GENOTYPE - PHENOTYPE CORRELATION OF ICAM-1 K469E POLYMORPHISM WITH SEVERITY OF RETINOPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

DISSERTATION SUBMITTED

BY

DR. SANJANA B. SINGH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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IN

OPHTHALMOLOGY

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

APRIL 2015

DEPARTMENT OF OPHTHALMOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

COIMBATORE

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I hereby declare that this dissertation entitled **"GENOTYPE - PHENOTYPE CORRELATION OF ICAM-1 K469E POLYMORPHISM WITH SEVERITY OF RETINOPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS"** is a bonafide and genuine research carried out by me under the guidance of **DR. JEEVAMALA MERCY JANAKI, D.O, DNB** Professor, Department of Ophthalmology, PSG Institute Of Medical Sciences And Research, Coimbatore in partial for the award of M.S Degree in Ophthalmology to be held in 2015. This dissertation has not been submitted in part or full to any other university or toward any other degree before this below mentioned date.

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April 3, 2013

To
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The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 8th February, 2013 in its expedited review meeting held at College Council Room, PSG IMS&R, between 2.30 pm and 4.30 pm, and discussed your application to conduct the study entitled:

"The genotype phenotype correlation of ICAMI K469E gene polymorphism and severity of retinopathy in patients with Type 2 diabetes mellitus"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Consent forms in English and Tamil
4. Budget
5. Data Collection Tool
6. CV

After due consideration, the Committee has decided to approve the above study.

The members who attended the meeting, at which your proposal was discussed, are listed below:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist Member - Secretary	Female	Yes	Yes
Dr Sudha Ramalingam	M.D	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
Dr D Vijaya	Ph D	Member – Basic Scientist	Female	Yes	Yes

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The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.

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3.7.13

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THE GENOTYPE PHENOTYPE CORRELATION OF ICAM1 K469E GENE POLYMORPHISM AND SEVERITY OF RETINOPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

INTRODUCTION

An emerging non communicable disease with increasing prevalence at an alarming rate throughout the globe is Diabetes Mellitus. 90% of the contribution to this emerging epidemic is made by Type 2 Diabetes Mellitus . India with the largest number of diabetic patients, is rightly called as **"**diabetes capital of the world"[1].

The most severe microvascular complication in Type-2 diabetes patients resulting in impaired visual function is Diabetic retinopathy[2] which is characterised by the presence of microaneurysms, superficial and deep haemorrhages, intra retinal microvascular abnormalities, venous beading, , hard exudates, cotton wool spots, neovascularistion of the retina. Late stages of diabetic retinopathy is complicated by pre retinal and intra vitreal haemorrhage leading to blindness.

In 2010 , number of diabetic patients with retinopathy worldwide was 126.6 million which is estimated to increase to 191 million by 2030[2] . India also face a alarming growth in the percentage of people with retinopathy due to diabetes[4], thereby an hike

in the percentage of diabetic patients who would have vision loss due to this condition. In India, Diabetic retinopathy is the sixth leading cause of blindness[4]. The age adjusted prevalence of diabetic retinopathy among rural and urban Indian population is 18%[5].

Environmental and genetic factors influence the development of diabetic retinopathy. Genetics factors are responsible for 25-50%[6] of the patients risk for developing diabetic retinopathy. The susceptibility to diabetic retinopathy and its variable incidence among individuals are largely determined by genetic factors which are not dependent upon diabetes duration and blood sugar control . Though polyol pathway, activation of protein kinase C,stress due to oxidative, growth factors upregulation, , advanced glycation of end products (AGEs) , adhesion molecules, etc have been propose cause the diabetic retinopathy , recently diabetic retinopathy is described as a retinal pathology which is associated with vascular neuroinflammation[7] which is has genetic influence.

Increase in the level of cytokines, other mediators of inflammation and angiogenesis promoting factors present in patients with diabetic retinopathy increase the expression of intracellular adhesion molecule 1 (ICAM-1) at endothelial cell surfaces ,there by mediating the processes of adhesion of leukocytes , migration across the endothelium and also interaction with integrins which are expressed on the surface of leucocytes ultimately leading to stasis of leukocytes within the retina. ICAM-1 in vascular endothelium mediates the process of adhesion of leukocytes to retinal

vascular endothelium of diabetic patients resulting in breakdown of blood–retina barrier, nonperfusion of capillaries and damage to the endothelial cells and its death which predisposes to apoptosis[8]. ICAM-1 also mediator of VEGF in the advanced stages of diabetic retinopathy[9] .

ICAM 1 genetic variants influence the expression of ICAM -1 at endothelial cell surface .It is associated with microangiopathies including Diabetic retinopathy and diseases of immune system like coronary artery disease, Graves disease, inflammatory bowel disease, diabetes mellitus Type1 and Behcet's disease [10-15]. The ICAM-1 K469E (rs5498) polymorphism in exon 6 causes increase adhesion of LFA-1 and Mac-1 on leucocytes to ICAM-1 on endothelial cells causing leukostasis[16] .Presence of this polymorphism also increases the serum ICAM levels[17].

Though studies establish a strong correlation between the presence of diabetic retinopathy and K469E (rs5498) polymorphism of ICAM-1 gene, the knowledge of the relationship between this polymorphism and severity of diabetic retinopathy is still lacking. This present study aims to investigate the association between K469E (rs5498) polymorphism of ICAM-1 gene and severity of diabetic retinopathy in South Indian population.

Aim

Primary Aim:

- To determine the association between K469E (rs5498) polymorphism of ICAM-1 gene and sight threatening diabetic retinopathy.

Secondary aim:

- To evaluate the relationship between K469E (rs5498) polymorphism of ICAM-1 gene and independent variable like sex, duration of diabetes, insulin requirements.

REVIEW OF LITERATURE

Definition of Diabetes Mellitus:

According to WHO, Group of disorders of metabolism characterized by high blood sugar levels h resulting from defective secretion of insulin, defective insulin action, or both[18] comprises diabetes mellitus.

Classification of Diabetes Mellitus:

Aetiopathological classification proposed by World Health Organisation is as follows:

1.Type 1 diabetes

Immune mediated

Idiopathic

2.Type 2 diabetes

3.Other specific types

Genetic defects of β-cell function

Genetic defects in insulin action

Diseases of exocrine pancreas

Endocrinopathies

Drug or chemical induced

Infections

Uncommon forms of immune-mediated diabetes

Other genetic syndromes sometimes associated with diabetes

4. Gestational diabetes mellitus.

Each of the above mentioned types of Diabetic Mellitus progresses through a stage of normoglycemia and hyperglycemia with the management differing in these stages. Most of the complications of Diabetes Mellitus develop only during the stage of hyperglycemia. The various stages of progression of Diabetes Mellitus can be classified as:

- Normoglycemic stage.
- Hyperglycemic stage.
- Impaired glucose regulation - Impaired Glucose Tolerance (IGT) and Impaired Fasting Glycaemia (IFG)
- Established Diabetes Mellitus - Those who don't require insulin
- Established Diabetes Mellitus - Those who require insulin for control
- Established Diabetes Mellitus - Those who require insulin for survival.

Different types of Diabetes Mellitus and their progression through the different glycemic stages is well depicted in the diagram below

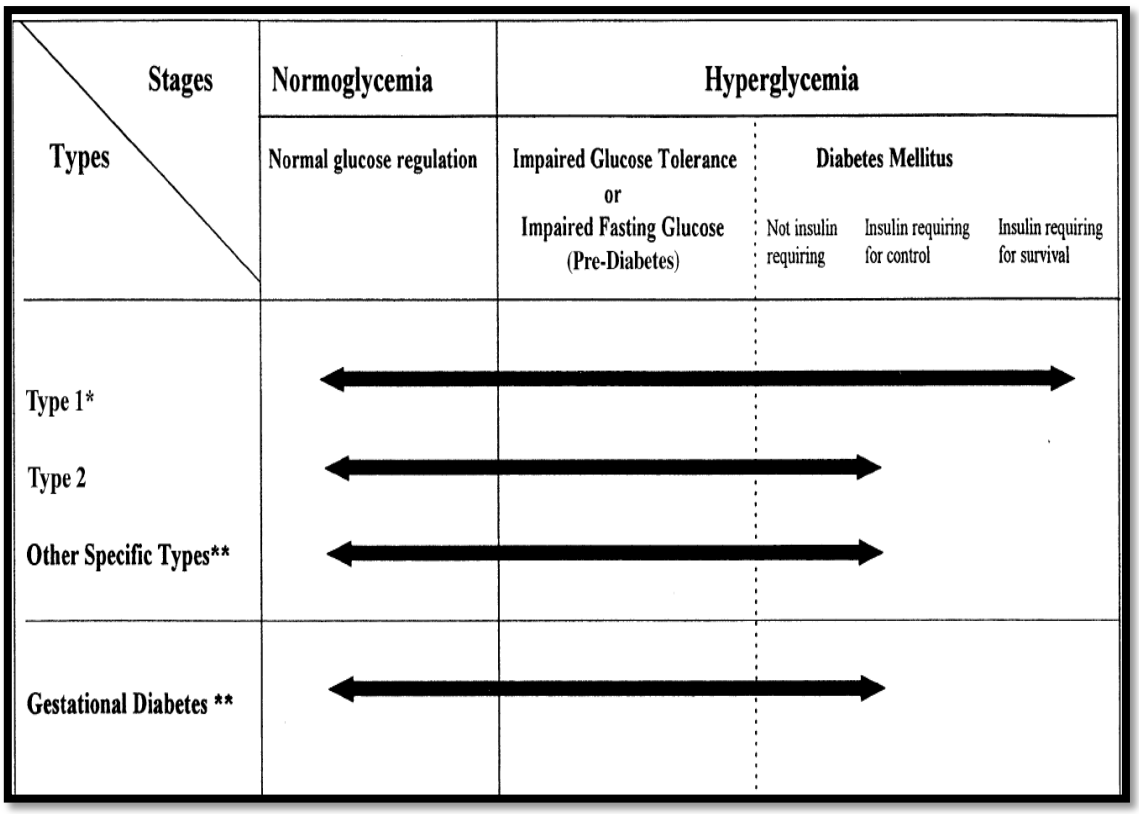


Figure 1:*These patients experience remissions without requiring continuous treatment known as ("honeymoon" remission); **in rare instances, these patients require insulin for survival.[18]

Epidemiology of Diabetes Mellitus:

According to WHO, the diabetes prevalance worldwide among any age group was predicted to rise from 2.8% in the year 2000 to 4.4% in the year 2030 given the current situation[18]. A probable increase in the number of patients with diabetes from 171 million in the year 2000 to 366 million in the year 2030[18] has been predicted to occur. Thus the number of diabetic patients will almost double by 2030.Though more number of women had diabetes than men, prevalence of diabetes was found to be slightly higher among men. Middle Eastern Crescent, India , and sub-Saharan Africa are the countries where the greatest relative increase was estimated to occur. Of these the greatest absolute increase in the number of diabetes patients was estimated in

India[18].5-10% of the diabetic population is compromised by Type 1 diabetes mellitus,a90-95% by type 2 diabetes and other types <1%[19].Hence in this study patients with Type 2 diabetes are taken into account since they contribute more to the diabetic epidemiology.

In India , the estimated total number of type 2 diabetic patients would increase from 50.8 million in 2010 to 87.0 million by 2030[20]. The prevalence of patients who had been already diagnosed with diabetes among urban areas was 5.6% and among rural areas it was 2.7% in India[21]. Prevalence of type 2 diabetes mellitus is on rise among the rural population as a result of socio-economic transition. The estimated number of patients with type 2 diabetes in India is shown in the following diagram.

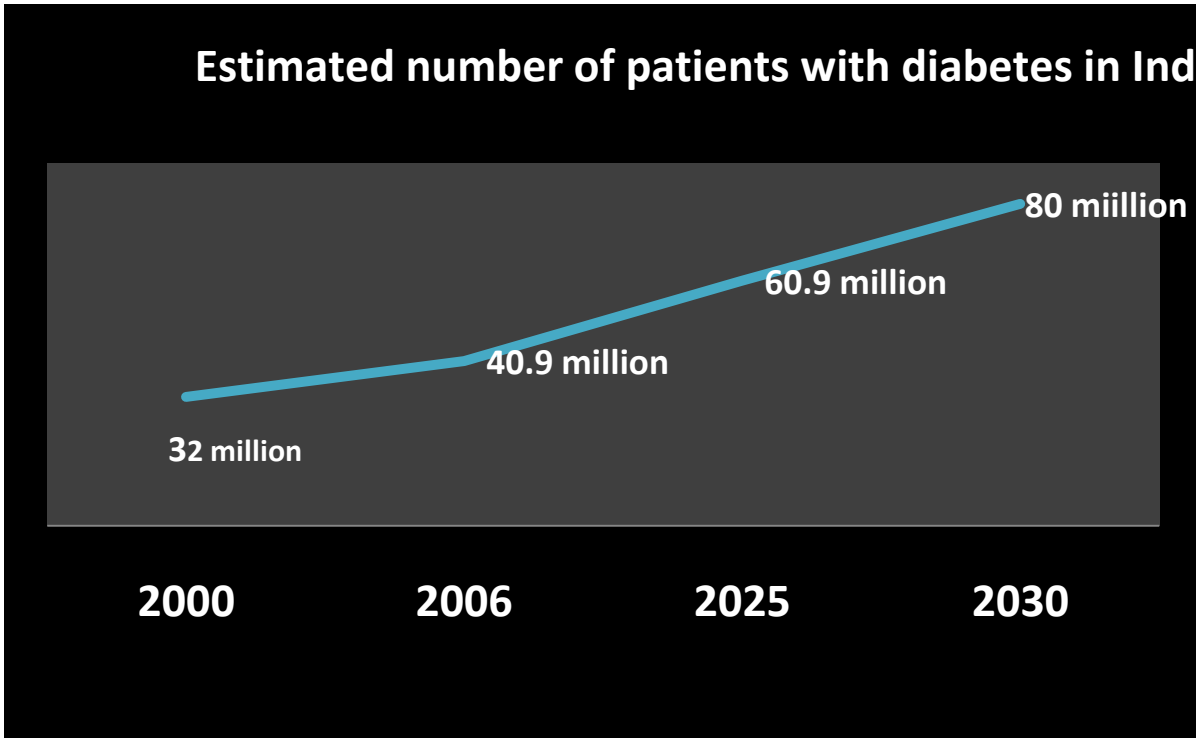


Figure 2: Estimated number of patients with type 2 diabetes in India upto year 2030[1].

According to the National Urban Diabetes Survey (NUDS), the prevalence of people with type 2 diabetes mellitus among the southern part of India was higher compared

to other parts of India. Among the southern parts of India, number of type 2 diabetic patients were highest in Ernakulam with the prevalence rate of 19.5% following this is Hyderabad with a prevalence rate of 16.6% followed by Chennai with the prevalence rate of 13.5% which is followed by Bangalore with the prevalence rate of 12.4%[22,24,25].

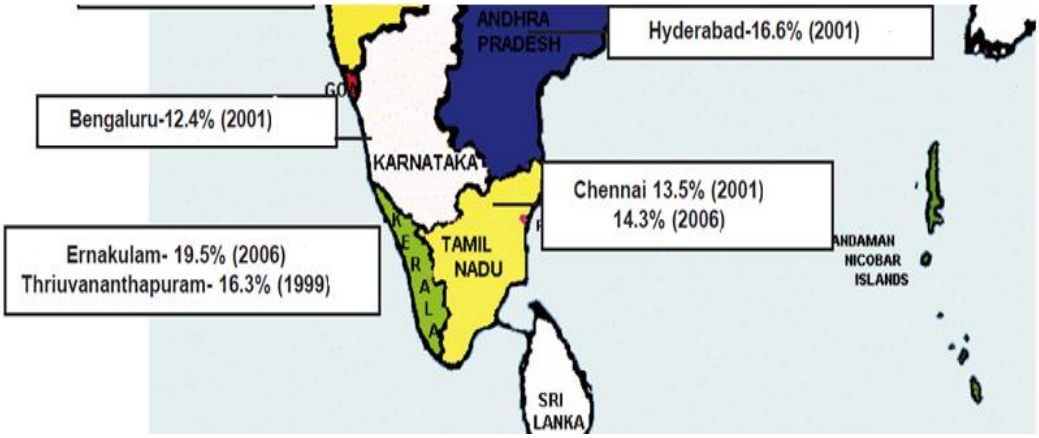


Figure:3: Type 2 diabetes prevalence in southern India[22,23,25,26].

According to the Chennai Urban Rural Epidemiology Study (CURES) the type 2 diabetes prevalence in Chennai in was 15.5 per cent. Type 2 Diabetes Mellitus prevalence in Chennai increased from 1989 to1995 by 39.8 per cent (8.3 to 11.6%); by 16.3 per cent (11.6 to 13.5%) between1995 to 2000 and by 6.0 per cent (13.5 to14.3%) between 2000 to 2004. Thus, the prevalence of type 2 diabetic patients within a span of 14 years has increased by 72.3 per cent which is significant [23].

Aging global population, ,a rising prevalence of obesity, sedentary lifestyles, unhealthy food habits, urbanization and genetic predisposition are the reasons for the predicted increase in the prevalence of Diabetes Mellitus[24].

Factors contributing to Diabetes Mellitus:

Obesity :

Obesity is an important factors driving the global diabetic epidemic. Though prevalence of obesity is relatively low in Asia compared to the western population, it is growing now due to the development in economic status and urbanisation. The Asians have greater tendency towards fat deposition in the abdomen and decreased muscle mass which termed as "metabolically obese" phenotype predisposes them to an increased chance for development insulin resistance[27] .

Food habits:

Foods rich in polyunsaturated fat and fibres decreases risk of developing type 2 diabetes mellitus, whereas foods with higher contents of trans fat and those containing higher glycemic load (GL) increases risk of type 2 diabetes. Sugar-sweetened beverages (SSBs) , intake of fats originating from animals, high caloric foods, less fibre consumption, and regular fast foods intake increases type 2 diabetes mellitus risk[28].

Physical activity:

Increased physical activity is associated with decreased risk of diabetes, whereas decreased physical activity increase diabetes mellitus risk[29].

Smoking:

Cigarette smoking increases the risk of developing type 2 diabetes mellitus and it is independent of other risk factors. When compared to non smokers, people who are currently smoking had 45% more chance having type 2 diabetes mellitus. The risk

of a smoker developing type 2 diabetes is directly related to the number of cigarettes he has smoked [30].

The mechanisms by which smoking predisposes people to type 2 diabetes risk is as follows: Cigarette smoking stimulates sympathetic nervous system activity which induces plasma cortisol secretion which results accumulation of visceral adipose tissue[31].Secondly smoking also decreases plasma testoterone levels in men and also has anti-estrogenic effect in women[32].Nicotine exposure in animal models in pre-natal and natal period resulted in dysfunction of beta cell and increased apoptosis of beta cell.

Alcohol :

Heavy alcohol intake is associated with increased risk of developing type 2 diabetes ,however light-to-moderate intake of alcohol reduces the risk of type 2 diabetes mellitus[33]. Simultaneous use of alcohol and tobacco which is common among Indians has an synergistic role on them developing type 2 diabetes[34]

Dietary habits, inflammation, and type 2 diabetes:

It has been found in the recent studies that underlying factor in the pathogenesis of type 2 diabetes is low grade systemic inflammation[35].Also it has been proved that elevated plasma levels of Intercellular adhesion molecule 1, tumour necrosis factor-alpha, interleukin-6 and C-reactive protein which are the major inflammatory cytokines increases risk of a person getting type 2 diabetes mellitus.Diet consisting of a high percentage of red meat, refined grains, and sugar sweetened beverages, butvery small percentage of coffee ,yellow vegetables, wine and cruciferous vegetables is

found to increase the level of above mentioned inflammatory cytokines in the plasma which in turn increases the type 2 diabetes mellitus risk[35].

Genetics of Type 2 Diabetes Mellitus :

Though type 2 diabetes mellitus is linked up with upto 40 genetic loci, they are responsible only for a moderate effect size and do not clinically predict the type 2 diabetes mellitus risk beyond the above mentioned traditional risk factors. And also there are notable variations in the location of these alleles and also in their frequency among different ethnic groups. Most of the diabetes susceptibility loci are associated with impaired function of beta cell, only a few are related to the development of insulin resistance. This emphasises the fact that beta cell dysfunction is the primary pathogenesis in diabetes mellitus[29].

Gene-environment interaction predisposing to Type 2 Diabetes Mellitus :

The hypothesis of thrifty genotype -

According to this hypothesis , for efficient functioning of metabolism and also for the storage of fat and energy during nutrient scarcity, there occurs a positive selection of certain genotypes called thrifty genotypes. This over expression of the so called thrifty genotypes results in type 2 diabetes mellitus and obesity[36].This hypothesis proposes that there exists a mismatch between genes of ancestral orgin and modern environment which thereby causes type 2 Diabetes Mellitus. This hypothesis explains the reason for the increased rate of type 2 Diabetes Mellitus among prima Indians. It suggests that in Indians there

occurs an evolutionary selection of the so called thrifty genes as a result of the repeated feast famine cycles predisposing them type 2 Diabetes Mellitus and obesity[37].However Southam et al[38], found no such thrifty genes responsible for either type 2 Diabetes Mellitus or obesity. He also found that there was no over expression or increased concentration of any susceptible genetic loci among a specific ethnic group.

The hypothesis of thrifty phenotype -

Thrifty phenotype hypothesis states that a mismatch exists between the intrauterine life and adult life is responsible for the development of various chronic disorders ,one among which is type 2 Diabetes Mellitus. Nutrition transition is also responsible for type 2 Diabetes Mellitus development. It states that as a result of fetal undernutrition , changes in the metabolism and structure (eg: decrease in the function and mass of beta cell and increase in the resistance to insulin) occurs that is required for survival in the early life which increases the chances of development of type 2 diabetes mellitus. This risk increases in the presence of nutritionally rich environment in later life[39].

This hypothesis is supported by the fact that “Asian Indian phenotype" with a greater circumference of waist and also greater ratio of waist to hip are more prone to get type 2 diabetes mellitus though the prevalence of obesity defined by body mass index(BMI) is lower among them[40].Also that Indian babies who are smaller at birth but are comparitively fatter than the Caucasian counterparts are called as "the thin fat Indian babies". The studies confirmed the fact that forerunner of adult diabetogenic phenotype is the persistence of this "thin fat phenotype " into childhood[41] .

Genes causing type 2 Diabetes Mellitus in Indians:

A variety of genes located on different chromosomes are involved in development of type 2 diabetes mellitus. These genes interact with a number of environmental factors to give birth to this disorder, which makes genetic analysis further complicated. Single gene defect occurs only in Maturity Onset Diabetes of the Young. Genes which are proved to protect against type 2 diabetes mellitus and resistance of insulin among Caucasians may not have the same protective effect among the Indians. Below is the list of few genes which are linked up with type 2 diabetes in Indians[42].

Type 2 diabetic genes	Association study results
***PPAR* γ(Pro12Ala)**	Pro12Ala polymorphism does not offer any protection against(Pro12Ala) diabetes or insulin resistance in South Asian population unlike in Caucasian population.
***PGC-1α*(Thr394Thr)**	Obesity and Type 2 diabetes mellitus are associated to it.
PC-1 (K121Q)	Related to type 2 Diabetes Mellitus development.
IRS-2 (Gly1057Asp)	By interacting with obesity D1057D genotype are susceptible to type 2 diabetes mellitus.
MODY genes	South Indians have different MODY3 mutations than that observed in Western populations. Ala98Val of HNF1A mutation is associated with younger age at onset.
***TCF7L2* polymorphism (rs12255372)**	Strongly related to Type 2 diabetes mellitus development in Indian population.

Figure :4- Genes associated with type2 diabetes mellitus.[42]

Diagnostic criteria for Type 2 Diabetes Mellitus:

The American Diabetes Association has recommended the following criteria for diagnosis of type 2 diabetes mellitus in 2012[43]:

HbA1C ≥6.5%[a,b]

OR

Fasting plasma glucose ≥126 mg/ dL (≥7.0 mmol/L)[b]

OR

Two-hour plasma glucose ≥200 mg/dL (≥11.1 mmol/L) during an oral glucose tolerance test; 75-g glucose load should be used

OR

A random plasma glucose concentration ≥200 mg/dL (≥11.1 mmol/L) in persons with symptoms of hyperglycemia/hyperglycemic crisis

[a] Test should be performed in a lab using a National Hemoglobin Standardization Program-certified method and should be standardized to the Diabetes Control and Complications Trial assay.;

[b] In the absence of unequivocal hyperglycemia, the first 3 criteria listed above should be confirmed using repeat testing.

