of atherosclerotic changes and in agreement with the study of **Arpaci et.al., 2015**[126]**.**

7)In our study, with reference to Table no. 9, mean serum magnesium level depend upon blood sugar control i.e. In patients with good control of blood sugar level's (FBS <130 mg/dl) mean serum magnesium is 1.38 mEq/liter, in fair control of blood sugar level's (FBS 130-150 mg/dl) mean serum magnesium is 1.18 mEq/liter and in poor control of blood sugar level's (FBS >150 mg/dl) mean serum magnesium is 0.90 mEq/liter and this association of blood sugar control with serum magnesium level's are clinically significant as p value is <0.05 and this is in agreement with the study of **Belhasan et. al., 2016**[127]**.**

8)In our study, with reference to Table no. 10, mean serum magnesium level is affected by the type of therapy given, whether oral hypoglycemic agent or insulin is taken by patient as we observed that patients treated by oral hypoglycemic agent (58 patients) the mean serum magnesium level is 1.38 mEq/litre and & in patients on insulin treatment (32 patients) it is 1.08 mEq/liter, which is lower than that with oral hypoglycemic drugs and this association of type of therapy with serum magnesium level's are clinically significant as p value is <0.05. This may be simply because of the fact that the patients on insulin therapy are more (21.87 %) with poor control of their blood sugar as compare to patients (8.6 %) on oral hypoglycemic drugs with poor control of their blood sugar.

9)In our study, with reference to Table no. 11, Group A cases with the mean serum magnesium level are toward higher side than the normal and thus is similar with **Dubey et al., 1975** [123] and may be simply because of dietary and bowel habits of the persons in this geographical region. While in group C and D (diabetic retinopathy) there is a marked decrease in the serum magnesium level. This is in agreement with the study of **P.Mc Nair et al., 1978**[18], who observed that hypomagnesemia is one of the risk factor in producing atherosclerosis of retinal vessels and thus affecting the stages of diabetic retinopathy. It has also been stressed that the magnesium has got the anti-atherosclerotic action by reducing cholesterol and other serum lipids. (**Dubey et al., 1975)**[123].

10) In this study mean serum magnesium level was decreased with a significant difference (P<0.05) between the diabetic without retinopathy group, NPDR and PDR group (with reference to Table no. 12, 13, 14, 15, 16) and suggested that level are decrease with increasing severity of diabetes retinopathy. This is in agreement with **P. Mc Nair et al. 1978**[18]**.** possible mechanism of hypomagnesemia may be as follows:

As suggested by **Stutzman et.al. 1953**[112] increased loss of magnesium in urine is due to Osmotic action of glucosuria lead to depress the net tubular reasborption of magnesium and may causes hypomagnesemia.

CONCLUSION

This thesis is an study to examine the serum magnesium levels among Non-diabetic and diabetic patients. Hypomagnesaemia is likely among patients with diabetes mellitus. Long term complications especially retinopathy may have hypomagnesemia as a contributing factor and hypomagnesemia is have negative association with stages of diabetic retinopathy. Hence it is prudent that serum magnesium levels are carefully monitored in diabetic patients. Future studies on the role of magnesium supplementation in diabetes in Indian population are recommended.

PROFORMA

DEPARTMENT OF OPHTHALMOLOGY
GOVT. MEDICAL COLLEGE AND ASSOCIATED GROUP OF HOSPITALS, KOTA

Case No.

Particulars Of Patients :

a. O.P.D. No:
b. Name :
c. Age:
d. Sex:
e. Occupation:
f. Address:

Complaints With Duration :

History Of Present Illness:

Personal History:

a)Smokers or Non-smokers
b)Alcoholic or Non-alcoholic

Family History:

If any member diabetic

Any Evidence Of:

1)Renal disease
2)Gastro-intestinal disorder
3)Pregnancy and lactation
4)Patients taking diuretics and antacid

Ocular/ophthalmic Examination :-

	Right Eye	Left Eye
1)Vision		
2)Lids		
3)Lacrimal Appratus		
4)Conjunctiva		
5)Cornea		
6)Anterior Chamber		
7)Iris		
8)Pupillary Reaction		
a)Direct		
b)Indirect		
9) Lens		
10)Vitreous		
11)Extraocular muscle movement		
12)IOP		
13)Fundus examination		
a)Indirect ophthalmoscopy		
b)90D biomicroscopy		
c) FFA and Fundus photography		
14) S/L Examination		
15)Any other Abnormality		

Biochemical Investigation:

1)Fasting blood sugar (mg %)
2)Postprandial blood sugar (mg%)
3)HbA1c level

4)Serum urea
5)Serum creatinine
6)Serum magnesium (mEq/ per Litre)
7)Urine analysis
a)Albumin
b)Sugar
c)Microscopic

Mode Of Treatment:
Inj. Insulin
Oral Antidiabetic drug

Duration Of Treatment

Duration Of Diabetes

Remark

सूचित सहमति पत्र
मैं .....पुत्री/पुत्र .....
होश पूर्वक चिकित्सक को प्रायोगिक अनुसंधान हेतु निम्नलिखित विशय के लिए दवा/प्रकिया की स्वंय पर प्रयोग की अनुमति देती हूँ ।

Topic: "ASSOCIATION OF SERUM MAGNESIUM LEVEL WITH STAGES OF DIABETIC RETINOPATHY" *इन प्रयोगो के समस्त लाभहानि के बारे में मुझे भलीभाँति समझा दिया गया है । मैं इस संस्थान में उपलब्ध समस्त सुविधाओं एवं सुरक्षा के आवरण सहित मानवता के हित में स्वंय पर किए जाने वाले प्रयोग की स्वीकृति देती हूँ । मुझे बता दिया गया है कि पूर्व में इस तरह के प्रयोग यद्यपि पूर्णतः सुरक्षित रहे हैं, फिर भी होने वाले समस्त लाभहानि की जिम्मेदारी स्वंय की होगी ।*

Informed Consent

I..... daughter/son of ..... with my full awareness and sound state of mind, present myself as an object for an experimental study of a drug effect/procedure for research purpose on following subject:

Topic: "ASSOCIATION OF SERUM MAGNESIUM LEVEL WITH STAGES OF DIABETIC RETINOPATHY" I have been well informed about all benefits and adverse effects of the experimental study. I give my consent in favour of humanity under cover of all available health facilities and protection of the institution. I have been informed that although earlier such type of experiments were safely performed in several institutions even after that I take responsibility for the all beneficial and adverse outcomes of the study.

Attendant's signature Patient's signature
Date: Date:

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