Pathogenesis of Diabetes Mellitus :

Resistance to insulin and dysfunction of beta-cell play role in the production of the hyperglycaemia in type 2 diabetics . Even attempts to maintain glucose within the normal range does not stop the disease progression. The progressive decline in function of beta cell is found to be cause for glucose intolerance .Hence impaired insulin secretion due to dysfunction of beta cell and resistance to insulin are the contributors to the pathogenesis of Type 2 diabetes mellitus as shown below:

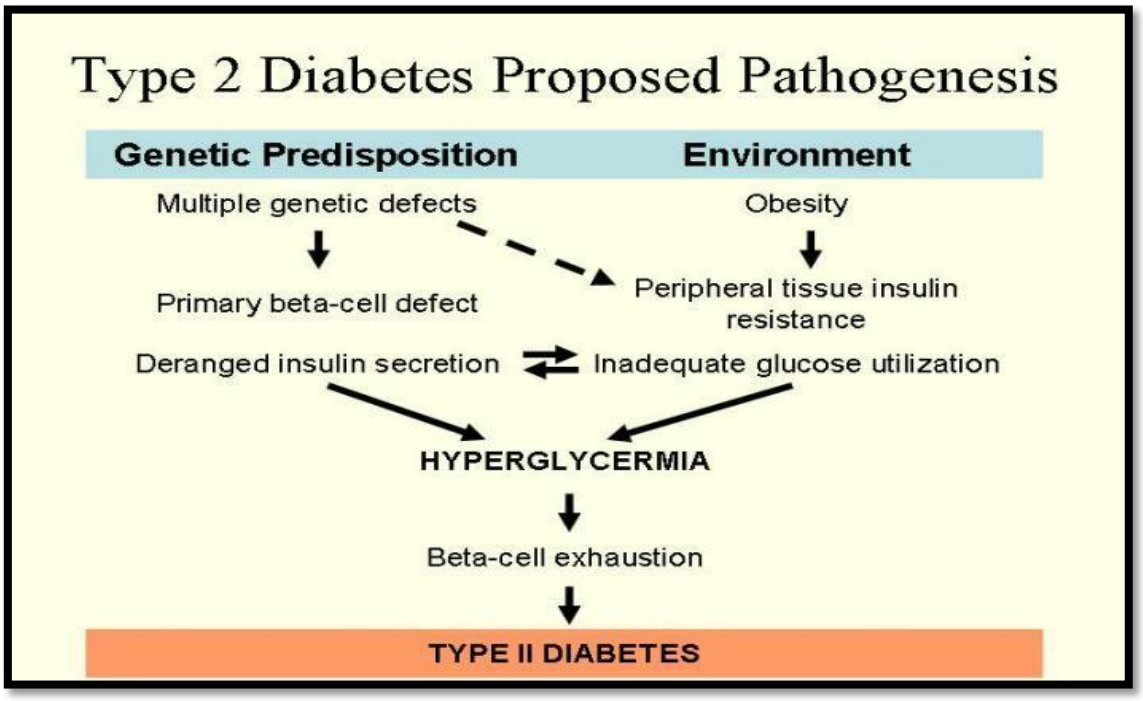


Figure 5: Pathophysiology of diabetes mellitus.

Impaired Insulin Secretion:

Even in the normoglycemic stage , beta cell dysfunction manifests as poor insulin response to intravenous glucose and non glucose secretatgogues ,alterations in the pulsatile release of insulin and ultra radian oscillatory insulin secretion. As the beta cell dysfunction progresses, patient develop Impaired glucose tolerance (IGT) due to

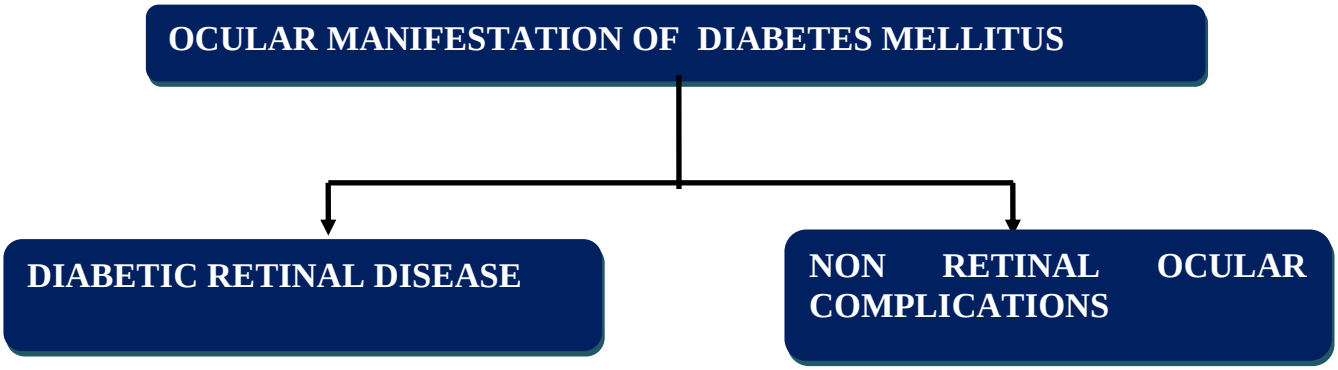
impairment of insulin secretion in the early phase in response to glucose, and a decrease in the secretion of additional insulin shortly after meals causing postprandial hyperglycaemia[44].Progression of impaired glucose tolerance results in increasing glucose toxicity and lipotoxicity, thereby resulting in more beta cell dysfunction which in turn results in permanent hypoglycaemia[45] .

Insulin resistance:

When there is a disproportion exists between the action exerted by insulin concentration in the body and its concentration in blood, it is defined as insulin resistance. Insulin resistance is seen prior to disease onset mainly in organs like liver and mucles. Insulin receptor belongs to the tyrosine kinase receptors family. Normally increased blood glucose levels causes the tyrosine kinase activity of the beta subunit of the receptor to be activated. It phosphorylates a substrate protien called Insulin Receptor Substrate (IRS-1) which in turn increases the affinity of glucose transporter(GLUT-4) molecules to outer membrane of insulin response tissue, therefore increasing glucose uptake from blood into these tissues. Various genetic factors and environmental factors causes insulin resistance..At cellular level insulin resistance is caused by decrease in insulin stimulated tryrosine kinase activity and also due to numerical and functional defect in glucose transporters[45].

Ocular manifestation due to type 2 Diabetes Mellitus:

Broad classification Ocular manifestations due to type2 diabetes is as follows:



Non retinal complications of eye :

1.Changes in visual function: a. Reduction of visual acuity:

This may be due to shifts in refraction, Papillopathy, Cataract, disorders of ocular surface, Ischemic optic neuropathy and Other diabetes related ocular changes.

b. Changes in refraction status[46]:

Hyperglycaemia channels the sorbitol pathway in the lens. Accumulation of sorbitol within the lens draws in more water resulting in increase in lens curvature ,thickness and refractive index thereby resulting in myopic shift. However hypoglycaemia resulting from treatment causes in flattening of lens and hence hyperopia.20-40% of diabetic patients report vision changes when first diagnosed.

c. Changes in colour vision:

Due to diabetic retinopathy ,Colour vision changes like the blue-yellow and red -green defects occur which correlate with the diabetes mellitus duration[47].

d. Accommodation dysfunction[48]:

- Transient reduction in accommodation in patients with uncontrolled sugar levels which improves on control of sugar levels.
- Also decrease in accommodation is noted among patients who had undergone pan retinal photocoagulation.

2.Visual field defects:Visual field defects among diabetes may occur secondary to[49]

- Primary open angle glaucoma.
- Neovascular glaucoma.
- Papillopathy
- Pre retinal or vitreous haemorrhages.
- Posterior vitreous detachment.
- Pan retinal laser photocoagulation.
- Ischemic optic neuropathy

3.Orbital lesions:

Diabetic patients are prone for orbital cellulitis. Orbitorhinomucormycosis caused by bread mould (mucor) develop due to severe acidosis and poor metabolic control

4. Lids:

- Ptosis is the most common lid lesion among diabetic patients which is due to isolated third nerve palsy. Levator Palpebral Superioris is sensitive to chronic hypoxia and hence ptosis. Poor diabetic control results in elevated serum lipids causing xanthelasmas of the lids.

- Recurrent hordeolum internum and externum.
- Chalazion

5.Abnormal Ocular movements:

Diabetes mellitus the cause of acute extra ocular muscle palsy in about 25-30% of people aged above 45 years[50]. Extra ocular motility disorder in diabetic patients occur due to mononeuropathy involving third,fourth and sixth cranial nerve[48]. However other causes of mononeuropathy has to be ruled out in these patients.1 % of diabetic patients were found to have ocular motility disorders and of these 41 % had third nerve palsy[51].

Third nerve palsy due to diabetes mellitus is characterised by acute painful ptosis with exotropia and hypotropia which become less painful as the duration increases. Sparing of the pupil is the most important feature differentiating diabetes mellitus related third nerve palsy from other surgical causes.Sixth nerve palsy is characterised by the presence of horizontal diplopia with esotropia and abduction deficit.Fourth nerve due to diabetes is associated with sudden onset vertical diplopia, vertical deviation increasing on looking down or looking away from the affected muscle and in head tilt towards the affected muscle side.

Extraocular motility disorders due to diabetes recovers within 3-6 months. Recurrences are common which may affect the same nerve or other nerves[48].

Conjunctiva:

- Microaneurysms in the conjunctiva.

- Lipid imbibition in the conjunctival capillaries.
- Decreased vascularity of the conjunctiva.
- Vasoconstriction
- Vessel distension.
- Increased tortuosity.
- Capillary proliferation.
- More susceptible to bacterial conjunctival infection.

Tear film[52]:

Abnormalities of tear film occur commonly with diabetes mellitus.Tear film stability is affected in persons with due to diminished tear film breakup time, thereby increasing the risk of ocular surface epithelial defects. Due to the involvement of the second division of the trigeminal nerve, corneal sensitivity is decreased causing decreased relex tear secretion.This increases the risk of neurotrophic keratitis. Long standing diabetic microvasculopathy may also affect blood supply to the lacrimal gland resulting in impaired lacrimation.

Cornea[57] :

- Prolonged corneal wound healing
- Recurrent epithelial due to reduction in hemi desmosomes among diabetic patients resulting in a weak corneal epithelial adhesion to the underlying stroma[53]
- Corneal ulcers
- Persistent epithelial defects.

- Superficial punctate keratitis.
- Gerontoxon
- Limbal vascularisation.
- Increased incidence of contact lens related microbial keratitis[52].
- Poor clearing of the stromal edema.
- Pigments at the back of the cornea from iris depigmentation.

Iris:

- Depigmentation[52] of the iris.
- Poor mydriasis due to dilator muscle damage.
- Pin point holes in the iris in retro illumination.
- Rubeosis iris starting from the pupillary border to involve the angle.
- Ectropion uvea as the fibrovascualar proliferation on the anterior surface of the iris contracts.

IOP and Glaucoma:

Mean IOP in diabetic population is greater than that in general population. Diabetic patients are more prone for developing two major types of glaucoma.

Primary open angle glaucoma:

5% of diabetic patients develop primary open angle glaucoma compared to only 2% in non diabetics. Primary open angle glaucoma are 1.6 to 1.7 folds common among diabetics than the non diabetics[54].Causes for the increased risk of primary open angle glaucoma among diabetics are as follows[55]:

- Diabetic microangiopathy affects the the anterior optic nerve head perfusion.
- Impaired autoregulation of posterior ciliary circulation among diabetics further impairs the optic nerve perfusion.
- Presence of concomitant cardiovascular risk factors more commonly among diabetics affects the vascularity of the optic nerve head.
- Diabetic patients are more prone to develop increased IOP and severe field loss.

Neovascular Glaucoma[56]:

This appears to be the sequelae of proliferative diabetic retinopathy, caused by the vascular endothelial growth factors diffusing from the posterior segment through the pupil and entering into the anterior segment .The fibrosis accompanying the new vessels in the angle may occlude the trabecular meshwork thereby impairing aqueous and cause secondary open angle glaucoma initially which later on progresses to secondary open angle glaucoma as the fibrovascular membrane contracts.

Lens:

Among diabetic patients , the major cause of defective vision is cataract. Cataracts among diabetic patients tend to occur earlier and progress at a faster rate compared to non diabetics. Increased Diabetes duration and uncontrolled hyperglycaemia are found to increase the risk of development of cataract [57].As a result of the above mentioned factors, advanced glycation end products (AGEs) deposit in the lens resulting in the formation of cataract among diabetic patients [57].Posterior subcapsular cataract and cortical cataracts have been reported to be more prevalent among diabetic patients

which may be also due to their increase use of statins[58]. Nuclear sclerosis and cortical cataract are strongly associated with type 2 diabetes mellitus[57].Reversible lenticular opacities due diabetes mellitus is attributed to uncontrolled blood sugar levels among diabetic patients. These bilateral dense bands of white sub capsular spots are called snowflake opacities[57].

Vitreous:

- Vitreous syneresis.
- Asteroid hyalosis
- Posterior vitreous detachment -Partial PVD increases the risk of proliferative diabetic retinopathy and retinal detachment.
- Vitreous haemorrhages from new vessels on the retinal surface projecting into the vitreous.

Optic disc:

a. Diabetic papillopathy:

Diabetic papillopathy diagnosed by the presence of hyperemic disc swelling in one or both the eyes .Defects in the visual field and afferent pupillary defect may or may not be associated with this.[59]. It should be differentiated from other causes of papilledema and optic disc swelling. Telangiectasia at the disc may be mistaken for proliferative diabetic retinopathy.

It is a risk factor for diabetic retinopathy proliferation[60]. It is now proposed as a mild and reversible form of Anterior Ischemic Optic Neuropathy[61].It is not associated with

degree of diabetic retinopathy or glycemic control.Visual acuity is moderately decreased and prognosis is very good. Most people regain vision by one year .Control of blood sugar ,systemic hypertension ,renal function improvement may play role in the resolution of vision .

Ischemic optic neuropathy:

Patients with diabetes mellitus are more likely to develop anterior ischemic optic neuropathy after the age of 67 years[62]. It is caused by microangiopathy of the optic nerve head.It is characterised by swelling, haemorrhage, sudden vision loss, afferent pupillary defect and visual field defects. It results in optic atrophy and decreased visual acuity. Diabetic papillopathy and anterior ischemic optic neuropathy can be differentiated by its younger age of onset of the former.25% of anterior ischemic optic neuropathy have diabetes[57].

Retina:

- Diabetic retinopathy
- Lipemia retinalis.

Ocular manifestations other than Diabetic Retinopathy depending upon its relation to diabetes mellitus can be broadly classified as follows[57]:

Ocular manifestations due to diabetes:

- Cataract.
- Anterior ischemic optic neuropathy
- Diabetic papillopathy

- Ocular movement disorders.

Ocular manifestations with diabetes as a risk factor:

- Primary open angle glaucoma.
- Neovascular glaucoma.
- Ocular ischemic syndrome.

Ocular manifestations with diabetes as a possible risk factor:

- Central/Branched Retinal vein occlusion
- Central/ Branched Retinal artery occlusion
- Retinal arteriolar occlusion.
- Corneal diseases.

Ocular conditions mimicking diabetic eye disease:

- Age related macular degeneration.
- Hypertensive retinopathy.
- Radiation retinopathy.
- Other causes of retinopathy like HIV/AIDS, Giant cell arteritis, , Bechet's disease, SLE, Wegener's granulomatosis, sickle cell disease, retinal telangiectasia etc.and sarcoidosis

OCULAR MANIFESTATION OF TYPE 2 DIABETES MELLITUS:

Fig6: Hordeolum Externum

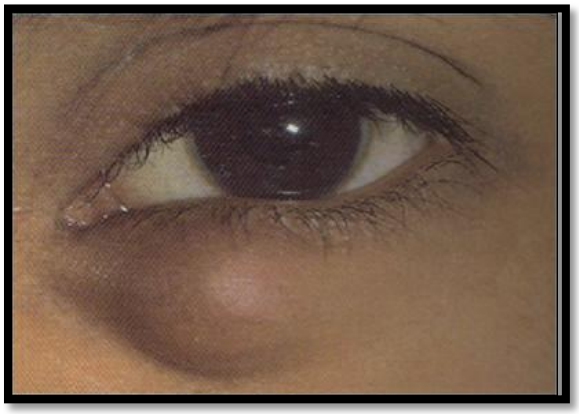


Fig 7: Chalazion

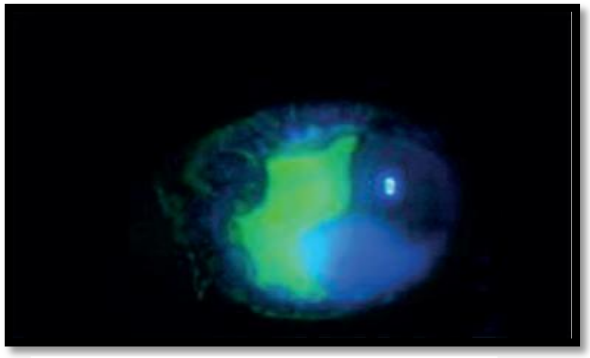


Fig:8 Corneal epithelial defects

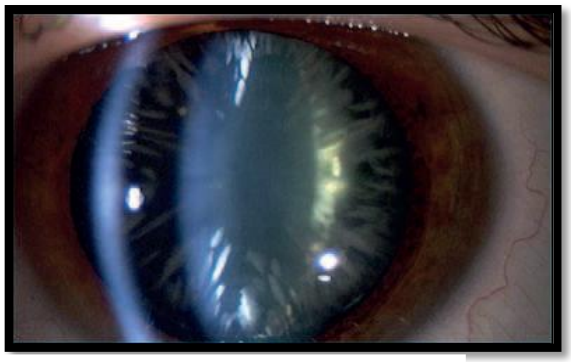


Fig:9 Snowflake cataract

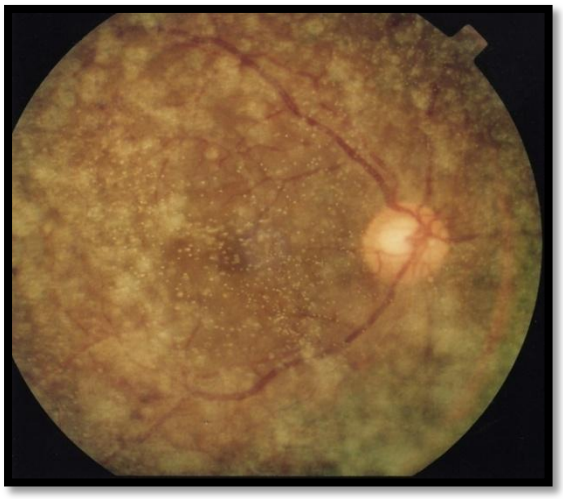


Fig :10 Asteroid Hyalosis.

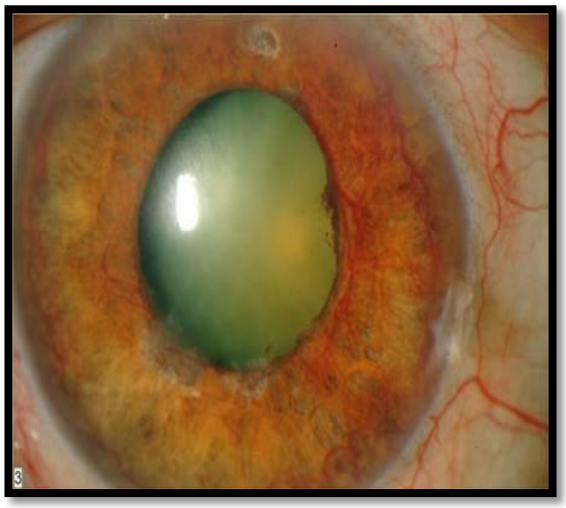


Fig 11: Rubeosis Iris.

Diabetic Retinopathy

Epidemiology of diabetic retinopathy:

The alarming rise in diabetic prevalence has lead to the emergence of diabetic retinopathy as the leading cause of blindness among the working age population. Among the various types of diabetes mellitus, most common form is type 2 diabetes mellitus .WHO estimates that 300 million will have type 2 diabetes mellitus by 2025.During the last two decades there has been increasing reports of diabetic retinopathy due to type 2 diabetes mellitus especially from Asia.

According to a study done in 2012, the number of people in the world with diabetic retinopathy is 93 million, of these 17 million have proliferative type of retinopathy due to diabetes ;Clinically significant macular edema was present in 21 million due to diabetes and 28 million had sight threatening retinopathy due to diabetes.[63]

Prevalance of Retinopathy due to diabetes among the western population:

[63]In the united kingdom the diabetic retinopathy prevalence is 30%,among these 3% had proliferative type of retinopathy and 6% had clinically significant macular edema due to diabetes..

In Europe, diabetic retinopathy prevalence is 32% in France,38.7% in Denmark,34% in Italy,34.2%in Russia and only 45% in Finland. The proliferative diabetic retinopathy prevalence among various European are as follows:3% in France,0.9% in Denmark,5% in Italy,2.75 in Russia. The prevalence of macular edema is highest in Denmark (12.8%) followed by France(5%)These differences may not be appropriate

due to the different methodologies employed. Retinal photographic studies among the whites reveal the diabetic retinopathy prevalence to be 40% and that of sight threatening retinopathy due to diabetes to be 6-8%.

In United states, the non-Hispanic whites had similar diabetic retinopathy prevalence as among white patients in Europe(40%). The incidence of new diabetic retinopathy among type 2 diabetic patients in United States is between 5% and10% per year. The diabetic retinopathy risk and likelihood of developing clinically significant macular edema were more common among Hispanics and African-Americans. The Hispanics were 3 times more prone to develop clinically significant macular edema whereas the Hispanic whites are 2.5 times more risk of developing CSME.

Prevalence of retinopathy due to Type 2 Diabetes mellitus in Asia[63]:

In Asia maximum number of diabetic retinopathy patients s are in India and China . The diabetic retinopathy prevalence among newly diagnosed type 2 diabetics in Pakistan (15%) ,Sri Lanka (15%),and Nepal (19.3%)is high compared to India. The diabetic retinopathy prevalence among the newly diagnosed Chinese urban population ranges from 21% to 21.9%.which is less common than those seen in rural China (33.5%). This is in contrast to clinical scenario in India where the diabetic retinopathy is more common in the urban population compared to the rural population. According to the Beijing Eye Study diabetic retinopathy was responsible for only 7.7% of blindness. This is mainly due to increased awareness, regular followup and prompt treatment.

Diabetic retinopathy prevalence among Type 2 Diabetics in Australia[63]:

5-year cumulative incidence of Diabetic Retinopathy (22.2%) was lower than the incidence (32.7%) of diabetic retinopathy after 4 years among non-insulin treated predominantly white type 2 diabetic population. This may be due to good awareness about the probelm among the public and better blood sugar control in Australia. According to the Melbourne Visual Impairment Project , incidence of Diabetic Retinopathy after 5 year period was 11%, with most patients with sight-threatening disease receiving treatment. Among various ethnic groups in Australia, annual incidence of sight threatening diabetic retinopathy is highest among Australian Aborigines (1.2%) and also the incidences of clinically significant macular edema is highest among this ethnic group.

Diabetic Retinopathy due to Type 2 Diabetes in India:

Among Indians the diabetic retinopathy prevalence among with type 2 diabetic patients is lower when compared to the whites. However India with the highest number of estimated diabetic population,has more number of patients affected by diabetic retinopathy[63]. Diabetic retinopathy has become the sixth cause of blindness in India. Studies based on retinal photography revealed a lower Diabetic Retinopathy prevalence among type 2 diabetics (18%) compared to the western population. And also in India the Diabetic Retinopathy prevalence among newly diagnosed type 2 daibetes is low (5-7%) compared to neighbouring areas[63]. Studies show that prevalence of diabetic retinopathy as more among urban (17.8-18%)population compared to the rural population(5-10%)[64,65,66].In India the rate of development of

clinically significant macular edema among patients with type 2 diabetes mellitus is high. According to the population based study done by Sankara Nethralaya,one third of the patients with type 2 diabetes mellitus had any degree of macular edema , however only 6.27% developed clinically significant macular edema (CSME)[65].Similar results were found in the Chennai Urban Rural Epidemiology Study (CURES) study, where Macular Edema prevalence was lower among newly diagnosed diabetics(1.1%) compared to the among known type 2 diabetics (6.3%) [64].

Diabetic retinopathy in Tamil Nadu:

According to a population based study done by evaluating fundus photographs done in Chennai by Shankar Nethralaya, the prevalance of diabetic retinopathy among the general population was 3.5% while that in type 2 diabetic patients was 18%[65]. According to the Chennai Urban Epidemiology Study (CURES) ,occurrence of diabetic retinopathy among general population was 17.6% with a prevalence of 20.8% among the known diabetics and 5.1% among the newly diagnosed diabetics[64]. Male gender, long duration of diabetes, known diabetics, proteinuria are the risk factors increasing the development of diabetic retinopathy in urban population in Tamil Nadu. An increase of 2% of glycosylated haemoglobin was associated with 1.7 times increase risk of diabetic retinopathy. As the duration of diabetes increases by 5 years the risk of developing diabetic retinopathy increased by 1.89 folds[64,65].

According to a population based cross sectional study done in rural Tamil Nadu by Shankar Nethralaya, the diabetic retinopathy prevalence type 2 diabetic patients is 10.3%. The prevalence of diabetic retinopathy was more among known diabetics

(13.1%) than in those who were newly diagnosed (2.8%).Prevalence of sight threatening diabetic retinopathy was 3.8% , with a prevalence of 5% among known diabetics and 0.6% among newly diagnosed diabetics[66]. Male gender, use of insulin, longer duration of diabetes mellitus, systemic hypertension, poor control of blood sugar are the risk factors for the diabetic retinopathy development among the rural population of Tamil Nadu[66].While comparing the data on the diabetic retinopathy prevalence between the rural and urban populations of Tamil Nadu ,higher prevalence of diabetic retinopathy in the newly diagnosed was found in urban population(6%) than the rural population(2.8%). However, the newly diagnosed diabetics had low prevalence of sight threatening diabetic retinopathy both in urban(0.4%) and rural population(0.6%)[65,66].Though when compared with other ethnic groups, the diabetic retinopathy prevalence is lower in South Indians, due to the huge diabetic population in Tamil Nadu, diabetic retinopathy is a major health probelm in Tamil Nadu[66].

Diabetic Retinopathy risk factors:

Duration of diabetes:

After 3-4 yrs of type 2 diabetes mellitus, 30% had developed retinopathy and 2% had developed proliferative retinopathy. After 20 years, 60% had developed retinopathy and 5% had developed proliferative diabetic retinopathy[67].

Hyperglycaemia:

According to The Diabetic Control and Complications Trial (DCCT), tight glycaemic control resulted in an initial worsening of diabetic retinopathy . However after three years of strict glycaemic control ,this pattern reversed as a result of which

progression of diabetic retinopathy decreased by 54%[68].In this study it was also proved that incidence of diabetic retinopathy decreased by 76% when blood sugar levels were maintained within normal limits[68]. Similar results were are seen in the United Kingdom Prospective Diabetes Study Group(UKPDS) , where a 25% decrease in the occurrence of diabetic retinopathy was seen in patients on intensive blood glucose control[69].

Glycosylated Haemoglobin levels:

In the WESDR study,the four and ten years incidence, course of progression of ,progression to proliferative type of diabetic retinopathy and development of macular edema were predicted by baseline glycosylated haemoglobin levels. Moreover, a decrease in the glycosylated haemoglobin levels over a period of 4 years lead to slowing down the progressive course of diabetic retinopathy and decrease in the occurrence of proliferative type of retinopathy[70].

Endogenous/Exogenous Insulin:

Though low levels of C-peptide in the blood was associated with severe retinopathy due to juvenile onset diabetes, severity of the retinopathy due to type 2 diabetes mellitus was not associated with blood C -peptide levels . And also dosage and type of exogenous insulin did not influence the diabetic retinopathy severity [71].

Age: There was a marked rise in the diabetic retinopathy frequency due to type 2 diabetes in those younger than 50 years. However after 50 years, there was little relationship between the severity and age of the patient. According to Framingham

Eye Study, the frequencies of diabetic retinopathy due to type 2 diabetes increases with age which is shown in the table below.

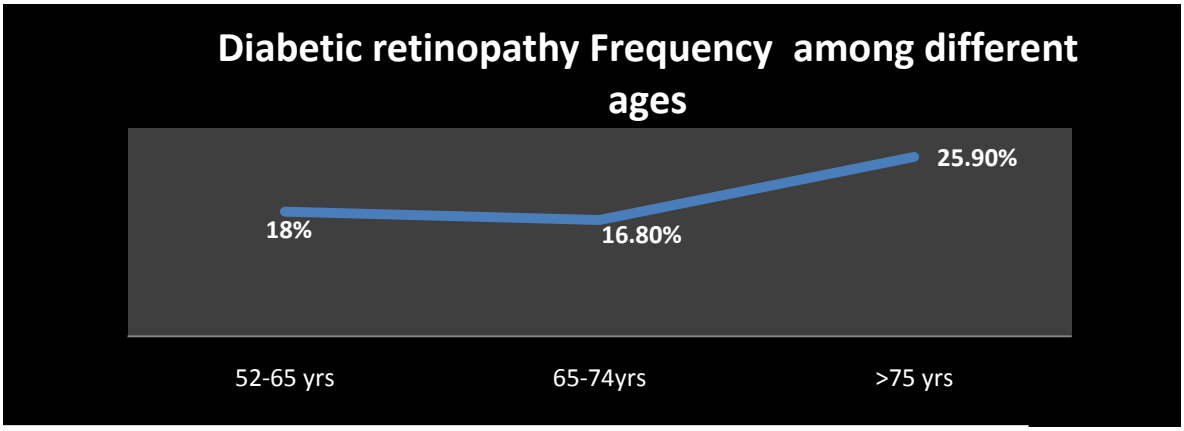


Figure:12: Frequency of diabetic retinopathy among different age groups[72]

Age at time of diagnosis:

Though no relationship was present between the age of diagnosis of type 2 diabetes mellitus and the diabetic retinopathy incidence or progression in Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), Minnesota eye study found that younger the age of onset of type 2 diabetes more the chance of diabetic retinopathy development [73].

Pregnancy and puberty:

During puberty increase in the sex hormones ,growth hormones, insulin resistance and systolic pressure may favour development of diabetic retinopathy especially among non insulin dependent diabetics. Hence the duration of diabetes in the prepubertal age is an important risk factor .Pregnant patients with type 1 diabetes have two times increased risk of developing proliferative diabetic retinopathy. Hence frequent retinal

examinations and aggressive treatment instituted to prevent loss of vision in this high risk situation.

Race:

Though the diabetic retinopathy prevalence among type 2 diabetics is low , the increasing number of diabetic patients in India has made diabetic retinopathy one of the leading cause of blindness.

Hypertension:

Increase in the presence and rapid progressive course of diabetic retinopathy and macular edema is seen among hypertensives[67]. Higher diastolic pressure is associated with higher incidence of macular edema in diabetic patients. Progression of retinopathy slows down by34% and visual acuity improves by 47% with strict control of hypertension[69]. According to Barbados Eye Study, When the systolic pressure increases by 10 mm Hg,the risk of developing diabetic retinopathy increased by 30% among African- Americans and also that antihypertensive treatment halved the risk[79].

Serum lipids:

Various studies confirm the association between the serum lipids and the presence of hard exudates in the retina. Increased serum lipids is associated with more chance of the patient having hard exudates in the retina. However this is more common among the insulin using population[67].

Renal disease:

Presence of any kidney disease may aggravate the progression of diabetic retinopathy. Co-existing hypertension, increased duration of diabetes may also confound the effects of renal disease on diabetic retinopathy. Presence of proteinuria among type 1 diabetic patients increases the chance of developing proliferative type of diabetic retinopathy. The prevalence of diabetic retinopathy increased from 7% at the onset of microalbuminuria to 29% after 4 years of onset[74]. Therefore regular eye checkups are advised in younger onset diabetic patients with gross proteinuria [74]. Patients with microalbuminuria showed greater prevalence with increased severity of diabetic retinopathy when compared to those with to microalbuminuria and normoalbuminuria.[75]

Anaemia:

Low haematocrit increases the risk of developing high risk proliferative diabetic retinopathy[76]. Patients with haemoglobin less than 12 g/dl has two fold increased chance of having diabetic retinopathy compared to those with higher haemoglobin[77].

Obesity:

Increased body mass index increases the chance of developing both diabetes and diabetic retinopathy. However presence of other factors increasing the chance of one getting diabetic retinopathy such as insulin resistance and uncontrolled blood sugar levels which accompany diabetic patients with obesity may predispose a patient with increased body mass index to develop diabetic retinopathy.

Exercise:

Women with increased physical activity are less prone for the development of proliferative type of retinopathy due to diabetes [67]. No such effect is seen in men[78]. However there has been concern that increased physical activity ahs detrimental effect on retinopathy and vision in advanced retinal disease due to elevated systolic pressure subsequently leading to vitreous haemorrhage and also further compromise in oxygen supply. However this has not been proved.

Nutritional factors:

There is no enough studies to recommend high dose antioxidant supplementation to prevent the incidence and progressive course of diabetic retinopathy among patients with Type 2 diabetes. According to San Luis Valley Diabetes Study, there was no protective effect offered by the antioxidants against the development of retinopathy among type 2 diabetic patients[80].

Smoking:

Though one may except high incidence of diabetic retinopathy among smokers due to ischemia and hypoxia, there is no consistent association between the two[81]. However smoking is strongly associated with cardiovascular disease and nephropathy among diabetics which may increase the incidence and progression of diabetic retinopathy .

Alcohol intake:

According to the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), young diabetic patients who consume alcohol are less prone to develop proliferative retinopathy. However after 6 years ,this association does not exist [82].

Socioeconomic status:

According to the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR),Women who were well educated had relatively lesser occurrence of diabetic retinopathy [67]. However poor socioeconomic status did not result in worsening of retinopathy. According to a study done in urban India, newly diagnosed old patients with diabetes in the upper or middle socioeconomic class had 86% higher risk of developing retinopathy than those belonging to the lower or extreme lower socioeconomic class[83]. However this association was statistically significant. Hence one can conclude that socioeconomic factors doesn't influence the course of diabetic retinopathy once the blood sugars under control.

Genetics of Diabetic Retinopathy:

The role of genetics in the development of diabetic retinopathy is evident from the fact that only 50% of patients with NPDR progress to develop PDR and also many diabetic patients never develop diabetic retinopathy. There are various candidate genes which are suspected to influence the occurrence of diabetic retinopathy by modifying the polyol pathway or by playing a role in the formation of advanced glycation end products (AGE) or by predisposing to hypoxia induced angiogenesis or

by predisposing the inflammatory changes . Few are the candidate genes are discussed below.

Aldose Reductase gene:

The aldose reductase pathway is the major factor influencing the occurrence of diabetic microvascular complications. The rate limiting enzyme of this pathway is Aldose reductase(ALR2).Normally only a very small fraction of glucose is metabolised through sorbital pathway. But during hyperglycemia as seen in diabetes, relatively large portion of glucose gets metabolise by this pathway resulting in the accumulation of sorbital intracellularly and resulting in osmotic stress which leads to a series of cellular changes cumulating in diabetic retinopathy

ALR gene (*AKR1B1)* is located on chromosome 7q35 which codes for aldose reductase(ALR2) is the most significantly associated with DR .Three single nucleotide polymorphisms are linked up with the diabetic retinopathy development and they are SNP rs9640883, the (CA)n microsatellite polymorphism located at 5' of the ALR gene, promoter SNP rs759853 [84].

Of the three alleles of (CA)n microsatellite ,z-2 allele is significantly associated with non-proliferative and proliferative Diabetic Retinopathy in both type 1 and type 2 diabetes. However the z+2 allele of the (CA)n microsatellite and allele T of single nucleotide polymorphism rs759853 has protective effect against Diabetic Retinopathy. The single nucleotide polymorphism rs9640883 predisposes to earlier onset of diabetes and so increased diabetic duration rather than a direct effect on increasing the risk of Diabetic Retinopathy.[84]

Human Leukocyte Antigens:

A strong association is present between human leukocyte antigen (HLA) and the occurrence and severity of diabetic retinopathy. According to a case control study done by Rand et al, HLA-DR 4/0 ,HLA-DR 3/0 and HLA-DR X/X is linked with proliferative diabetic retinopathy due to type 2 diabetes. However HLA DR 3/4, HLA DR 3/X and HLA DR 4/X is not associated proliferative type of diabetic retinopathy[85].

Vascular Endothelial Growth Factor (VEGF) Gene[86]:

VEGF is an endogenous cell specific mitogen which results in development of retinopathy among diabetics. Due to hypoxia and hyperglycaemia which occurs in diabetic retinopathy, VEGF expression is stimulated which results in increased VEGF in blood and also increased expression of VEGF receptors in diabetic retina cumulating in formation of new vessels. The VEGF gene is present in chromosome 6 (6p21.3) and alternate splicing of its exon results in two families of VEGF proteins.SNPs in the promotor region of the VEGF gene has been known to influence diabetic retinopathy development. The Single nucleotide polymorphism of the VEGF gene predisposing to one population to diabetic retinopathy may not predispose another population of different ethnicity to diabetic retinopathy risk.

In the Japanese population , the CC allele at the C(-634)G region in the VEGF gene predisposes them to diabetic retinopathy . The C allele of this single nucleotide polymorphism is associated with higher serum levels of VEGF, higher expression of

VEGF-A receptors in the human retina and also increased development of macular edema[86].

This C allele single nucleotide polymorphism association with diabetic retinopathy has not been confirmed in the Caucasian population. On the other hand, GG genotype of this single nucleotide polymorphism results in diabetic retinopathy among Caucasians. Further CA genotype of promoter -2578 polymorphism of the VEGF gene and SRp552994 polymorphism of one of the splicing factors which control alternative splicing thereby disrupting the balance in the proportion of pro and anti angiogenic VEGF isoforms influence the occurrence of diabetic retinopathy in Caucasian population. Among Indians ,the CG genotype at the C(-634)G region increases the occurence diabetic retinopathy[87].

Receptor for Advanced Glycation End Products Gene[86]:

Non-enzymatic glycation of protein and lipids resulting from uncontrolled blood sugar level leads to the formation of Advanced Glycation End products (AGES) which act on specific receptors for advanced glycation end products (RAGE) causing direct tissue damage by initiating pro inflammatory cascade. The gene coding for RAGE belonging to the immunoglobin super family located on chromosome 6p21.3 increase the risk of developing diabetic retinopathy.

Though -374T/A polymorphism which up regulates RAGE transcription by binding to a transcription binding site,Gly82Ser polymorphism and -429T/C polymorphism are associated with diabetic retinopathy in few independent studies, meta-analysis of these three polymorphism revealed no association with diabetic retinopathy. However,

the 1704T allele among East Asian populations and 2245A allele among Malaysian population was associated with an increased risk of DR[88] .

Endothelial Nitric Oxide Synthetase gene(eNOS):

Formation of nitric oxide from L-arginine is catalysed by endothelial nitric oxide synthetase and this enzyme is coded by a gene located in the chromosome 7q35-26 and its polymorphism is associated with increased risk of developing diabetic retinopathy .From various meta analysis it has been concluded that

- Association of a/b polymorphism of intron 4 eNOS gene ,C allele of T-786C polymorphism and G894T polymorphism with diabetic retinopathy could not be confirmed[89].
- 4a allele of 4b/a polymorphism and C allele of T-786 polymorphism decreases the risk of diabetic retinopathy[89].

Angiotensin-I Converting Enzyme (ACE):

Increased expression of Angiotensin -I Converting Enzyme has adverse effects on retinal blood flow and vascular structure resulting in neovascularisation. The gene for this enzyme is located in chromosome 17q23

- Insertion/Deletion polymorphism has been associated with proliferative diabetic retinopathy but not with non proliferative diabetic retinopathy among the caucasian population.
- ACE 2350 G/A polymorphism is also associated with diabetic retinopathy among Chinese population[90].

Erythropoietin gene(EPO):

Erythropoietin is an angiogenic factor expressed in retina and kidney with its gene located at chromosome 7q21. Initially it acts as a neuroprotective element in the retina and protects against diabetic retinopathy but in advanced stages it acts synergistically with VEGF and worsens diabetic retinopathy., T allele of promoter SNP rs1617640 predisposes European-American type 1 diabetic population proliferative diabetic retinopathy in the presence of diabetic nephropathy but not with proliferative diabetic retinopathy in the absence of nephropathy[86].

Peroxisome Proliferator Activated Receptor -γ_2 (PPARγ_2) gene:

A1a allele of the Pro12Ala polymorphism has protective effect against the development retinopathy due to type 2 diabetes among the Caucasians. However it offers no protective effect among the Asian population against the development of retinopathy due to type 2 diabetes mellitus[86].

Interacellular Adhesion Molecule (ICAM) gene polymorphism and Diabetic Retinopathy

ICAM-1 also known as CD54 belongd to immunoglobulin super family and is expressed by leukocytes and endothelial cells. ICAM 1 is a transmembrane glycoprotein with 5 extracellular domains with amino terminus, a single transmembrane domain and a carboxy cytoplasmic domain. The secondary structure of this protein is beta sheet[91].

Regulation of ICAM-1[92]:

ICAM -1 expression is regulated in a cell specific manner by the following pathways[92]

- The NFκB pathway.
- JAK/STAT and IFN-γ
- AP-1 and MAP Kinase
- Protein kinase C pathway.

These pathways regulate ICAM expression at the level of transcription. The NFκB pathway is the most common inducer of the ICAM -1 in cells which is prompted by proinflammatory cytokines such as TNF-α and IL-1β activate NIK via different receptors. Interferon -γ has a signalling effect on the transcription of ICAM-1 through JAK/STAT pathway[93] .Other growth factors and cytokines activate the AP-1 promoter of the ICAM-1 through a complex pathway. However this pathway offers a more controlled expression of Icam-1 than the TNF-α and IL-1β promoted pathway when induces by uncontrolled factors like stress or oxidants. Protein kinase C (PKC) indirectly activates the expression of ICAM-1 and it is required for the expression of ICAM-1 by mediators like TNF-α, IL-β,INF-γ and other stress factors.

The primary receptor for ICAM-1 are intergrins though they do not contain a RGD (Arg-Gly-Asp) motif to promote intergrin binding.By binding with these intergrins ICAM-1 mediate cell-cell interactions and allow for signal transduction[91].ICAM-1 specifically bind to two intergrins of β_2 subunit namely LFA-1 and Mac-1 on the leukocyte surface and thereby mediate T-cell function and activation and leukocyte-endothelial cell interaction[94].

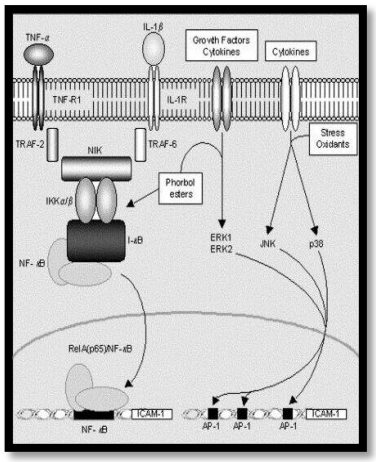


Figure:13- NFkB or AP-1 MAPK regulatory pathways of ICAM-1[93].

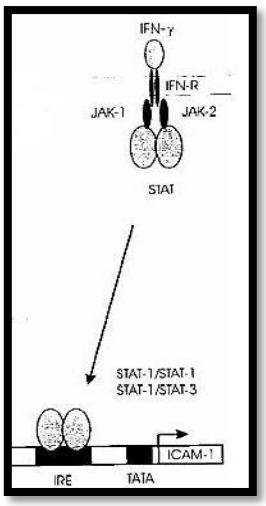


Figure:14-JAK/STAT regulatory pathway of ICAM-1[92].

Function of ICAM-1:

ICAM-1 and LFA-1 interaction is the primary step in the antigen recognition by the CD_8 cells which allows time for the T cell receptor to align with the MHC-II and peptide complex. A successful T cell receptor interaction will increase the adhesive forces and commence the effector function. ICAM-1 along with the MHC-I in the antigen presenting cells play a similar role in CD_4 cells activation[91].

Selectins like P, E and L initially instigate upon the leukocyte a rolling behaviour over the endothelial cells.ICAM-1 ,whose expression is upregulated by inflammation interacts with the leukocyte LFA-1 and MAC-1 and stablises the interaction. The arrested leukocytes begins diapedesis with teh help of protein called PECAM which is present in both endothelial cells and intercellular junctions of endothelial cells. Though ICAM-1 intergrin interaction is not specific, ICAM-1is specifically upregulated by certain cytokines, thus controlling the nature of inflammatory response[95].ICAM-1is shed by the cells and seen in the plasma as sICAM which is elevated in a number of pathological conditions.Other ligands for ICAM-1 include

plasmodium falciparum affected RBCs,CD_{43} ,soluble molecular fibrinogen, matrix factor hyaluron.ICAM-1 acts as a receptor for rhino viruses[91].

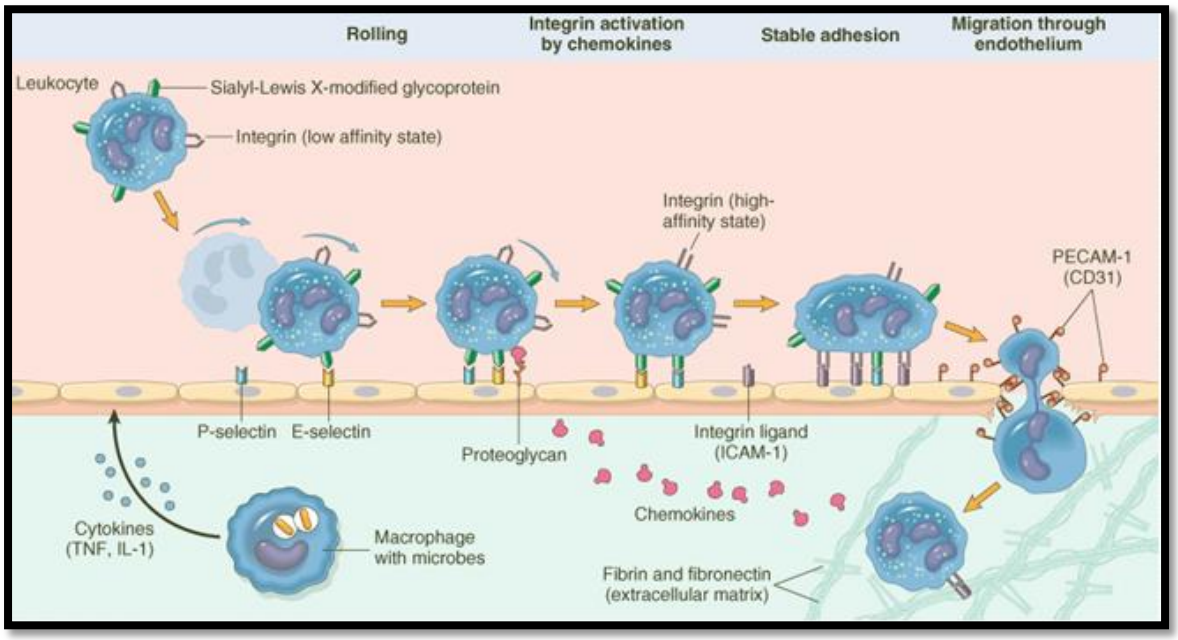


Figure15:ICAM mediated stable adhesion and diapedesis of leukocytes.

ICAM-1 Gene:

ICAM-1 gene is a protein coding gene located in the chromosome 19p13.2 with the size of 15781 base pairs and has a plus strand orientation. It starts from 10,381,511 base pairs from *pter* and ends at 10,397,291 base pairs from *pter*

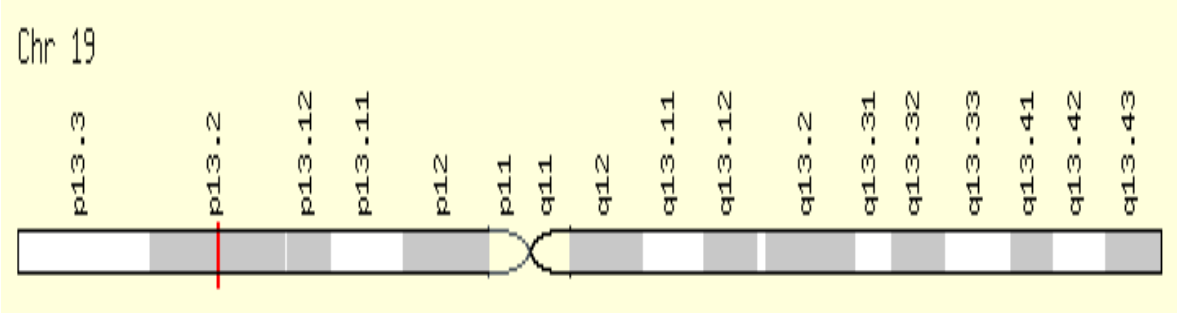


Figure 16: ICAM-1 gene location at chromosome 19p13.2

Single nucleotide ICAM gene polymorphism :

Single Nucleotide Polymorphism(SNP) is a variation in a genetic sequence of DNA that occurs in the genome when there is alteration of a single nucleotide . It has been proposed that these are point mutation which are evolutionarily successful to recur in a considerable portion of population of a species. For example nucleotide cytosine may be replaced by nucleotide thymine in a stretch of DNA. Always SNP has two alleles and their frequency vary among different ethnic groups. Normally SNP occur once in every 300 nucleotides in an individual DNA and they are found between the genes. Mostly they occur in a non -coding region. When they occur within the coding region ,they alter the gene function and lead to a particular disease. Most SNPs do not have a phenotypic effect. But SNPs involving the coding region may predict the individual susceptibility to certain drugs or environmental factors such as toxins and also susceptibility to a particular disease[96].

Recently it has been found out that K469E and G241R are the two polymorphisms of the ICAM-1 gene causing several microangiopathies like diabetic retinopathy, vascular dementia ,stroke, peripheral arterial occlusive disease and also immune mediated diseases like Type 1 diabetes, Graves disease, Behcets disease, Coronary artery disease and Inflammatory Bowel Disease. Howver they are related only to diabetic retinopathy and not diabetes[99].

The K469E ICAM polymorphism change gene function by two mechanisms:

- As a result of the K469E polymorphism, 5th immunoglobulin like domain of ICAM-1 which has lysine changes to glutamine thereby influencing

dimerisation and hence altering structure thereby increasing the affinity to the leukocytes[97].

- The K469E polymorphism produces alternatively spliced short isoforms (ICAM-1s) which has only extracellular domain influences signal transduction of ICAM and intercellular contact. It also increases the apoptosis resulting in endothelial injury. The exhausted regenerative capacity of the endothelium along with diabetes induced defective endothelial repairing capacity results in vascular damage and macrophages influx which further amplifies the ischemic nature of diabetic retinopathy[97].All these cumulates in exacerbation of diabetic retinopathy as shown in the picture below.

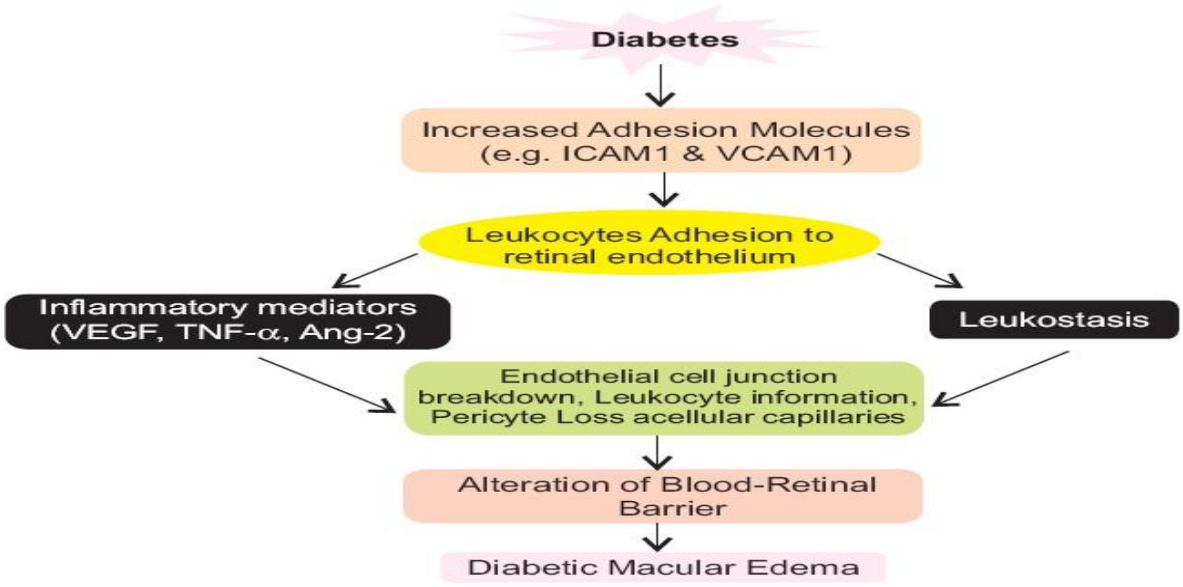


Figure:17-Role of ICAM in diabetic retinopathy.

K469E ICAM gene polymorphism and diabetic retinopathy association studies:

[97]Vinitha et al studied ICAM-1 K469E polymorphism among Indian Type 2 Diabetes patients with retinopathy and without retinopathy . They concluded that allele A of rs5498 in ICAM-1 predisposes the patient to develop retinopathy due to type 2 diabetes mellitus by decreasing the folding rate of the protein and thereby its structure and function.

[98]Petrovic and his colleagues studied the association of ICAM-1 K469E polymorphism and proliferative type of retinopathy due to diabetes in Caucasian population and concluded that EE genotype of the K469E ICAM-1 polymorphism predisposes the Caucasians to develop diabetic retinopathy.

[99]Liu L found that K469E ICAM-1 gene polymorphism among Chinese diabetic patients is related to type 2 diabetic retinopathy.

[100]Balasubbu S evaluated the association of nine candidate gene polymorphisms of type 2 diabetic retinopathy among Indian patients one among which is ICAM-1 gene. However only rs2070600 polymorphism of RAGE gene was associated with type 2 diabetic retinopathy among Indian patients. In this study ,ICAM-1 gene was not found to be influencing the development of diabetic retinopathy.

[101]Kamiuchi K et al evaluated the role of ICAM-1 and PECAM in predisposing to Type 2 diabetic retinopathy. In their study, only patients with ICAM-1 469KK genotype was predisposed with type2 diabetic retinopathy.

Pathogenesis:

Biochemical Mechanisms related to diabetic retinopathy pathogenesis:

1.Aldose reductase theory[84]:

Excess glucose in blood in Type 2 diabetes mellitus

↓

Sorbital /Aldose reductase pathway intracellularly

↓

Excess sorbital intracellularly which is osmotically active.

↓

Accumulation of water intracellularly.

↓

1. Pericyte loss
2. Adverse effects on retinal photoreceptor function and retinal blood flow
3. Vasodilation.

2. Advanced Glycation End product (AGE) theory[88]:

Irreversible non enzymatic modification of proteins, lipids and nucleic acids by reducing sugars and sugar derived products results in the formation of Advanced Glycation End products (AGE) and this reaction is called Maillard reaction. Intial glycation is reversible in which a sugar non enzymatically binds to amino groups of proteins, lipids and nucleic acids which results in stable Amodori products which undergo further reaction to form AGE.Since this is slow process , it affects tissues with a slow protein turnover, like the basement membrane. Cellular effects of AGE is

mediated through the receptors for AGE (RAGE) which activate a number of kinases which results in cellular dysfunction.

3.Protein Kinase -C theory[102]:

Hyperglycaemia, AGE, Reactive oxygen intermediates activates Protein Kinase -C(PKC) .Increased blood sugar levels by activating glycolytic pathway and thereby increasing the intracellular levels of glyceraldehyde-3-phosphate which in turn promotes de-novo synthesis of Diacyl glycerol(DAG) which activates protein kinase-C aiding in the development of diabetic retinopathy by

- mediating the functions of VEGF.
- Blood retinal barrier breakdown
- Neovascular proliferation.

4.Reactive Oxygen Intermediates theory[102]:

Increased oxidative phosphorylation due to hyperglycaemia, auto-oxidation of glucose, advanced glycation end products and sorbitol pathway results in free radical production. Increase in the amount of free radicals results in decrease in nitric oxide synthetase which leads to vasoconstriction, decreased leukocyte adhesion, decrease endothelial cells barrier function and causes cellular proteins damage which leads to diabetic retinopathy.

5.Angiogenic factors theory[102]:

Vascular Endothelial Growth Factor(VEGF) is apeptide secreted by extravascular tissues like glia ,retinal pigment epithelium(RPE), macrophages and T cells and its

secretion is upregulated by hypoxia. It is soluble in aqueous and vitreous and it is potently angiogenic .It causes

- Breakdown of blood retinal barrier by disrupting zonula occludens
- induces capillary endothelial proliferation resulting in neovascularisation.
- Other factors like Insulin-like growth factors and fibroblast growth factor has only synergistic effect. Factors inhibiting angiogenesis are pigment epithelial derived factor and angiostatin ,which has a reciprocal relationship to VEGF.

6.Insulin receptors and Glucose transporters[102]:

The pericytes and endothelial cells of the retinal microvasculature have insulin receptors. These receptors are required for glucose uptake by the endothelial cells and insulin upregulates glycogen synthesis. Insulin may also act as a growth factor in unphysiologically high concentration as seen in type 2 diabetes mellitus.

There are 5 glucose transporters in the body, of these GLUT-1,GLUT-2,GLUT-3 are present in the retina.GLUT-1 in present in the endothelial cells, Retinal pigment epithelium , mullers cells, nerve fibre layer and in the photo receptor cell bodies.GLUT-2 is present in apical ends of mullers cells and GLUT-3 is found in the plexiform layers .It has been found that GLUT-1 concentration is increased 18 folds in diabetic patients which might permit greater glucose influx thereby initiating glucose mediated damage to the cell.

Newer concepts in pathogenesis: Traditionally it has been believed that the hall mark of diabetic retinopathy is microvascular occlusion and leakage leading to tissue hypoxia and edema. However newer concepts question these concepts.

1.Extravascular changes in diabetic retinopathy[103]:

Decreased blue-yellow colour perception, reduced oscillatory potentials on the b-wave of ERG and nerve fibre defects in the red free photographs in the preclinical stage stresses upon the fact that neurosensory dysfunction occurs long before vascular changes. These changes are due to abnormal glutamate metabolism resulting in neuronal cell death which points towards a malfunction of muller cells and astrocytes which are extravascular tissue. Also that VEGF is secreted by extravascular tissues like the retinal pigment epithelial cells, glial cells.

2. Involvement of outer retina[103]:

- ✓ Retinal pigment epithelium is the source of angiostatic and angiogenic factors.
- ✓ Highest rate of metabolism of the rod photoreceptors precipitates hypoxia by depleting oxygen in inner retina.

Hence rods and the retinal pigment epithelium are responsible for the earlier involvement of retina in diabetic microangiopathy compared to rest of the body.

Diabetic retinopathy as an inflammatory disease[103]:

The following points support diabetic retinopathy as an inflammatory disease

- Presence of two signs of inflammation i.e swelling and loss of function.

- All microscopic signs of inflammation i.e vasodilatation, altered blood flow, fluid and protein exudation and leucocytosis.
- Evidence showing the presence of leukostasis , leukocytes clogging the capillaries and apoptosis ,all of which is mediated by ICAM-1
- Increased VEGF and other inflammatory mediators

Histopathological changes occuring in diabetic retinopathy:

1. Thickening of Capillary basement membrane: This is caused by

1. Glycation of capillary basement membrane collagen.
2. decreased proteoglycan which inhibits collagen production.
3. Vacuolization of capillary basement membrane.

All of the above results in increased platelet adhesion, poor nutrient diffusion and decreased growth factor binding which are now freely circulating to induce angiogenesis[103].

2. Loss of microvascular intramural pericytes:

Pericytes are the muscles of the capillaries which maintain their tone. As a result of sorbitol accumulation and free radicals formation, the pericytes are destroyed resulting in the formation of microaneurysms which are focal dilatation of weakened capillaries. In addition capillary basement membrane is thickened which is normally inhibited by the pericytes[103].

3.Damage to the endothelial cells:

Capillary endothelium is damaged by the accumulation of sorbitol, Advance glycation end products and glucose which is accentuated in the later stages of diabetic retinopathy by the accelerated blood flow. Damage to the capillary endothelium results in breaching of the inner blood retinal barrier causing leakage, leukoctye migration and adhesion to the capillary wall with loss of its cellularity[103].

4.Hematological Changes:

Following are the changes in the blood cells which results in thrombus formation in the capillaries and widespread retinal ischemia resulting in neovascularisation

- RBCs which are less deformable
- WBC which are less deformable and which are activated
- Platelets with a tendency to aggregate easily
- Defective fibrinolytic mechanism[103].

5.Neovascularisation:

Neovascularisation occurs due to retinal hypoxia resulting from capillary occlusion.It may be intraretinal or preretinal. Intra Retinal Microvascular Abnormalities (IRMAs) are shunts that run from arterioles to venules within the retina. It is an attempt to revascularise the ischemic retina when am imbalance develops between the angiogenic and antiangiogenic factors[104].

The histopathological changes occuring in diabetic retinopathy is shown in the figure below

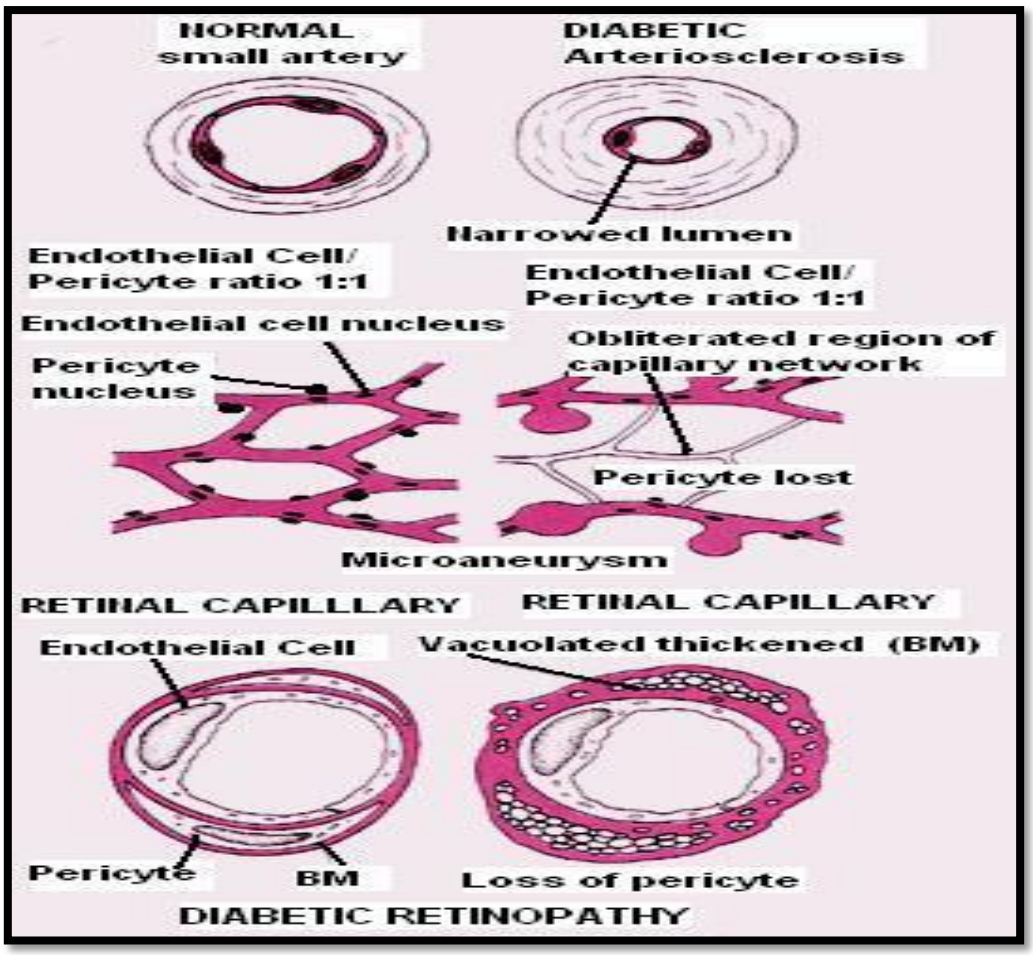


Figure:18 -Histopathologiacl changes in diabetic retinopathy.

Clinical features of Diabetic Retinopathy on fundus examination:

1.Microaneurysms:

Microaneurysms are localised outpouchings in the capillary wall formed either by the focal dilatation of capillaries where the pericytes are absent or by the fusion of two arms of the capillary loop and seen mainly in the inner nuclear layer where there are capillary non perfusion. Cellular microneurysms are formed by the endothelial cell proliferation due to the loss of pericytes. Hence a microaneurysms may either leak plasma or may be thrombosed. They are often visualised as red dots resembling dot haemorrhages temporal to the fovea. Hyperflouresence is seen in the early phase of

fundus flourescein angiography which is followed by diffuse hyperflouresence due to leakage in late phase[104].

2.Retinal haemorrhages[104]:

- Retinal nerve fibre layer haemorrhages: arise from superficial precapillary arterioles and are flame shaped due to the arrangement of nerve fibre layer.
- Intraretinal haemorrhages: are located in the compact middle layers of the retina and arise from the venous end of capillaries.
- Deeper dark round haemorrhages: which are located in the middle retinal layers are haemorrhagic retinal infarcts and marker of retinal neovascularisation.

3.Exudates:

Exudates are composed of lipoprotein and lipid filled macrophages in the outer plexiform layer and represent chronic retinal oedema .They are exacerbated by [10]hyperlipidemia. In fundus examination they are seen as waxy yellow lesions with distinct borders that are seen in clumps/rings around a leaking microaneurysms in the posterior pole and tend to incrse with time if there is chronic leakage .If the leakge stops they are absorbed spontaneously. Hardexuadtes in FFA show a blocked flouresence[104].

4.Diabetic macular oedema[104]:

This is the common cause o visual impairment among type 2 diabetics. Extensive capillary leakage leads to diffuse macular oedema and focal capillary leakage results

in focal macular oedema. The fluid is present initially between the outer plexiform and inner nuclear layer and later progress to involve the entire thickness of retina or may result in Cystoid macular oedema. Due to capillary leakage ,the macular oedema is seen in fundus flourescence angiogram as hyperflouresence in late phase or assumes as petalloid pattern if cystoid macular oedema is present. Ocular tomogram shows retinal thickening or cystoid spaces. It is of three types :

- Focal maculopathy : well circumscribed area of thickening with complete or incomplete ring of exudates with fundus flourescein angiogram(FFA) showing focal hyperflouresence with good macular perfusion.
- Diffuse maculopathy: Severe retinal oedema seen as diffuse retinal thickening obliterating the landmarks and may be associated with cystoid changes. In FFA, late diffuse hyperflouresence and if cystoid macular edema is present flower petal appearance is seen.
- Ischemic maculopathy: Fovea appears normal with loss of vision.On FFA,capillary non perfusion of fovea Foveal Avascular Zone (FAZ) enlargement is seen.

5.Clinically Significant Macular Oedema[104]: is defined according to ETDRS as

- Thickening of the retina which is within 500 µm of the macular centre.
- Hard exudates which is within 500 µm of the macular centre with adjacent thickening of the retina which lies outside 500 µm .
- Retinal thickening which is one or more disc diameters(1500 µm), part of which lies within one disc diameter of the macular centre[104].

6.Cotton wool spots:

Cotton wool spots are microscopic infarcts of the nerve fibre layer composing of the neuronal debris which result from the disrupted axons with swollen ends known as cystoid bodies. The neuronal debris is removed by phagocytosis or autolysis. They are seen as small ,whitish, fluffy lesions obscuring the blood vessels in the post equatorial retina.FFA shows blocked fluorescence[104].

7.Venous changes[104]:

The xtend of retina showing venous changes represents the area prone for neovascularisation. The venous changes seen are:

- Generalised venous dilatation
- Generalised venous tortuosity
- venous looping
- venous beading(focal narrowing and segmentation)

8.Arterial changes:

- Sub retinal arteriolar dilatation.
- Peripheral narrowing of arterioles.
- Silver wiring and obliteration of arterioles.

9.Intraretinal Microvascular Abnormalities:(IRMAs)are arteriolar-venous shunts that bypasses the capillary bed and run from the retinal arterioles to the venules adjacent to capillary non perfusion areas. They are seen as fine irregular ,red intraretinal lines that run from arterioles to venules without crossing the major vessels. In FFA, it is seen as focal hyperflourescence with adjacent capillary dropout but without leakage[104].

10.Proliferative diabetic retinopathy[104]

The main pathology behind PDR is neovascularization of retina which is an attempt to re-vascularize hypoxic retina. Angiogenic growth factors are elaborated by hypoxic retinal tissue promote neovascularization on the retina and optic nerve head and occasionally on the iris.Development of retinopathy is associated with the net balance between the activity of VEGF and endostatin. In order for PDR to develop more than one-quarter of the total surface area of retina has to be non-perfused. It occurs in the following forms.

New blood vessels at optic disc (NVD) i.e neovascularization on or within one disc diameter of the optic nerve head .

Newblood vessels elsewhere (NVE) i.e neovascularization along the course of the major vessels.

FFA shows hyperfluorescence during the later stages due to intense leakage of dye from neovascular tissue.

Clinical assessment :

1. Severity of PDR is determined by the area covered with new vessels in comparison with the area of the disc as follows:

- NVD is severe when more than one-third disc area in extent and mild if less
- NVE is severe when more than half disc area in extent and mild if less .

2.Flat new vessels are more responsive to laser therapy than elevated vessels.

3.Fibrous proliferation associated with neovascularization increases risk of tractional

4.High risk factors for severe loss of vision within 2 years, if untreated:

- Mild NVD with haemorrhage carries a 26% risk of losing vision, which is reduced to 4% with treatment.
- Severe NVD without haemorrhage carries a 26% risk of losing vision, which is reduced to 9% with treatment.
- Severe NVD with haemorrhage carries a 37% risk of visual loss, which is reduced to 20% with treatment. retinal detachment .
- Severe NVE with haemorrhage carries a 30% risk of visual loss, which is reduced to 7% with treatment..

Clinical features of Diabetic Retinopathy:

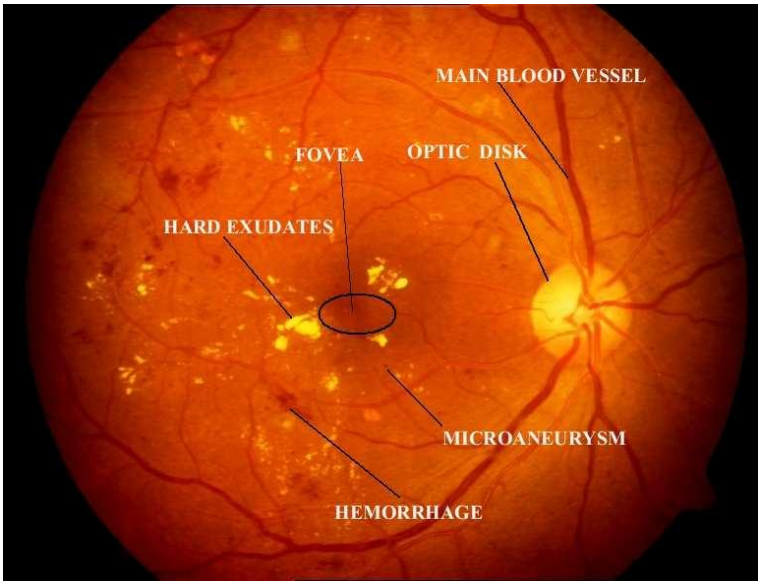


Figure:19- showing diabetic fundus with microaneurysms , hard exudates, dot and blot and superficial haemorrhages.

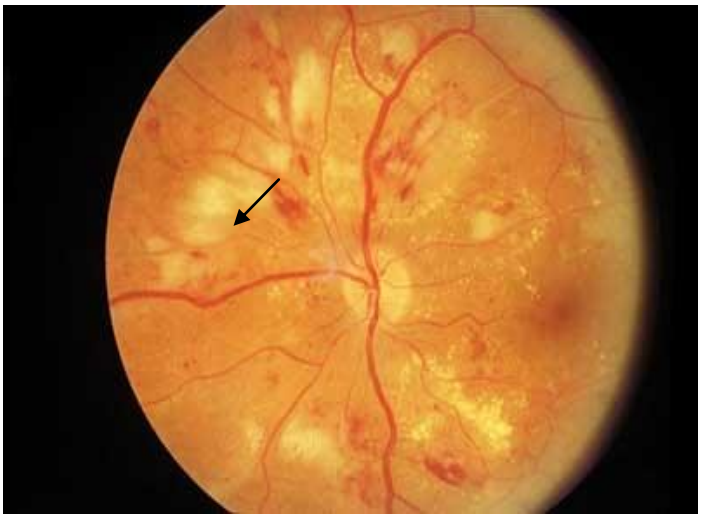


Figure:20- Fundus picture showing Cotton Wool Spots(black arrows)

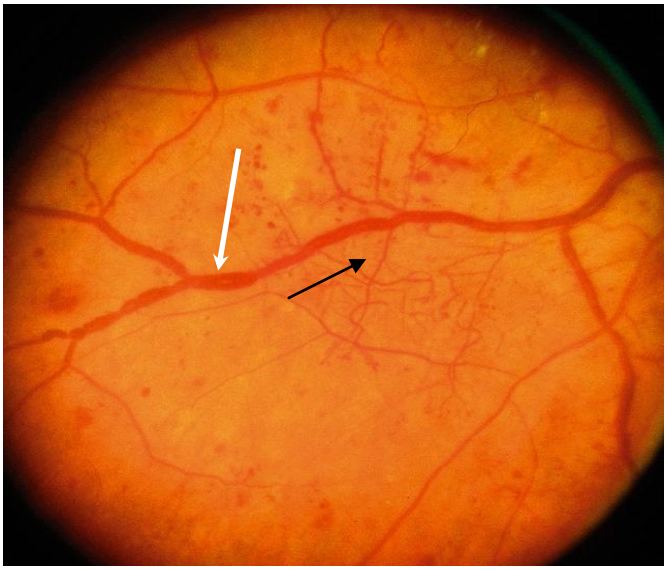


Figure :20- IRMAs(black arrow) and venous beading(white arrow)

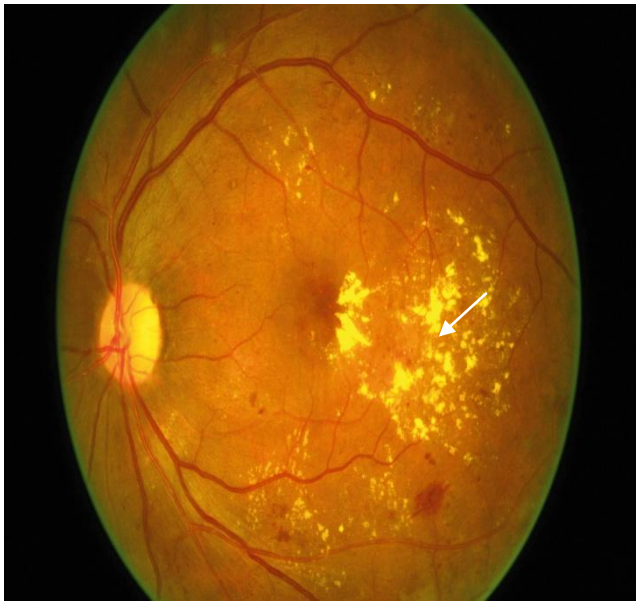


Figure:22-Clinically significant maculare edema with hard exudstes within 500µ from the center of fovea.

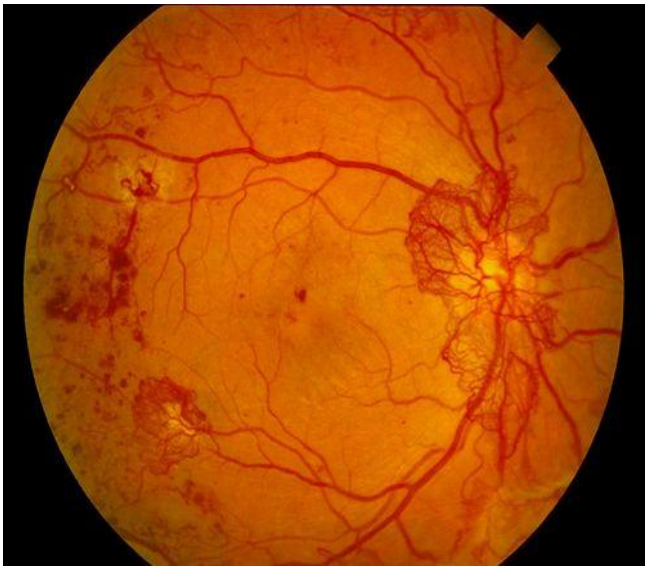


Figure:23-Showing neovascularisation of disc and neovascularistion elsewhere

Diabetic retinopathy Classification based of ETDRS modification of the Airlie House Classification:

This classification evaluates seven field - thirty degree nonsimultaneous stereo colour fundus photographs. It is used to assess baseline status of retinopathy due to diabetes and progressive course of disease. This classification which is to be used in the study are summarized below:

Disease Severity Level	Findings Observable upon Dilated Ophthalmoscopy
Mild nonproliferative retinopathy	At least one microaneurysm **AND** Criteria not met for more severe retinopathy
Moderate nonproliferative retinopathy	Hemorrhages/ microaneurysms ≥ standard photograph 2A(Fig;25) **AND/OR** Cotton wool spots , venous beading, or intraretinal microvascular abnormalities (IRMA) definitely present; **AND** definition not met for severe retinopathy.
Severe nonproliferative retinopathy	Cotton Wool Spots, venous beading, and IRMAs definitely present in at least two of photographic fields 4 to 7; **OR** two of the preceding three lesions present in at least two of fields 4 to 7 and hemorrhages and microaneurysms present in fields 4 to 7 > 2A(fig:25) in at least one of them; **OR** IRMAs t in each of fields 4 to7 and > 8A(fig:25) in at least two of them; **AND** definition not met for early proliferative retinopathy or high-risk proliferative retinopathy (see below)

Early proliferative retinopathy	New vessels **AND** Criteria not met for more severe retinopathy
High-risk proliferative retinopathy	New vessels on or within one disc diameter of the optic disc (NVD) ≥ standard photograph 10A(fig:26) (about 1/4-1/3 disc area), with or without vitreous or preretinal hemorrhage; **OR** vitreous and/or preretinal hemorrhage accompanied by new vessels, either NVD < standard photograph 10A(fig24) or new vessels elsewhere (NVE) ≥ one-quarter disc area

Figure:24- Diabetic retinopathy classification[107]

Standard fundus colour photographs for diabetic retinopathy grading

Standard photograph 2A, the standard for hemorrhages/microaneurysms. Eyes with severe NPDR have this degree of severity of hemorrhages and microaneurysms in all four midperipheral quadrants.

Standard photograph 6A, less severe of two standards for venous beading. Two main branches of the superior temporal vein show beading that is definite, but not severe.

Standard photograph 8A, the standard for moderate IRMA. Patients with severe NPDR have moderate IRMA of at least this severity in at least one quadrant.

Figure:25

Reprinted with permission from the Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie House classification. ETDRS report number 10. Ophthalmology 1991;98:786-806.

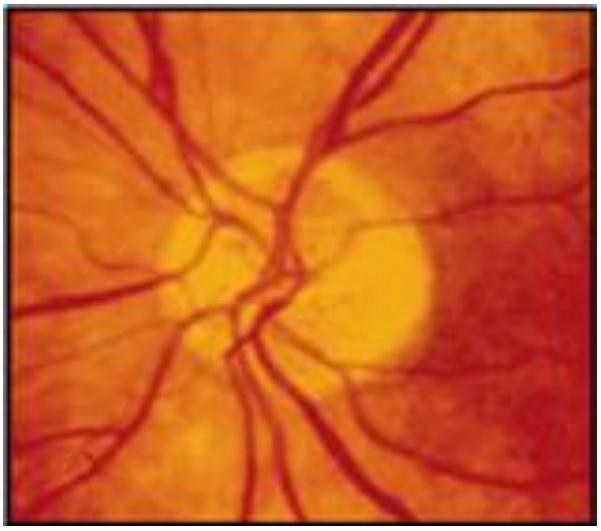


Figure:26:Standard photograph 10A:Neovascularisation of disc.

Investigations:

Fundus Fluorescein Angiography[103]:

Though not recommended for grading diabetic retinopathy,FFA is used for guiding treatment of clinically significant macular oedema. Other important indications for FFA are

1.Featureless retina: In clinical scenarios where new vessels are present without evidence of severe NPDR or presence of white thread like arterioles in mild NPDR,FFA may reveal the presence of unsuspected new vessels and also extensive capillary non perfusion.

2.Asymetrical diabetic retinopathy: where the fellow eye has proliferative diabetic retinopathy, FFA picks up hidden new vessels.

3.Macular ischemia: suspected macular oedema due to clinical features like subnormal vision, sclerosed macular arterioles,blot hamorrhages and soft exudates at the macula can be confirmed by FFA.

4.Poor visibility due to asteroid hyalosis.

5.To differentiate severe NPDR from PDR:In severe NPDR, non leaking IRMAs are seen along with extensive capillary drop outs in contrast to PDR where leaking new vessels are seen in FFA.

6.To confirm diabetic papillopathy: where in FFA, vascular filling defects of the disc and choroid are absent unlike Non arteritic AION and late leakage of disc is intramural unlike that of neovascularisation of the disc.

Salient features of diabetic retinopathy in FFA:

1.Mid-aretriovenous phase or capillary phase: clinical features visible are

- Capillary non perfusion areas.
- Macular ischemia characterised by enlargement of foveal avascular zone, irregularity of the borders of foveal avascular zone.
- Leaking microaneurysms which can be differentiated from solid microaneurysms and dot haemorrhages.
- IRMA s which do not leak can be differentiated from new vessels which leak.

2.Late phase:

- Intraretinal haemorrhages become prominent and pre retinal and intravitreal leaks from the new vessels give a classic cotton ball hyperflourescence.

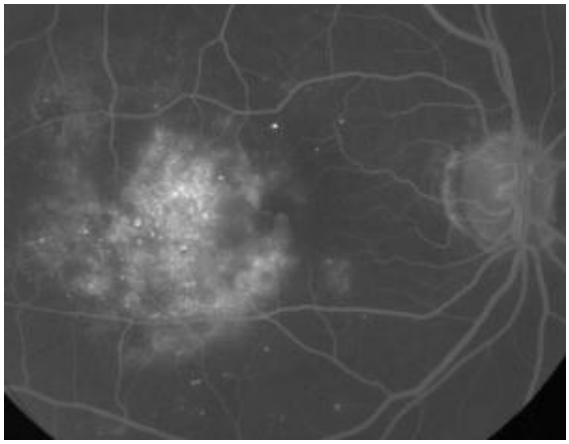


Figure:27 Late FFA picture showing diffuse leakage in CSME

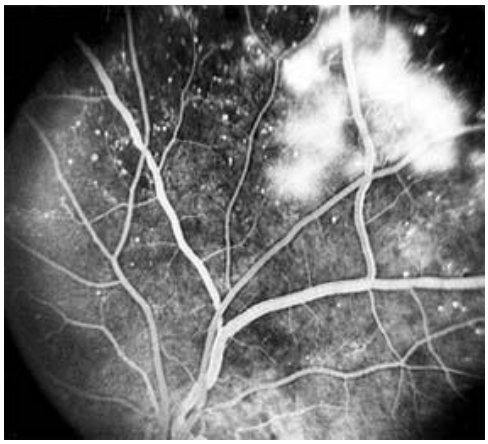


Figure:28 FFA showing leakage from new vessels.

B mode ultrasonogram[103]:

- in advanced diabetic retinopathy with opaque media.
- Gives information about the macular status and the extend of posterior vitreous detachment, retinal detachment and areas of traction.

Optical Coherence Tomography[103]:

OCT is based on the principle of interference of reflected infra red rays from the tissue.The OCT gives a high resolution histopathological view of the macula and are therefore primary tool of macular assessment .It is used to assess the role of vitreomacular traction and hence the need for laser photocoagulation and as alternative for FFA to asses and follow up CSME .However neither the sites of leakage as in FFA nor the macular status in opaque media as in USG can be assesd using OCT and it is always an alternative to the above two.

Electroretinogram[103]:

Used only as research tool .Abnormalities in oscillatory potential precedes the vascular changes in diabetic retinopathy. Multifocal ERG is records responses from individual areas and hence used to predict focal retinal areas that would develop edema.

Management of diabetic retinopathy:

Diabetic control:

Unless the systemic diabetes is under control, one cannot treat or impede the progression of diabetic retinopathy. The few of treatment of diabetes are as follows:

1.Insulin therapy:

- Rapid acting preparations
- Intermediate acting preparations
- Long acting preparations
- Human Insulin

2.Diet therapy: 35kcal/kg body weight/day and 0.8-1gm protein/kg body weight/day and fat content to be 30% or less of total calories with saturated fat - 7-10%.

3.Exercise therapy.

4.Oral hypoglycemics:

- Sulphonyl ureas –Glipizide, Glimepride, Glyburide
- Biguanides – Metformin
- Meglitinides – Nateglinide, Repaglinide

- Thiazolidinediones – Pioglitazone, Rosiglitazone
- Glucosidase Inhibitors – Acarbose, Miglitol
- DPP-IV Inhibitors - Sitagliptin

Also the ophthalmologist should make sure the diabetic patients HbA_1C is maintained close to 7g/dl, blood pressue close to 130/80 mm Hg, total cholesterol within 200 mg/dl and haemoglobin >13g/dl with no traces of protein in urine.

Management of different grades of Diabetic Retinopathy :

Grades of Diabetic Retinopathy	Management.
No diabetic retinopathy	Review every 12 months
Very mild NPDR : Microaneurysms only	Review every 12 months
Mild NPDR	Review range 6-12 months, depending on severity of signs, stability, systemic factors, patient's personal circumstances
Moderate NPDR	Review in approximately 6 months
Severe NPDR	Review in 4 months
Very Severe NPDR 2 or more of the criteria for severe	Review in 2-3 months
Low risk PDR	Customize the treatment according to the patient, if not treated review in 2 months.
High risk PDR	Immediate treatment.
Advanced diabetic eye disease: Tractional retinal detachment, Vitreous hemorrhage and neovascular glaucoma	Immediate treatment.

Figure:29: Different grades of diabetic retinopathy treatment[104].

Treatment specific for Diabetic Retinopathy

- Laser Photocoagulation
- Surgical – Vitrectomy, Intravitreal injections

Laser photocoagulation[103]:

Mechanism of action:

Laser increases the temperature of the tissue to about 20^0 C ,thereby causing coagulative necrosis and intracellular protein denaturation resulting in photocoagulation. The target is melanin pigment in RPE and haemoglobin in capillaries and microaneurysms. In PDR, laser treatment converts the hypoxic retina into anoxic retina thereby decreasing the release of angiogenic factors and also increases oxygen supply to inner retina by destroying the RPE and photoreceptors. Laser induced debridement of RPE induces reproliferation of both inner and outer retianl barrier and therefore better function resulting resolution of macular edema.

Laser Wavelength selection:

Argon green(514 nm),Nd:YAG(green,532nm),Dye yellow(577 nm), Kryton Red(647nm) and diode (infrared 812 nm) can be used for both macular edema and proliferative diabetic retinopathy. However Argon blue-green is contraindicated in macular edema.

Modes of laser delivery:

- Using slit lamp using standard goldmann 3 mirror lens or a panfundoscope.

- Indirect ophthalmoscopic laser delivery method.
- Endoscopic laser delivery using fibreoptic tubes during vitrectomy.
- Transscleral diode laser for cyclocoagulation in neovascular glaucoma.

Indications:

1.Clinically significant macular edema

- Grid laser- for diffuse leakage
- Focal laser- for focal leakage

2.Paramacular edema

3.Early/high risk proliferative diabetic retinopathy.

4.Severe/very severe Non Proliferative Diabetic Retinopathy-in case of poor compliance, fellow eye with proliferative diabetic retinopathy, other uncontrolled systemic diseases, pregnancy, pending cataract surgery, uncontrolled diabetes.

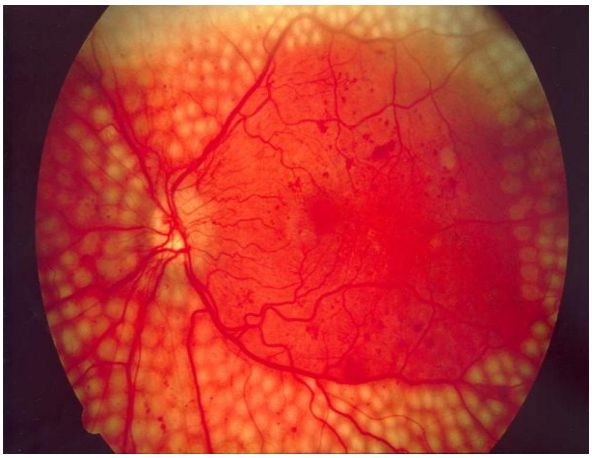


Figure:30- Fundus picture fresh laser marks of scatter retinal photocoagulation

Parameters: ETDRS protocol for Pan Retinal Photocoagulation[105]

Spot size	**500microns**
Exposure time	**0.1s**
Intensity	**Moderate**
Number of shots	**1200 – 1600**
Location	**Diameter of shot separation, >2DD out of fovea to the equator**
Number of sessions	**At least 2**
Treated lesions directly	**New vessels which 2DD in extrapapillary location**
Indications for new treatment	**Areas of new vessels in the extrapapillary region , high risk PDR, recurrence**

Figure:31-ETDRS Pan retinal photocoagulation protocol.

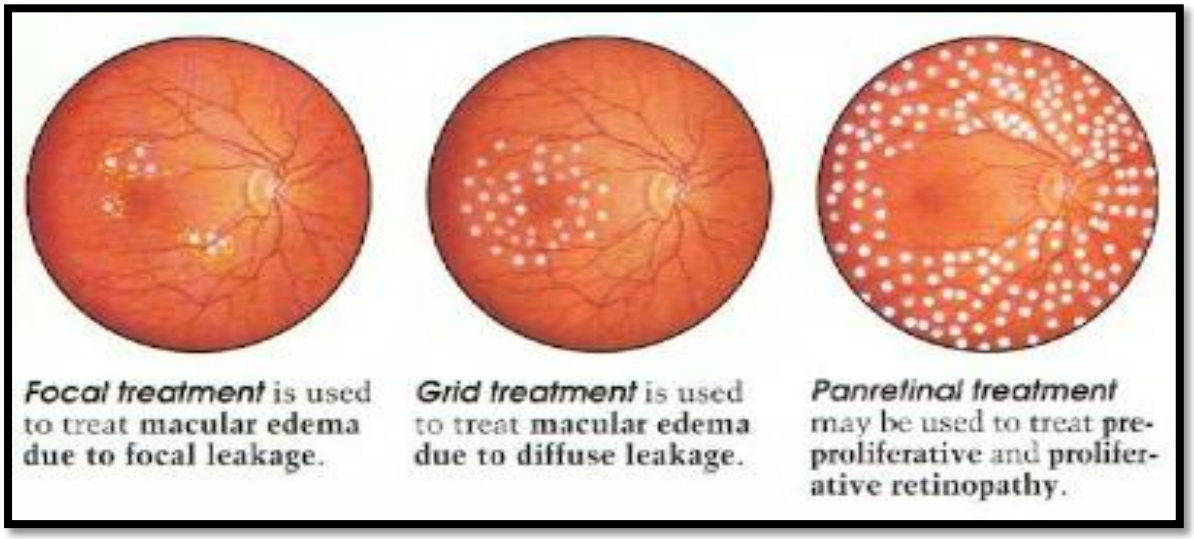


Figure:32- Different patterns of laser photocoagulation.

Modified ETDRS protocol for focal /grid photocoagulation in CSME[102]

Treatment parameter	Focal laser	Grid laser
Area treated	**500-3000µ from the centre of the macula**	**500-3000µ superiorly ,nasally and inferiorly from the macular centre.** **500-3500 µ from the macular centre. not within 500µ of disc.**
Spot size	**50µ**	**50µ with two burn width apart with barely visible burn intensity.**
Burn duration	**0.05to 0.1 seconds**	**0.05-0.1 seconds**

Figure:33-ETDRS photocoagulation protocol for CSME.

Side effects:

A. After laser treatment of Diabetic macular edema:

1. Transient increase in edema.
2. Paracentral scotomas.
3. Photocoagulation scar expansion
4. Inadvertant foveolar burns.
5. sub retinal fibrosis
6. Choroidal neovascular membranes.

B. After panretinal photocoagulation:

1. Peripheral field loss.
2. Macular edema.
3. Pain
4. Pre-retinal fibrosis/ Tractional Retinal detachment.
5. Choroidal detachment

Surgical Treatment:

Vitrectomy[103]:

When optimal glucose control and photocoagulation fails to stop the progression of diabetic retinopathy then one should consider Pars plana vitrectomy. Whenever possible pan retinal photocoagulation should be done before doing pars plana vitrectomy.

Indications:

1. Chronic persistant vitreous haemorrhage.
2. Epimacular membrane
3. Tractional retinal detachment involving the macula.
4. Neovascularisation not responding to photocoagulation
5. Rhegmatogenous retinal detachment alone or if complicating a tractional retinal detachment.
6. Diabetic macular edema caused by vitreo macular traction.
7. Rarely in neovascular glaucoma to clear media opacities before giving PRP.

Objectives and techniques:

- Removal of vitreous opacities.
- Removal of anteroposterior and tangential vitreoretinal and epiretinal membrane induced traction.

  -Segmentation technique to relieve antero posterior traction

  -Delamination technique to remove tangential traction.

Intravitreal injections[102]:

Anti-VEGF:

1. Bevacizumab (Avastin): is 149 kD full-length, humanized, monoclonal recombinant antibody against VEGF isoforms , it also blocks VEGFR tyrosine kinase receptors in the endothelial cells and thereby inhibits neovascularisation and vascular leakage .It's dose intravitreally is1.25 mg/0.05 ml. It has been prove to decrease the OCT macular thickness and improves vision. However it increases systemic blood pressure.

2. Ranbizumab(Lucentis):It is affinity matured humanized 48 kD, monoclonal recombinant antibody fragment .It is given in a dose of 0.5 mg/0.05 ml intravitreally.

3.Pegatinib(Lucentis): It is a 28 kD RNA pegylated aptamer and specifically blocks VEGF-165 isoforms and given in adose of 0.3mg/0.09ml intravitreally. However it lacks the efficacy of Avastin and Lucentis.

4.VEGF trap:It is formed by the fusion of ligand binding elements of VEGF receptor extracellular components and Fc portion of IgG1.

Steroids: Intravitreal Triamcinolone injection is given to treat diabetic macular edema not responding to focal or grid laser photocoagulation. It is given in a dose of 4mg in 0.1 ml . It predisposes the patient to increased intraocular pressure.

Eye Examination protocol for early detection of diabetic retinopathy[106]:

Type of diabetes	Recommended initial eye examination	Routine follow up
Type 1	5 years after onset or during puberty	Yearly.
Type 2	At time of diagnosis	Yearly.
Pregnancy with pre-existing Diabetes mellitus	Prior to pregnancy for counselling	Early in 1st trimester. Each trimester or more frequently Six weeks postpartum

Figure 34:Eye examination protocols for diabetics.

Materials and Methods

- It is a prospective descriptive analytical study
- **Study population:**

  This is a hospital based study involving a cross section of inpatients and outpatients of Ophthalmology department of PSG Hospitals with clinical diagnosis of Type 2 diabetic retinopathy over a period of 12 months from June 2012 to June 2013.

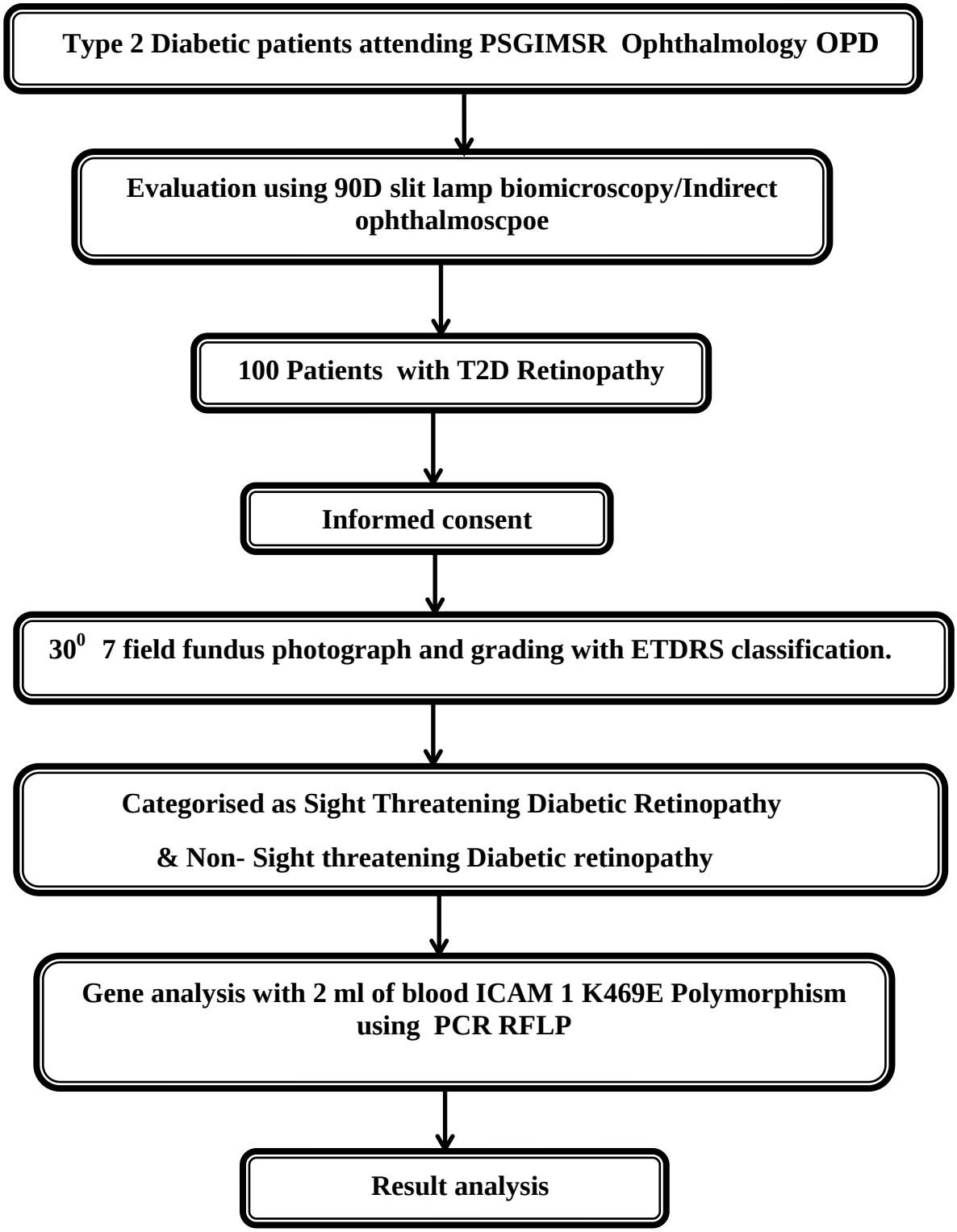
  - ✓ Inclusion criteria:
    1. Type 2 Diabetes With retinopathy
    2. ≥40 years of age
    3. Duration of diabetes more than 5 years.

  - ✓ Exclusion criteria:
    1. Type 1 Diabetes
    2. <40 years of age
    3. other retinal disease
    4. Type 2 diabetes without retinopathy
    5. Terminally ill patients

- **Sample size:**

  Due to money and time constraint convenient sample of 100 patients with type 2 diabetic retinopathy are included in this study.

❖ **Study design:**



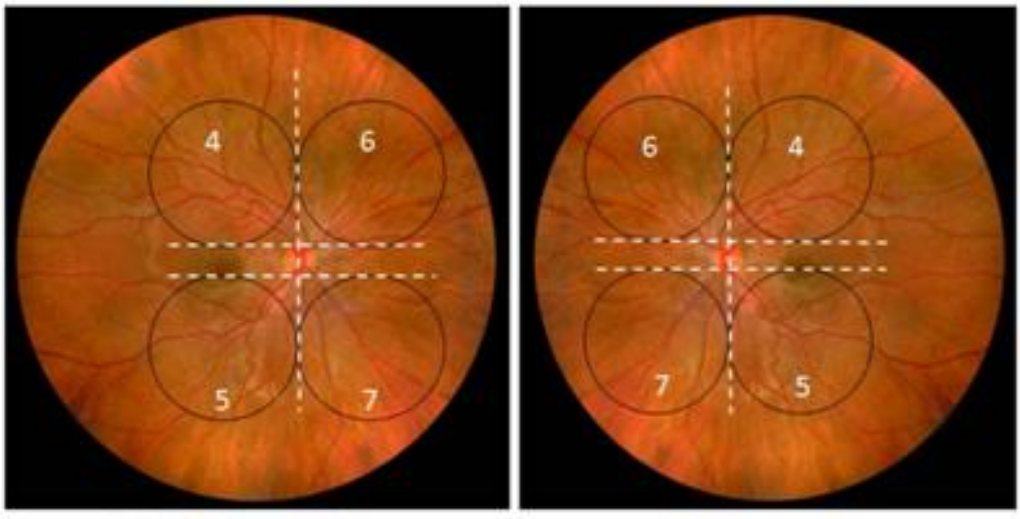
❖ Methodology:

- Type 2 diabetic patients are evaluated for the presence of retinopathy by using 90D slit lamp biomicroscopy 20 D and with indirect opthalomoscopy for the presence of diabetic retinopathy
- Type 2 diabetic patients with retinopathy who fit into the inclusion criteria are selected.
- 30^0 7 field colour fundus photographs are taken .The area to be covered in each field is as follows:
    - ✓ **Field 1: Optic disc**; the intersection of the mires is at the optic disc centre.
    - ✓ **Field 2: Macula**; the intersection of the mires is at the macula.
    - ✓ **Field 3:Temporal to the macula**; the field is so positioned that the macula lies in the nasal edge of this field.
    - ✓ **Field 4: Superior temporal**; a horizontal line which pass through the superior optic disc border is tangential to this fields lower edge and a vertical line through the optic disc centre is tangential to its nasal edge.
    - ✓ **Field 5:Inferior temporal**; a horizontal line which pass through the inferior optic disc border is tangential to this fields upper edge and a vertical line through the optic disc centre is tangential to its nasal edge.
    - ✓ **Field 6: Superior Nasal**; a horizontal line which pass through the superior optic disc border is tangential to this fields lower edge and a vertical line through the optic disc centre is tangential to its temporal edge.

- ✓ **Field 7:Inferior nasal**; a horizontal line which pass through the inferior optic disc border is tangential to this fields upper edge and a vertical line through the optic disc centre is tangential to its temporal edge.

Right eye of the 1, 2 and 3 fields

Left eye of the 1, 2 and 3 fields



Right eye and left eye fields 4, 5, 6, and 7

Fig:36-Fields 1,2,3 of the ETDRS 7 field fundus photographs for Diabetic Retinopathy grading

- Their diabetic retinopathy graded according to ETDRS modification of the Airlie House Classification by comparing the photographs taken with standard ETDRS colour photographs 2A,6A,8A,10A and grading done independently by three ophthalmologists
- 25 patients each having mild ,moderate ,severe and proliferative diabetic retinopathy respectively are enrolled in the study which accounts for the total sample size of 100.
- For convenience purpose early PDR and high risk PDR are clubbed together into a single group called "Proliferative Diabetic Retinopathy"
- These 4 categories are further divided into 2 groups namely Non-Sight Threatening Diabetic Retinopathy (NSTDR) and Sight Threatening Diabetic Retinopathy (STDR) depending upon the course of the grade of retinopathy to cause defective vision in the near future.
- Sight Threatening Diabetic Retinopathy(STDR) is defined as those with Severe Diabetic Retinopathy, Proliferative Diabetic Retinopathy and any Diabetic Retinopathy with Clinically Significant Macular Edema
- Non Sight Threatening Retinopathy is defined as those with Mild and Moderate Diabetic Retinopathy
- 2ml of blood is drawn from each of these patients for the gene anlaysis to determine the presence of ICAM 1 K469E Polymorphism.
- Gene analysis:
  - ✓ Extract DNA from the peripheral blood samples by conventional phenol chloroform method .

- ✓ The genomic region flanking the K469E polymorphism in exon 6 of ICAM-1 amplified with forward (50 -CTTGAGGGCACCTACCTCTG-30) and reverse on(50 -CATTATGACTGCGGCTGCTA-30) using PCR .
- ✓ RFLP will be done to identify the mutation

- Data so obtained is entered in the excel sheet and statistically analysed using SPSS software.

STEPS IN GENE ANALYSIS

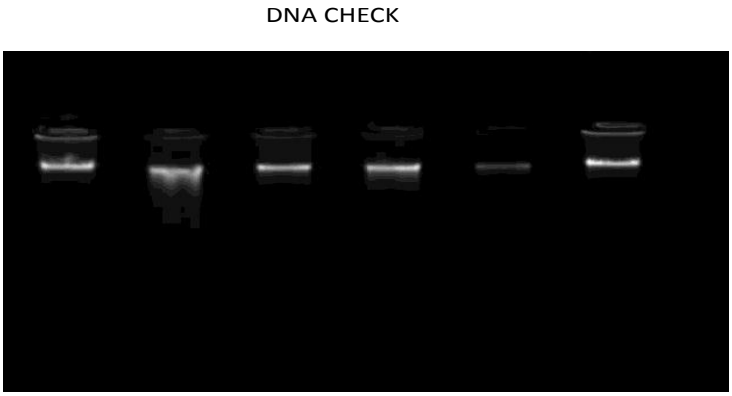


Figure:37 DNA Extraction

PCR CHECK

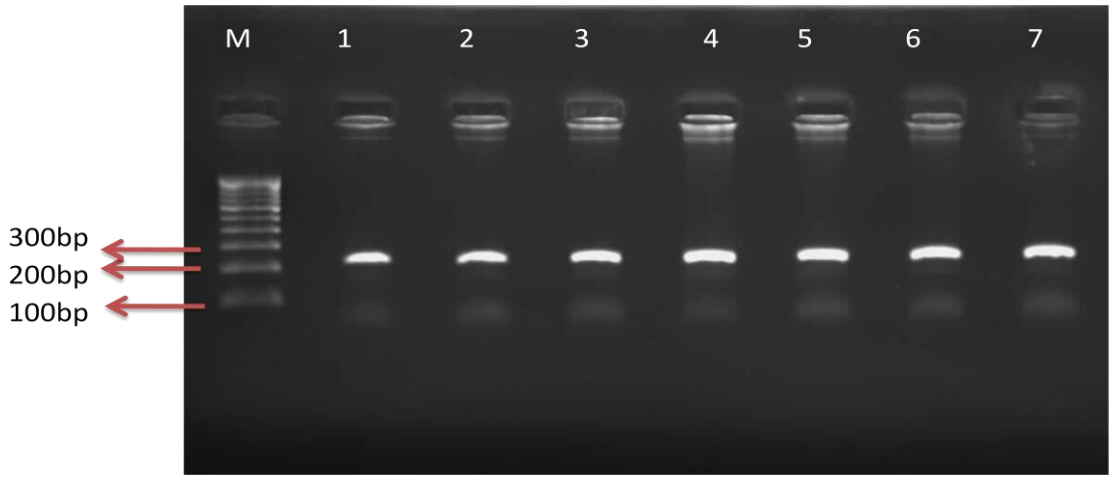


Figure:38 PCR to amplify the gene

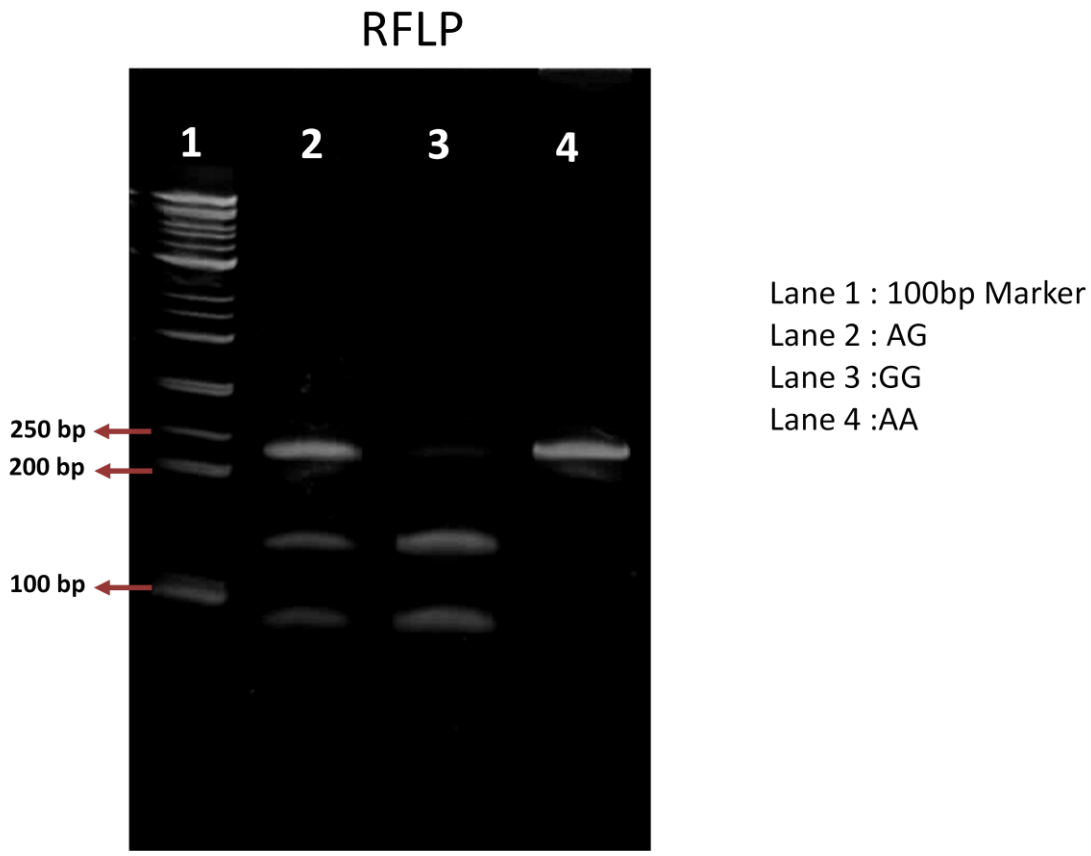


Figure 39:using restriction enzyme BstUI to identify the Single nucleotide polymorphism.

RESULTS

Demographic Profile of the study population:

1.Age distribution of the study population:

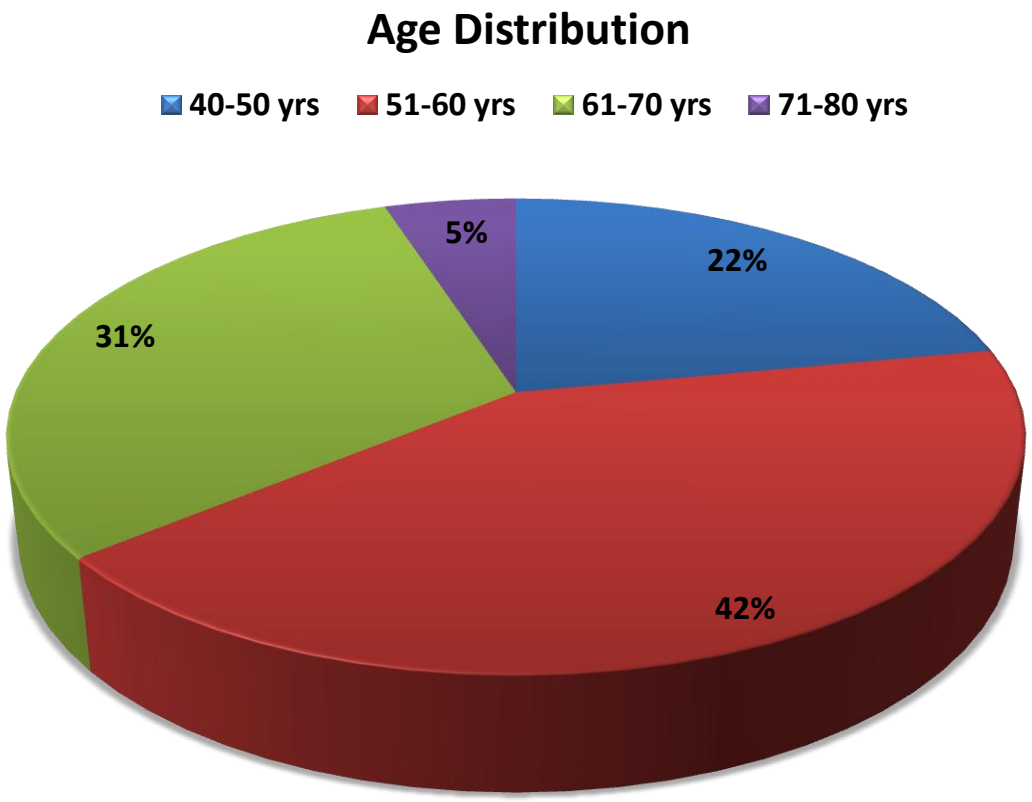


Figure 40:-Age distribution of the study population.

In this study only patients who had been diagnosed with diabetes after 40 years were included to exclude patients with Type 1 diabetes which has its onset before 30 years of age. Majority of the study population in this study were in the age group 51-60 years as seen from the pie chart above.

Sex Distribution:

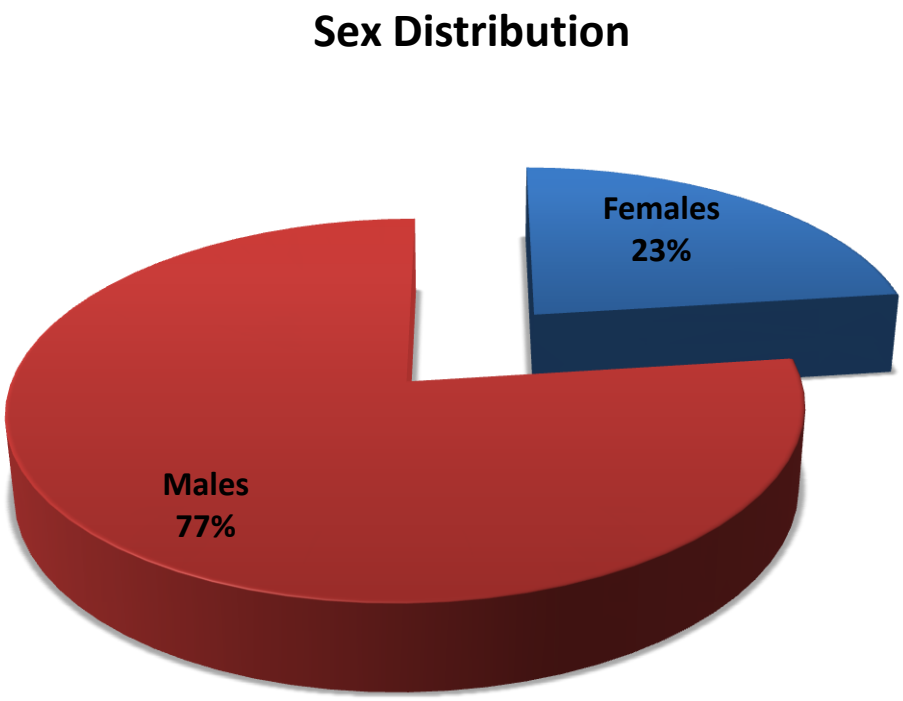


Figure 41: Sex Distribution of the study population.

Among the 100 Type 2 diabetic patients involved in my study,77% were males and 23% were females as shown in the pie chart above.

Duration of Diabetes among study population:

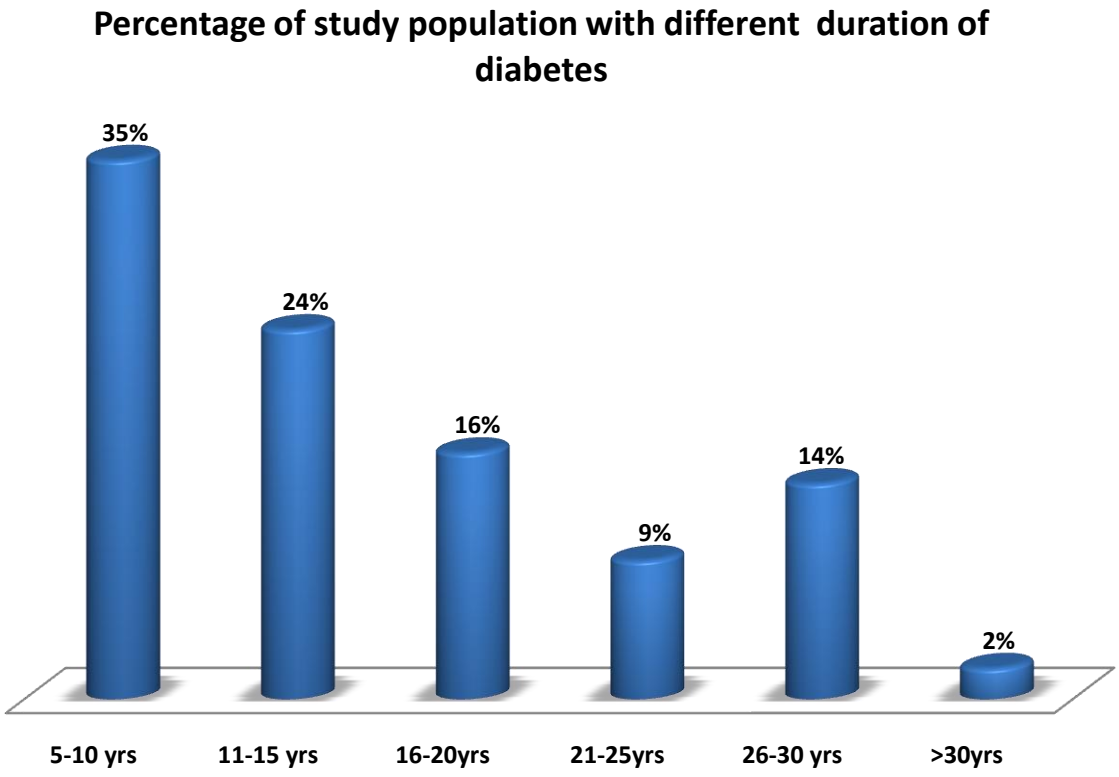


Figure:42-Diabetic duration among study population

In this study only patients who are older than 40 years and have Type 2 diabetes for more than 5 years are included. Of the 100 patients studied majority (35%) had diabetic duration within the range of 5-10 years.

Treatment for Type 2 diabetes among the study population:

Majority of the study population were on treatment with oral glycemics for blood sugar control. Only 29% were on insulin treatment for Type 2 diabetes . None of them were on diet control only.

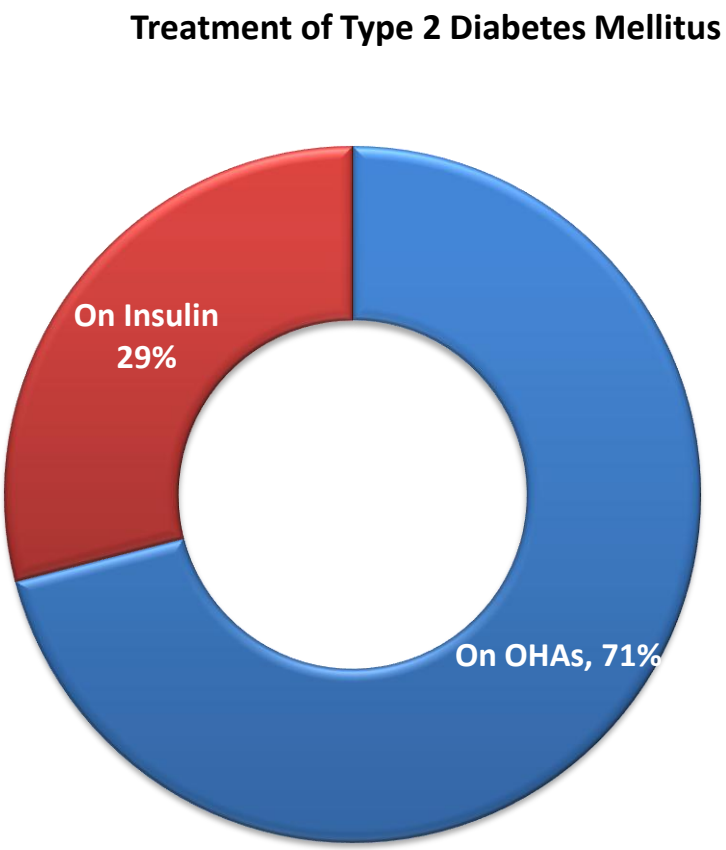


Figure:43: Treatment of Type 2 diabetes among the study population.

Presence of other co-morbidities among the study population:

In our study , almost half of the patients with Type Diabetic Retinopathy had no co-morbidities and the other half had co morbidities. Other co-morbidities associated with diabetic retinopathy type 2 include systemic hypertension , Cerebrovascular accident ,Ischemic vascular diseases, Peripheral Vascular Diseases etc. The percentage of study population with and without co-morbidities is shown in the picture chart below.

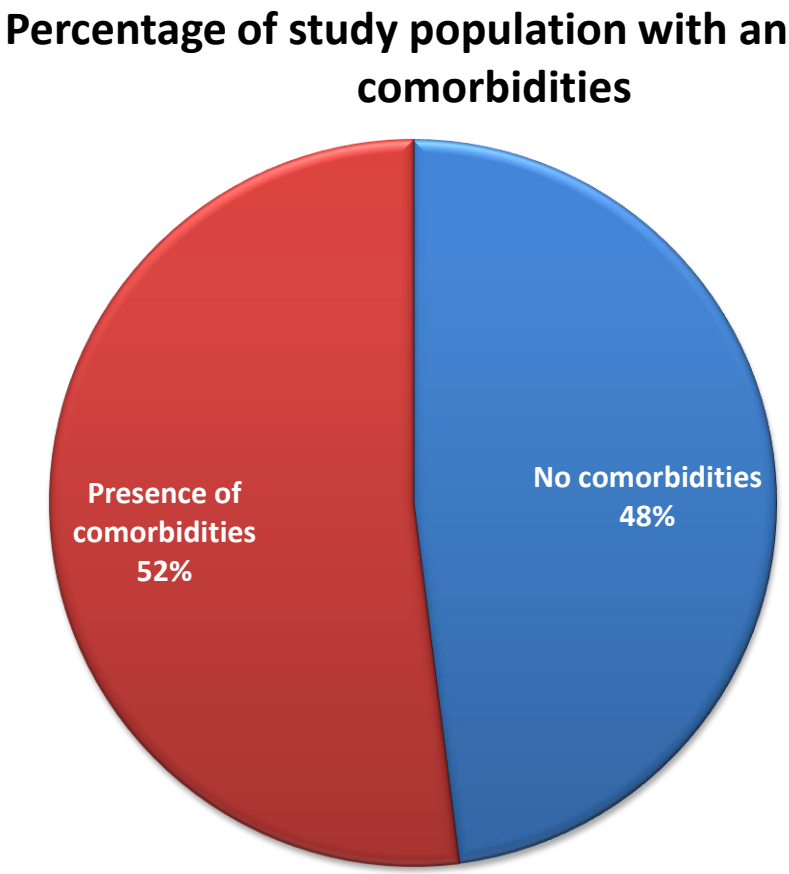


Figure 44:Presence of comorbidities among study

Severity of Diabetic Retinopathy among study population:

After grading the patients with diabetic retinopathy according to ETDRS classification, Early and High risk proliferative diabetic retinopathy has been clubbed into a single category as "Proliferative Diabetic Retinopathy", thereby making up to 4 categories in this study namely Mild Non Proliferative Diabetic Retinopathy group, Moderate Non Proliferative Diabetic Retinopathy group, Severe Non Proliferative Diabetic Retinopathy group and Proliferative Diabetic Retinopathy group.

In our study we enrolled 25 patients in each category .Incidence of CSME in our group was 9%.7 patients in the Severe NPDR group had CSME and one patient in each of the Mild and Moderate NPDR group had CSME. Among the 25 patients in the Proliferative Diabetic Retinopathy group ,only 3 had high risk characteristics.

Frequencies of different grades of Diabetic Retinopathy among different age groups

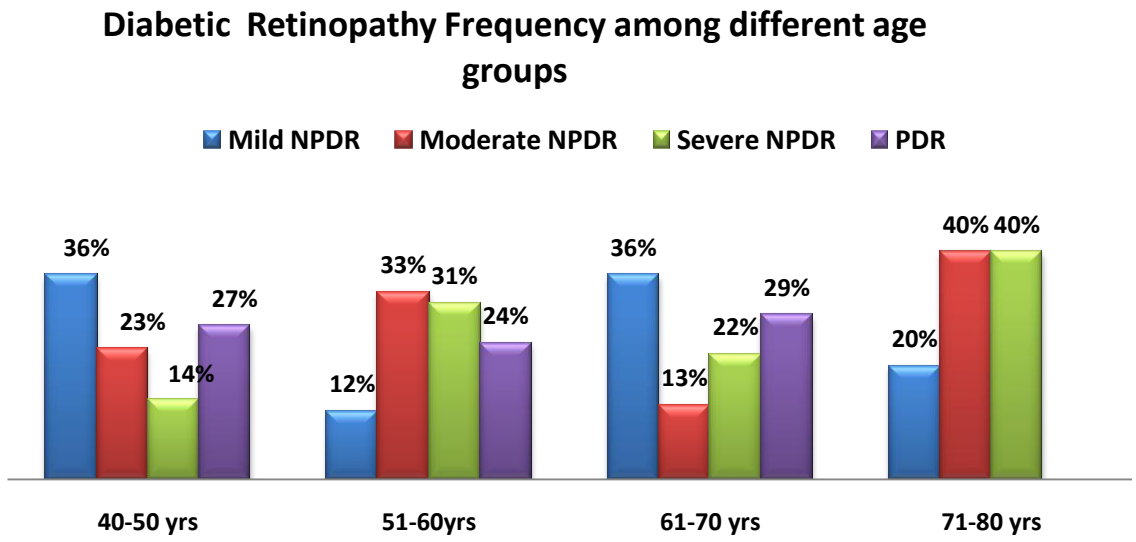


Figure 45: Frequencies of different grades of Diabetic Retinopathy

Different grades of diabetic retinopathy is somewhat equally distributed among all the age groups and there was no statistically significant relation between them .

Gender differences in the frequency of diabetic retinopathy:

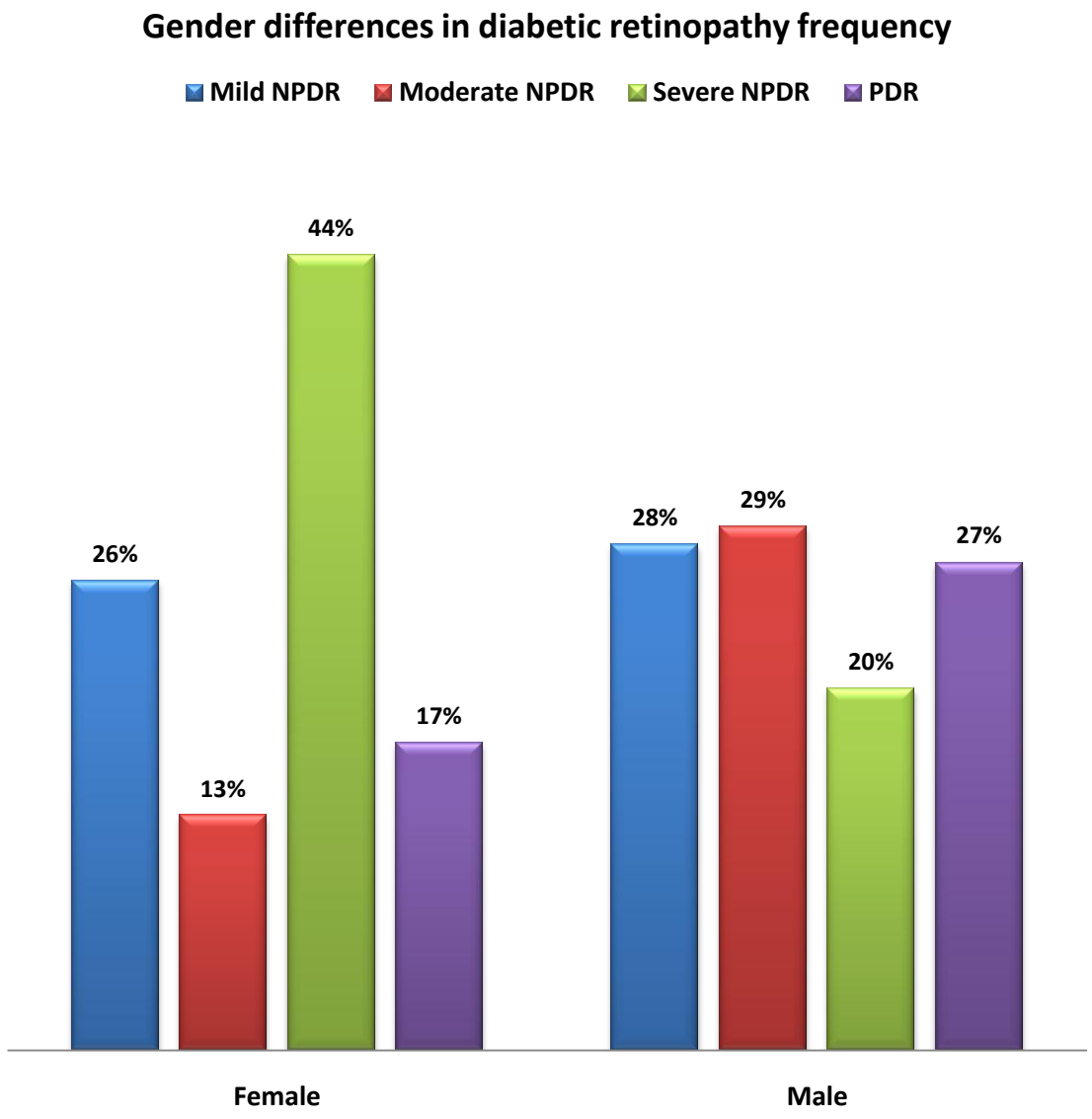


Figure 46: Gender differences in diabetic retinopathy frequency

There was no statistically significant relationship between severity of diabetic retinopathy and the gender differences.

Prevalence of Sight threatening Retinopathy among the study population:

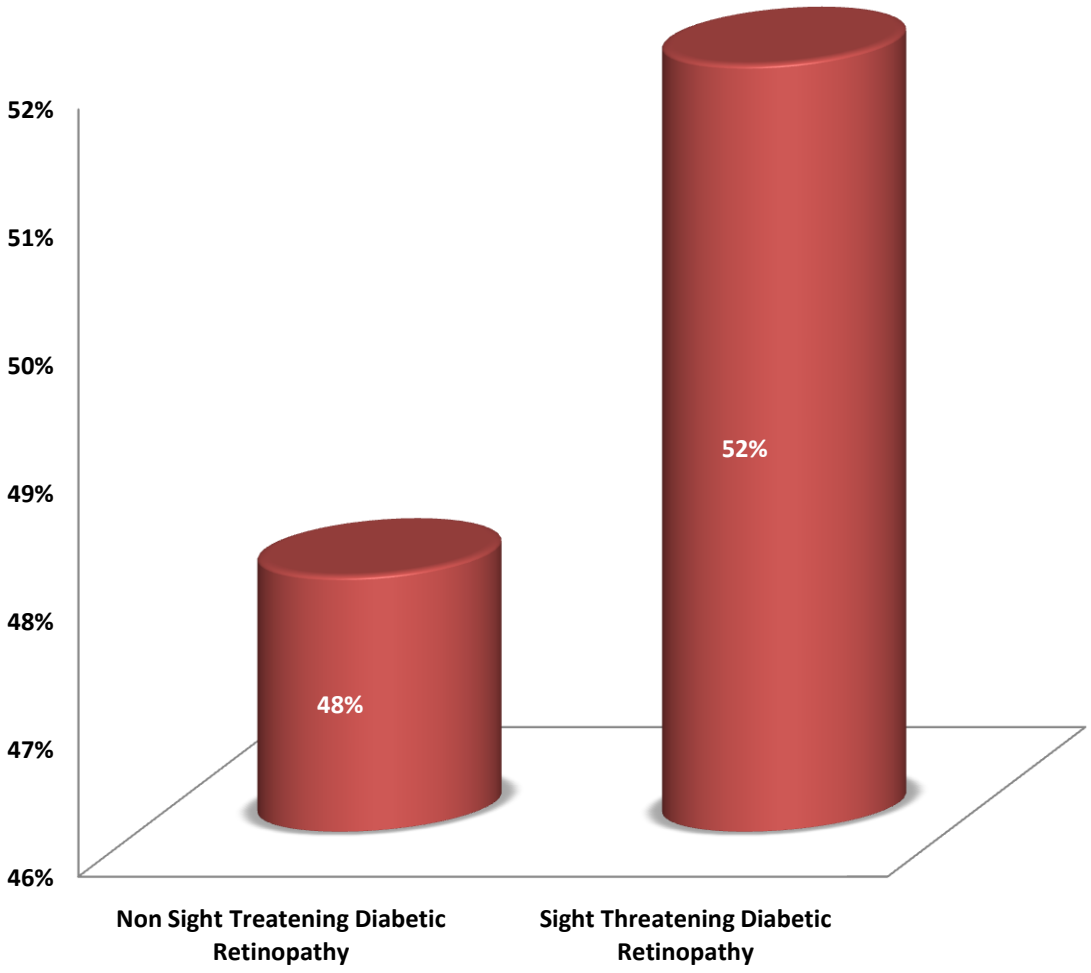


Figure 47: Prevalence of Sight Threatening and Non Sight Threatening Diabetic Retinopathy among the study population.

In our study, 25 patients had Proliferative Diabetic Retinopathy ,25 patients had Severe Non Proliferative Diabetic Retinopathy and 2 patients outside these two groups had Clinically Significant Macular Edema accounting for the 52% prevalence of Sight Threatening Diabetic Retinopathy .

Gender differences among Sight Threatening and Non Sight Threatening Diabetic Retinopathy:

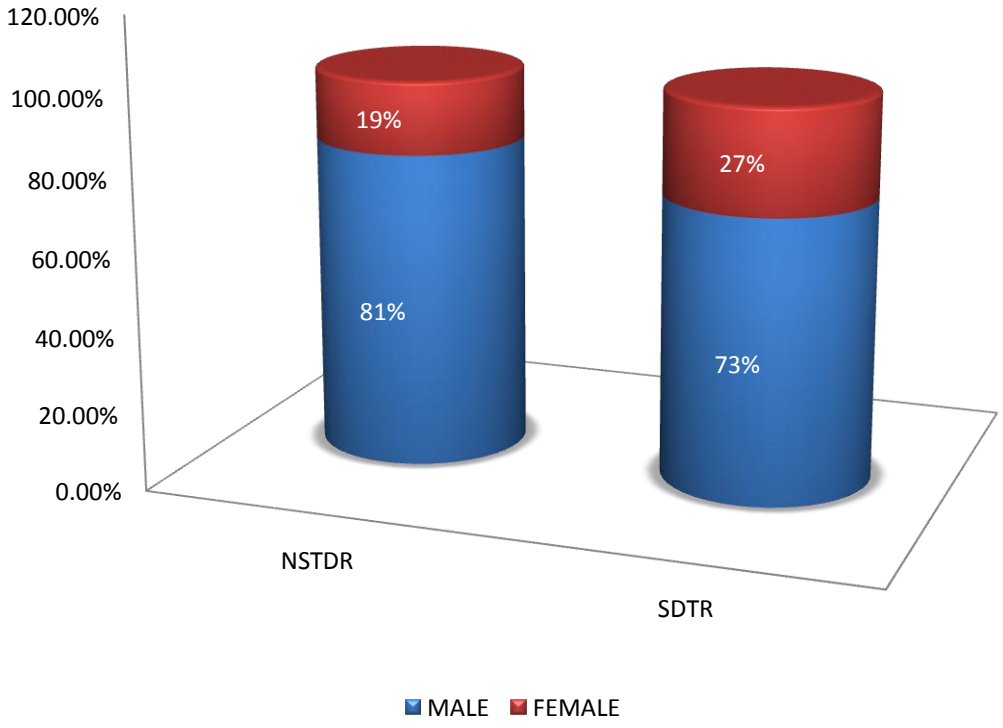


Figure:48:Gender distribution among Sight Threatening Diabetic Retinopathy(STDR) and Non Sight Threatening Diabetic Retinopathy(NSTDR)

The males constituted 81.25% among Non sight threatening daibetic retinopathy and 73% of those with sight threatening diabetic retinopathy.

Insulin requirement among patients with Sight Threatening Diabetic Retinopathy(STDR) and Non Sight Threatening Diabetic Retinopathy (NSTDR)

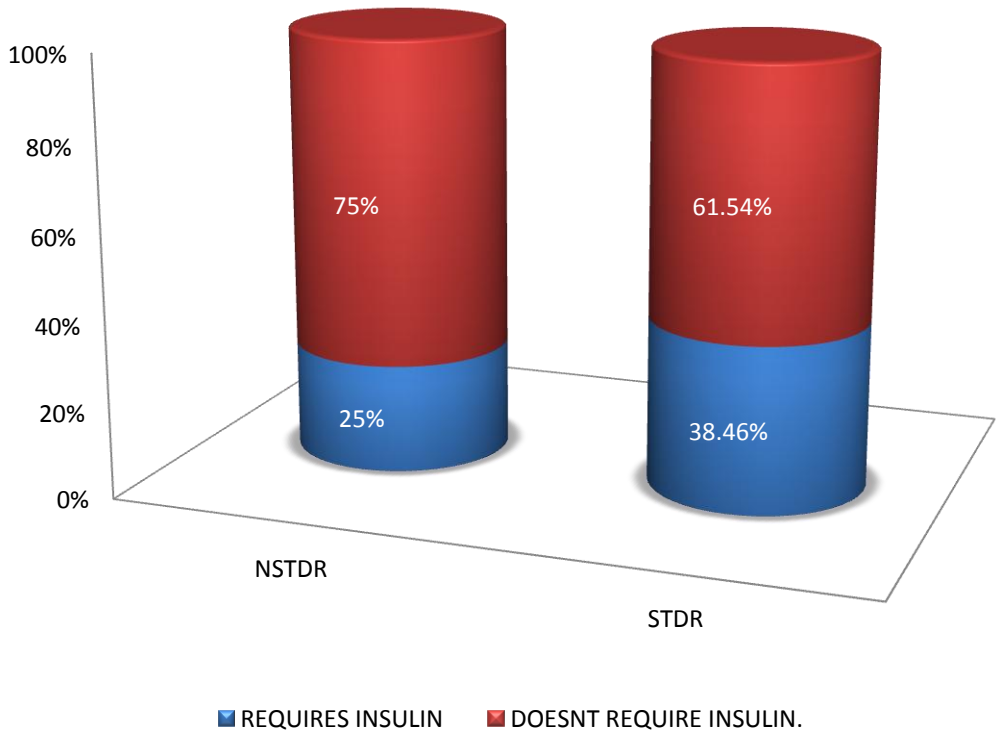


Figure:49: Insulin requirement among patients with Sight Threatening Diabetic Retinopathy(STDR) and Non Sight Threatening Diabetic Retinopathy(NSTDR).

The above graph depicts that insulin requirement among the Sight Threatening Diabetic Retinopathy(STDR) is 61.54% which is less than insulin requirement among those with Non Sight Threatening Diabetic Retinopathy (NSTDR)(75%).

Co existing co-morbidities among patients with Sight Threatening Diabetic Retinopathy(STDR) and Non Sight Threatening Diabetic Retinopathy (NSTDR)

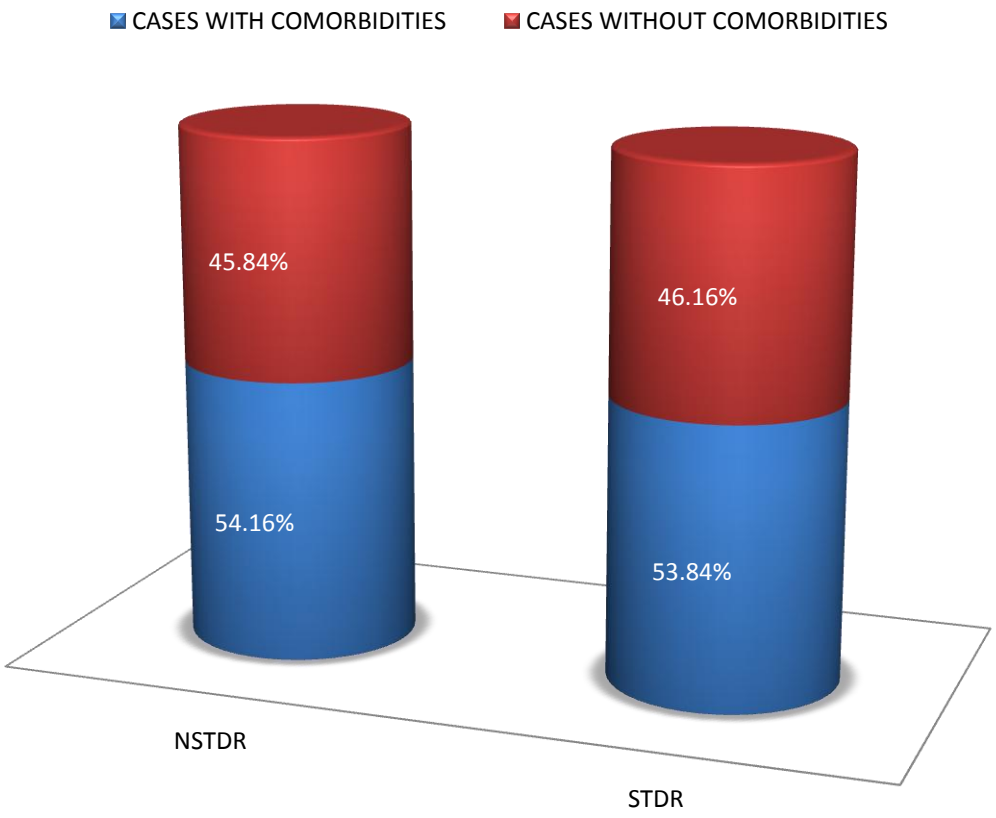


Figure 50:Co-morbidities among those with Sight Threatening Diabetic Retinopathy(STDR) and Non Sight Threatening Diabetic Retinopathy (NSTDR)

46.16% of those people with Sight Threatening Diabetic Retinopathy(STDR) and 45.84% of those with Non Sight Threatening Diabetic Retinopathy (NSTDR) had co-morbidities.

Distribution ICAM-1 K469E Polymorphism among the study population.

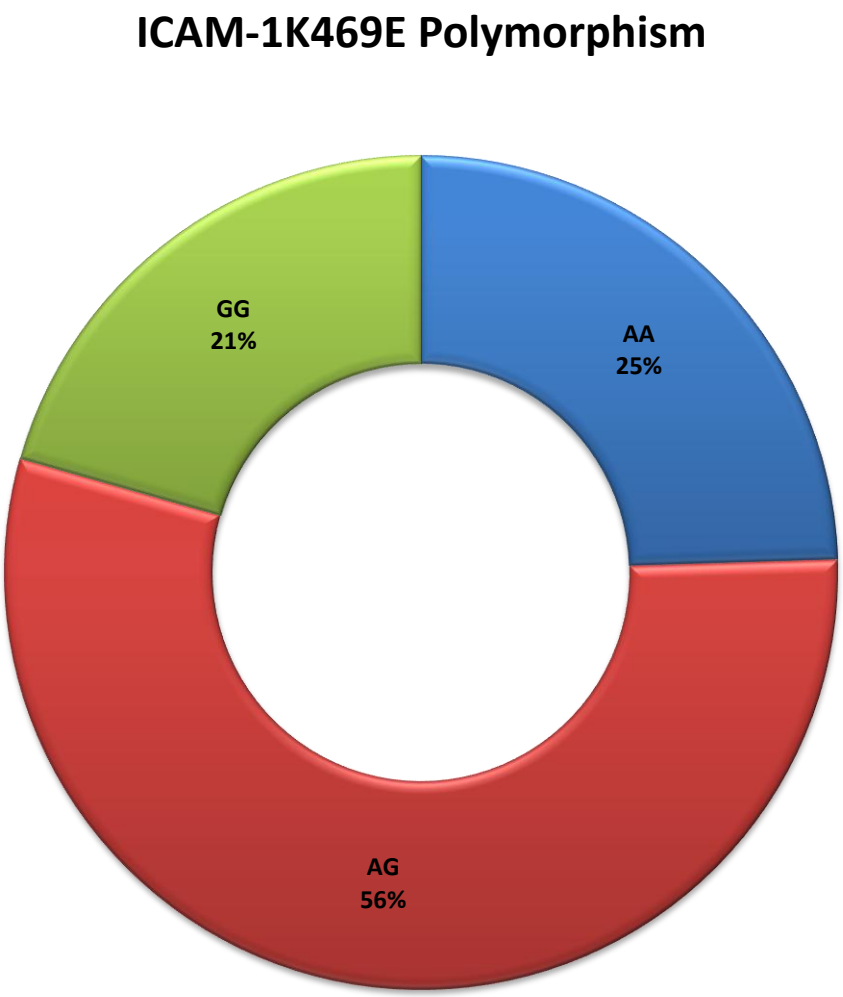


Figure 51:Distribution of ICAM-1 K469 E polymorphism among study population.

The heterozygous AG genotype was more common among the study population with a prevalence of 56% followed by AA genotype with a prevalence of 25% and then GG with a prevalence of 21%

Distribution of ICAM-1 K469E Polymorphism among patients with Sight Threatening Diabetic Retinopathy(STDR) and Non Sight Threatening Diabetic Retinopathy (NSTDR).

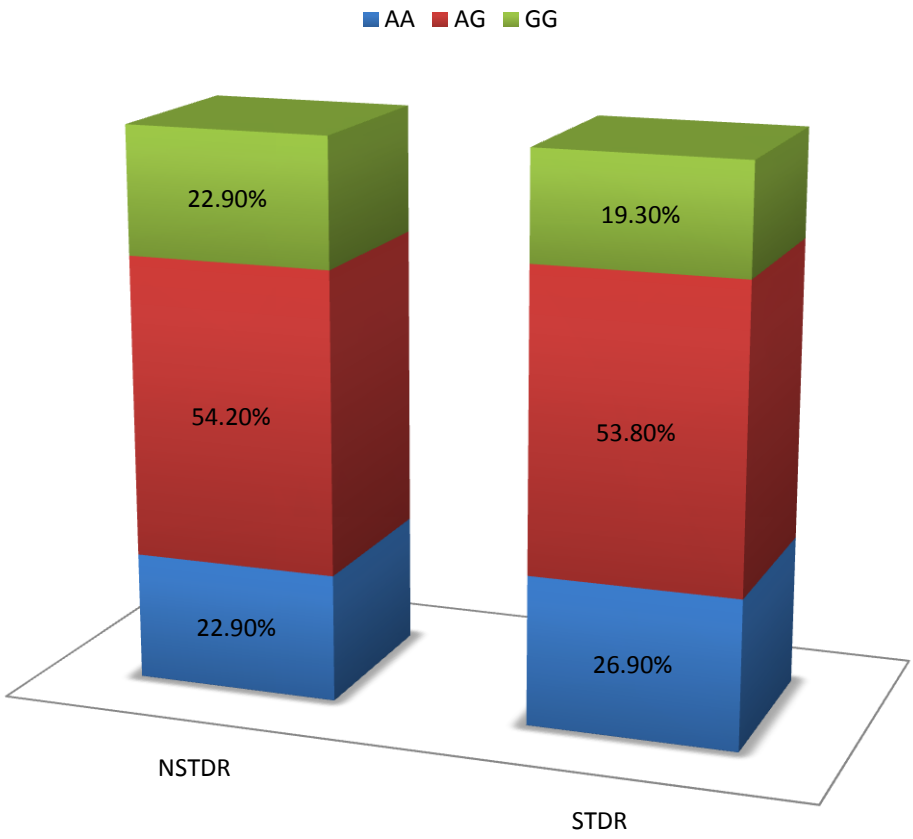


Figure:52: ICAM-1 K469E Polymorphism among patients with Sight Threatening Diabetic Retinopathy (STDR) and Non Sight Threatening Diabetic Retinopathy (NSTDR).patients with Sight Threatening Diabetic Retinopathy (STDR) and Non Sight Threatening Diabetic Retinopathy (NSTDR

Table 35: FEATURES OF THE PATIENTS IN THE STUDY:

Characteristics	NSTDR	STDR	P value
Age(years)	59.02 + 7.8	59.35+6	0.815
Males(n,%)	39(81.25)	38(73.07)	0.334
Duration(years)	10.79+8.3	11.90+5.4	0.434
Co-morbidities(n,%)	26(54.16)	28(53.84)	0.975
Eye manifestations(n,%)[$]	20(60.42)	42(80.76)	**0.026***
Insulin requirement(n,%)	12(25)	20(38.46)	0.151
FBS (mg/dl)	160.2+57.14	175.56+71.073	0.236
HbA1c(%)	8.14+2.15	8.26+1.94	0.151

[$]Presence of Ocular manifestation of diabetes other than retinopathy with M+ SD .* $p<0.05$ which is statistically significant.

FBS- Fasting blood sugar; **HbA1c**-Glycosylated Haemoglobin; **ICAM-1**-Intercellular Adhesion Molecule, **STDR**-Sight Threatening Diabetic Retinopathy ; **NSTDR**-Non Sight Threatening Diabetic Retinopathy.

Table 36:Distribution of ICAM-1 K 469E polymorphism among non sight threatening and sight threatening retinopathy

Genotypes	NSTDR	STDR	P value
AA (n,%)	11(22.9)	14(26.9)	0.640
AG(n,%)	26(54.2)	28(53.8)	0.974
GG(n,%)	11(22.9)	10(19.3)	0.651
A Allele(n,%)	48(50)	56(53.9)	
G allele(n,%)	48(50)	48(46.1)	0.5713

STDR-Sight Threatening Diabetic Retinopathy ; **NSTDR**-Non Sight Threatening Diabetic Retinopathy. **ICAM-1**-Intercellular Adhesion Molecule

Table 37:Logistic regression analysis of NSTDR and STDR groups among the ICAM -1 K469E Polymorphism genotypes and various clinical variables keeping severity of diabetic retinopathy as the dependant factor:

Clinical Variables	AA		AG		GG	
	OR(95% CI)	p	OR(95% CI)	P	OR(95% CI)	P
Age	1.031(0.93 to 1.178)	0.651	1.001 (0.926 to 1.081)	0.981	1.006 (0.898 to 1.126)	0.921
Sex	1.227(0.167 to 9.017)	0.840	0.540 (0.163 to 1.785)	0.313	0.900 (0.49 to 16.594)	0.944
Diabetes Duration	1.016(0.869 to 1.188)	0.839	1.030 (0.955 to 1.111)	0.444	1.010 (0.904 to 1.128)	0.866
Co- morbidities	0.625 (0.127 to 3.066)	0.562	1.035 (0.346 to 3.095)	0.951	1.944(0.322 to 11.756)	0.469
Eye manifestations[$]	0.333(0.063 to 1.752)	0.194	0.194 (0.052 to 0.720)	**0.014***	2.500(0.191 to 32.802)	0.485
Insulin requirement	0.133(0.013 to 1.346)	0.088	0.317 (0.093 to 1.085)	0.67	4.8(0.682 to 33.798)	0.115
FBS	0.999 (0.989 to 1.008	0.804	1.003 (0.993 to 1.013)	0.537	1.016(1.000 to 1.034)	0.56
HbA1c	0.894(0.607 to 1.317)	0.570	0.933 (0.721 to 1.207)	0.599	1.960(1.076 to 3.570)	**0.028***

[$] Presence of Ocular manifestation of diabetes other than retinopathy.* $p<0.05$ which is statistically significant.

FBS- Fasting blood sugar; **HbA1c**-Glycosylated Haemoglobin; **ICAM-1**-Intercellular Adhesion Molecule

Table 38:Logistic Regression analysis of the STDR group with sequential addition of clinical covariates and ICAM - 1 K469E Polymorphism genotypes as dependent variable.

Clinical Variables	AA Vs AG		AA Vs GG		GG Vs AG	
	OR(95% CI)	**p**	**OR(95% CI)**	**p**	**OR(95% CI)**	**P**
Unadjusted	1.182 (0.456 to 3.066)	0.731	1.400(0.437 to 4.488)	0.571	0.844(0.308 to 2.316)	0.742
Age	1.192 (0.458 to 3.102)	0.718	1.422(0.441 to 4.582)	0.555	0.844(0.308 to 2.316)	0.742
Age+ Gender	1.251 (0.476 to 3.289)	0.649	1.399(0.429 to 4.563	0.578	0.940 (0.334 to 2.650)	0.907
Age + Gender +DD	1.228 (0.465 to 3.242)	0.678	1.364 (0.403 to 4.613)	0.618	0.982 (0.344 to 2.8)	0.973
Age + Gender +DD + Insulin	1.272 (0.468 to 3.460)	0.637	1.375(0.405 to 4.670)	0.610	1.004(0.347 to 2.902)	0.994
Age + Gender +DD+ Insulin + FBS	1.265 (0.464 to 3.447)	0.646	1.431(0.413 to 4.959)	0.572	0.902(0.301 to 2.698)	0.853

Age + Gender +DD + Insulin +FBS+ HbA1c	1.248 (0.454 to 3.430)	0.668	1.437(0.414 to 4.989)	0.568	0.902(0.302 to 2.701)	0.854
Age + Gender +DD+ Insulin +FBS+ HbA1c+ co- morbidities	1.246 (0.453 to 3.428)	0.670	1.413(0.397 to 5.027)	0.594	0.885(0.285 to 2.748)	0.833
Age + Gender +DD + Insulin +FBS+ HbA1c+ co- morbidities+ Eye manifestations	1.440(0.501 to 4.138)	0.499	1.579(0.420 to 5.930)	0.499	0.753(0.233 to 2.436)	0.636

DD- Diabetes Duration**; FBS**- Fasting blood sugar; **HbA1c**-Glycosylated Haemoglobin; **ICAM-1-** Intercellular Adhesion Molecule.

Result analysis of the Tables 35-38:

- **Table 35:**shows the baseline characteristic of the study population among Non sight threatening diabetic retinopathy and sight threatening diabetic retinopathy with their statistical significance. Only ocular manifestations of diabetes other than retinopathy had p value <0.05 and was significantly associated with sight threatening diabetic retinopathy.

- **Table 34:** shows the frequency distribution of genotypes and alleles of ICAM-1 polymorphism among Non sight threatening diabetic retinopathy and sight threatening diabetic retinopathy with their statistical significance. There was no statistically significant association between them .

- **Table 35:** Logistic regression analysis of NSTDR and STDR groups among the ICAM -1 K469E Polymorphism genotypes and various clinical variables keeping severity of diabetic retinopathy as the dependant factor. However in the table only the odds ratio and p value of sight threatening retinopathy is shown.AG genotype of ICAM-1K469E polymorphism among the STDR group is significantly associated with other ocular manifestation with a p value of 0.014. Elevated HbA1c among AG genotype of ICAM-1K469E polymorphism is signifacntly associted with sight threatening retinopathy with a p value of 0.028.

- **Table 38:** Logistic Regression analysis of the STDR group with sequential addition of clinical covariates and ICAM -1 K469E Polymorphism genotypes as dependent variable. Here AA genotype is considered normal and taken as the reference group. However in GG Vs AG,GG is considered the reference group. Unadjusted analysis id done between the sight threatening diabetic retinopathy as independent variable and genotype as the dependent variable .Then other clinical covariates are sequentially added.

Discussion:

Our study population consisted of 100 patients with diabetic retinopathy who were classified into four groups of 25 each depending upon the severity of diabetic retinopathy. The diabetic retinopathy severity is determined using the ETDRS Classification of 30 degree 7 field fundus photograph. In our study, only 9% had Clinically Significant Macular Edema (CSME) as determined by ETDRS. Among the 25 patients with proliferative diabetic retinopathy, only 4 patients had high risk characters like vitreous haemorrhage, Retinal detachment, post PRP un stable PDR.

In this study, Sight Threatening Diabetic Retinopathy is defined as presence of proliferative diabetic retinopathy or severe non proliferative diabetic retinopathy or presence of clinically significant macular edema. Non sight threatening retinopathy is defined as the presence of either mild or moderate non proliferative diabetic retinopathy[108]. The overall prevalence of sight threatening diabetic retinopathy among the study population is 52% and the prevalence of Non Sight diabetic Threatening Retinopathy among the study population is 48%.

The mean age of the study population with Sight Threatening Diabetic Retinopathy is 59.35+6 years while that of those with Non Sight Threatening Diabetic Retinopathy is 59.02 + 7.8 years. Thus the mean age of both the groups are the same thereby eliminating the confounding effect of age on the severity of diabetic retinopathy.

Majority of the study population were males(77%) constituting 81.25% of those with Non Sight Diabetic Threatening and 73.07% of those with Sight Threatening Diabetic Retinopathy. However this slightly high prevalence of Non Sight Threatening Diabetic Retinopathy among male gender was not statistically significant. This observation in our study is supported by Wisconsin Epidemiologic Diabetic Retinopathy(WESDR) where it is proved that there is no association between the sex and the incidence and progression of Type 2 Diabetic Retinopathy[67]. However in studies done in South India proved that men are at greater risk than women in developing diabetic retinopathy[108].

The mean duration of diabetes among the patients with Sight Threatening Diabetic Retinopathy is 11.90+5.4 years which is only marginally more than the mean duration of diabetic among patients with Non Sight Threatening Diabetic Retinopathy which is 10.79+8.3 years. This means that the severity of diabetic retinopathy is not affected by the duration of diabetes among the study population. However studies in South India[108] show that the severity of diabetic retinopathy increases as the duration of diabetes increases which is also supported by international studies like WESDR [67] . The role of duration of Type 2 diabetes in determining the severity of diabetic retinopathy is lacking in this study population mainly due to the fact that most patients are uncertain about their onset of diabetes and also few have their Type 2 diabetes diagnosed only after they develop complications due to diabetes like nephropathy, retinopathy etc.

Presence of Co-morbidities like hypertension, Cerebral Vascular Accidents, Peripheral Vascular Diseases, Nephropathy, Ischemic heart disease, anaemia etc worsen the severity of diabetic retinopathy[67]. In our study, the contributions from the coexisting co-morbidities to worsen the diabetic retinopathy is missing with only 54.16% of patients with Non Sight Threatening Diabetic Retinopathy and 53.84% of patients with Sight Threatening Diabetic Retinopathy having co-morbidities.

Ocular manifestations due to diabetes other than retinopathy among our study population is significantly greater among those with Sight Threatening Diabetic Retinopathy compared to those with Non Sight Threatening Diabetic Retinopathy. This observation can be substantiated by the fact factors like uncontrolled blood sugar, prolonged diabetic duration ,genetic variations predisposing patient to severe Sight threatening diabetic retinopathy may also predispose them to other microvascular complications resulting in extraocular motility disturbances, Open angle glaucoma and also metabolic disturbances that leads to lens changes.

Percentage of patients requiring insulin among the sight threatening diabetic retinopathy group was 38.46% as compared to only 25% requiring insulin among the Non sight threatening diabetic retinopathy group but however the difference in the insulin requirement between the two groups was not statistically significant. This was similar to the WESDR study where no association was found between the requirement of exogenous insulin and the severity of diabetic retinopathy[67]. But in a recent study

done in rural South India, it has been found out that use of insulin is linked up with increased diabetic retinopathy risk[108].

Though the mean Fasting Blood Sugar (FBS) levels are slightly higher among the patients with Sight Threatening Diabetic retinopathy compared to those with Non Sight Threatening Diabetic Retinopathy, this difference was not clinically significant. This is similar to a study done in South India which no relationship between the levels of fasting blood glucose and incidence and severity of diabetic retinopathy[109]. However according to studies like The Diabetic Control and Complications Trial (DCCT)[68] and the United Kingdom Prospective Diabetes Study Group(UKPDS)[69] , the progression of diabetic retinopathy is retarded by tight glycemic control.

The mean glycosylated haemoglobin levels among patients with Non Sight Threatening Diabetic Retinopathy and Sight Threatening Diabetic retinopathy are similar .In our study the progression of diabetic retinopathy to sight threatening type is not influenced by glycosylated haemoglobin. This is in contrast to the results of WESDR study where a significant increase in the proliferative type of diabetic retinopathy was associated with elevated glycosylated haemoglobin levels[70].

Three different genotypes as a result of ICAM-1 K469E polymorphism noted in our study population are AA,AG,GG. Of these AA is the wild type one which is present in majority of the population. The presence of G allele in place of one A or both A alleles result in heterozygous or homozygous gene mutant.GG is the recessive

genotype present only in small proportion of the population and responsible for certain diseases.

The frequency distribution of AA,AG and GG in our study population is 25%,56%,21% respectively .The genotype distribution in our study is quite similar to the genotype composition among the patients with diabetic retinopathy in a study conducted by Sankara Nethralaya where the frequencies of AA,AG,GG were 30.2%,46.2% and 23.6% respectively[97].

Among the patients with Non Sight Threatening Diabetic Retinopathy,22.9% had AA genotype,56.2% AG genotype and 22.9% had GG genotype. The frequencies of three different genotypes among the patients with Sight Threatening Diabetic Retinopathy are 26.9%- AA,53.8%-AG,19.3% -GG. Hence in both the groups the frequency of AG genotype is higher than the other two genotypes. However there is no statistically significant difference in the distribution of genotypes among the two groups.

The frequency of A allele in our study population was 52 % and that of G allele was 48% which similar to the allele frequency among the study population with diabetic retinopathy in two studies done in South India done independently by Sankara Nethralaya [97]and Aravind Eye Hospital[100].

Among the Sight Threatening Diabetic Retinopathy group the frequency of A allele was 53.9% and that of G allele was 46.1 %.The frequency distribution of A allele was 50% and that of G allele was 50% among the Non Sight Threatening Group. No significant difference in the allele frequency was found between the two groups.

Multivariate analysis between the patients with Sight Threatening Diabetic Retinopathy and Non Sight Threatening Diabetic retinopathy among the three genotypic variants of the ICAM-1 K469E polymorphism and various clinical covariates revealed that factors like age ,sex, duration of diabetes ,co-existing co-morbidities ,insulin requirement and fasting blood sugar did not influence the presence of Sight Threatening Diabetic retinopathy among the three genotypes. However according to a population based study done in Sankara Nethralaya[97] , the age of the patient among the patients with GG and AG genotypes and insulin requirement among those with any of the genotype influence the development of diabetic retinopathy. Though age of the patient and insulin requirement among diabetic patients along with genotypic variants of ICAM -1 Polymorphism influence the diabetic retinopathy development ,they have no effect on the development of sight threatening diabetic retinopathy. However due to small sample size in the present study, further evidence is required to support this observation.

The logistic regression analysis also revealed a negative association between the presence of ocular manifestations of diabetes other than retinopathy and AG genotype among patients with Sight Threatening Diabetic Retinopathy. Since various

other systemic and local factors other than that due to diabetes might be involved in the development of these ocular manifestations, this observation in the present study has to be confirmed by further studies.

The analysis reveals that a unit rise in the glycosylated haemoglobin increases the risk of developing sight threatening diabetic retinopathy by 1.96 times among patients with GG genotype of ICAM-1K469E Polymorphism. The south Indian study done by Sankar Nethralaya also proves that elevation of glycosylated haemoglobin increases the risk of a diabetic patient with any genotypic variant of ICAM-1 K469E polymorphism developing diabetic retinopathy by 1.32 to 1.48 folds[97]. However in their study they did not find the association between the severity of diabetic retinopathy among the genotypic variants of ICAM-1 K469E polymorphism and the glycosylated haemoglobin levels. The above observation indicates the fact that though elevated glycosylated haemoglobin levels among diabetic patients with any of the genotypic variants of ICAM-1 K469E polymorphism predisposes them to develop diabetic retinopathy, the presence of GG genotype among diabetic patients with elevated glycosylated haemoglobin level increase the susceptibility of their developing sight threatening diabetic retinopathy.

Logistic Regression analysis performed with genotypic variants of ICAM-1K469E polymorphism as the dependent variable with sequential addition of various clinical variables.Significant p value as not observed after adjusting for all the clinical covariates for any of the genotypic variants of ICAM-1 K469E Polymorphism.

However odds ratio >1 though statistically not significant, is associated with GG phenotype compared to AA or AG phenotype which might indicate that G allele is the risk allele for developing Sight Threatening Diabetic Retinopathy which requires further study with large sample size to support this observation.

Hence in this study no association was observed between any of the genotypes of ICAM-1K469E polymorphism and the development of sight threatening diabetic retinopathy after adjusting for various clinical confounding factors. However the unadjusted risk of developing sight threatening diabetic retinopathy increased by 1.96 times among diabetic patients with GG genotype of ICAM-1K469E polymorphism with elevated glycosylated haemoglobin levels in this study population.

Similar results were obtained in study done in Aravind Eye Hospital, where no association was found between ICAM-1 K469E Polymorphism and proliferative diabetic retinopathy[100] .Hence it is evident that though ICAM K469E polymorphism predisposes patient to develop diabetic retinopathy, it has no role in the development of sight threatening diabetic retinopathy.

Conclusion:

Thus we conclude that

- Age, sex, duration of diabetes, insulin requirement, Co-existing co-morbidities, Fasting blood sugar levels and glycosylated haemoglobin levels were not associated with severity of diabetic retinopathy in our study.
- Ocular manifestations of diabetes other than retinopathy were significantly more among the patients with sight threatening diabetic retinopathy.
- Frequency of AA genotype of ICAM -1 K469E Polymorphism is 25%,that of AG genotype is 54% and that of GG genotype is 21% in our study population. The frequency of A allele in our study population was 52 % and that of G allele was 48%.
- There was no significant difference in the frequency of alleles and genotype of ICAM -1 K469E Polymorphism among sight threatening and non sight threatening retinopathy.
- The unadjusted risk of developing sight threatening diabetic retinopathy increased by 1.96 times among diabetic patients with GG genotype of ICAM-1K469E polymorphism with elevated glycosylated haemoglobin levels in this study population.

- No association was observed between any of the genotypes of ICAM-1K469E polymorphism and the development of sight threatening diabetic retinopathy after the confounding effect of various clinical factors were removed.

- **Though ICAM K469E polymorphism predisposes patient to develop diabetic retinopathy, it has no role in the development of sight threatening diabetic retinopathy.**

Need for the study:

The association of ICAM-1 K469E polymorphism with diabetic retinopathy has been proved in caucasian and chinese population. However two studies done in South India show conflicting results and these South Indian studies did not find the association of this polymorphism with the Sight threatening diabetic retinopathy. Hence, in this study we have found the association between the severity of diabetic retinopathy and ICAM-1 K469E polymorphism. If there is a significant association between the Sight Threatening Diabetic Retinopathy and any of the genotypic variant of ICAM-1K469E polymorphism ,patients can be checked for the specific genotype of this polymorphism at the time of diagnosis of type 2 diabetes and can be followed up frequently to prevent the sight threatening complications of diabetes.

Limitations of the study:

- ✓ It is hospital based study so the study population tends to be more heterogenous compared to a population based study.
- ✓ Small sample size involved in this study.
- ✓ Due to money constraint and non uniformity of the clinical data available, not all clinical confounding factors of diabetic retinopathy could be included in this study.

REFERENCE

1. V. Mohan, S. Sandeep, R. Deepa, B. Shah & C. Varghese.Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res 125, March 2007, pp 217-230.
2. Mohamed, Q., M.C. Gillies, and T.Y. Wong, Management of diabetic retinopathy: a systematic review. JAMA, 2007. 298(8): p. 902-16.
3. Zheng, Y., M. He, and N. Congdon, The worldwide epidemic of diabetic retinopathy. Indian J Ophthalmol, 2012. 60(5): p. 428-31.
4. Rani PK, Raman R, Sharma V, et al. Analysis of a comprehensive diabetic retinopathy screening model for rural and urban diabetics in developing countries. Br J Ophthalmol 2007;91:1425–9.
5. Rani PK, Raman R, Agarwal S, et al. Diabetic retinopathy screening model for rural population: awareness and screening methodology. Rural Remote Health 2005;5:350.
6. Simó-Servat O, Hernández C, Simó R. Genetics in diabetic retinopathy: current concepts and new insights. Curr Genomics. 2013 Aug;14(5):289-99.
7. Liou GI. Diabetic retinopathy: role of inflammation and potential therapies for anti-inflammation. World J Diabetes 2010;1:12–18.
8. Barouch FC, Miyamoto K, Allport JR, Fujita K, Bursell SE, Aiello LP *et al.* Integrin-mediated neutrophil adhesion and retinal leukostasis in diabetes. *Invest Ophthalmol Vis Sci* 2000; 41: 1153–1158.
9. Joussen AM, Poulaki V, Qin W, Kirchhof B, Mitsiades N, Wiegand SJ *et al.* Retinal vascular endothelial growth factor induces intercellular adhesion molecule-1 and endothelial nitric oxide synthase expression and initiates early diabetic retinal leukocyte adhesion *in vivo*. *Am JPathol* 2002; 160: 501–509
10. Kretowski A, Wawrusiewicz N, Mironczuk K. Intercellular adhesion molecule 1 gene polymorphisms in Graves' disease. *J Clin Endocrinol Metab* 2003; 88: 4945–4949.

11. Kim EH, Mok JW, Bang DS, Lee ES, Lee SN, Park KS. Intercellular adhesion molecule-1 polymorphisms in Korean patients with Behcet's disease. *J Korean Med Sci* 2003; 18: 415–418.

12. Papa A, Danese S, Urgesi R, Grillo A, Guglielmo S, Roberto I *et al.*Intercellular adhesion molecule 1 gene polymorphisms in inflammatory bowel disease. *Eur Rev Med Pharmacol Sci* 2004; 8: 187–191.

13. Auer J, Weber T, Berent R, Lassnig E, Lamm G, Eber B. Genetic polymorphisms in cytokine and adhesion molecule genes in coronary artery disease. *Am J Pharmacogenomics* 2003; 3: 317–328.

14. Nejentsev S, Guja C, McCormack R, Cooper J, Howson JM,Nutland S *et al.* Association of intercellular adhesion molecule-1 gene with type 1 diabetes. *Lancet* 2003; 362: 1723–1724.

15. Joussen AM, Murata T, Tsujikawa A, Kirchhof B, Bursell SE,Adamis AP. Leukocyte-mediated endothelial cell injury and death in the diabetic retina. *Am J Pathol* 2001; 158: 147–152.

16. Gundel RH, Letts LG. Adhesion molecules and the modulation of mucosal inflammation (chapter 3). In: Goldie R, ed.,Immunopharmacology of epithelial barriers. London: Elsevier, 1994;273p.

17. Pare G, Ridker PM, Rose L, et al. Genome-wide association analysis of soluble ICAM-1 concentration reveals novel associations at the NFKBIK, PNPLA3, RELA, and SH2B3 loci. PLoS Genet 2011;7:e1001374.

18. Wild S, Roglic G, Green A, Sicree R, King H.Global prevalence of diabetes: Estimates for the year 2000and projections for 2030. *Diabetes Care* 2004; *27* : 1047-53.3

19. American Diabetes Association. "Diagnosis and classification of diabetes mellitus." *Diabetes care* 33.Supplement 1 (2010): S62-S69

20. *IDF Diabetes Atlas, 4th edition.* International Diabetes Federation,2009.

21. Mohan V, Pradeepa R. Epidemiology of diabetes in different regions of India. *Health Administrator* 2009;22:1–18.

22. Ramachandran A, Snehalatha C, Kapur A, Vijay V, MohanV, Das AK, *et al*. Diabetes Epidemiology Study Group inIndia (DESI). High prevalence of diabetes and impairedglucose tolerance in India: National Urban Diabetes Survey.*Diabetologia* 2001; *44* : 1094-101.14

23. Mohan V, Deepa M, Deepa R, Shantirani CS, Farooq S,Ganesan A, *et al*. Secular trends in the prevalence of diabetes and glucose tolerance in urban South India - the Chennai Urban Rural Epidemiology Study (CURES-17). *Diabetologia* 2006; *49*: 1175-8.

24. Sicree R, Shaw J, Zimmet P.Diabetes and impaired glucose tolerance. In:Gan D, editor.Diabetes Atlas.International Diabetes Federation.5th ed. Belgium: International Diabetes Federation; 2011

25. Raman Kutty V, Joseph A, Soman CR. High prevalence oftype 2 diabetes in an urban settlement in Kerala, India.*Ethn Health* 1999;*4*: 231-9.

26. Menon VU, Kumar KV, Gilchrist A, Sugathan TN, SundaramKR, Nair V, *et al*. Prevalence of known and undetecteddiabetes and associated risk factors in central Kerala - ADEPS.*Diabetes Res Clin Pract* 2006; *74* : 289-94.

27. Lear SA, Humphries KH, Kohli S,Chockalingam A, Frohlich JJ, BirminghamCL. Visceral adipose tissue accumulation differs according to ethnic background: results of the Multicultural Community Health Assessment Trial (M-CHAT). AmJ Clin Nutr 2007;86:353–359

28. De Munter JS, Hu FB, Spiegelman D,FranzM, van DamRM.Whole grain, bran,and germ intake and risk of type 2 diabetes:a prospective cohort study and systematic review. PLoS Med 2007;4:e261.

29. Hu FB(2011) Globalization of Diabetes:The role of diet, lifestyle, and genes.Diabetes Care 34:1249–1257, 2011

30. Willi C, Bodenmann P, Ghali WA, Faris PD, Cornuz J. Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2007;298:2654–2664

31. Grassi G, Seravalle G, Calhoun DA, Bolla G, Mancia G. Cigarette smoking and the adrenergic nervous system. Clin Exp Hypertens A 1992;14:251–260.

32. Meikle AW, Liu XH, Taylor GN, Stringham JD. Nicotine and cotinine effects on 3 alpha hydroxysteroid dehydrogenase in canine prostate. Life Sci 1988;43:1845–1850.

33. Koppes LL, Dekker JM, Hendriks HF,Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005;28:719–725.

34. Gupta PC, Maulik PK, Pednekar MS, Saxena S. Concurrent alcohol and tobacco use among a middle-aged and elderly population in Mumbai. Natl Med J India 2005;18:88–91.

35. Schulze MB, Hoffmann K, Manson JE,et al. Dietary pattern, inflammation, and incidence of type 2 diabetes in women. Am J Clin Nutr 2005;82:675–684; quiz 714–715.

36. Neel JV. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"?1962. Bull World Health Organ 1999;77:694–703; discussion 692–693.

37. Southam L, Soranzo N, Montgomery SB,et al. Is the thrifty genotype hypothesis supported by evidence based on confirmed type 2 diabetes- and obesity susceptibility variants? Diabetologia 2009;52:1846–1851.

38. Speakman JR. Thrifty genes for obesity, an attractive but flawed idea, and an alternative perspective: the 'drifty gene' hypothesis. Int J Obes (Lond) 2008;32:1611–1617.

39. Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull 2001;60:5–20.

40. Deepa R, Sandeep S, Mohan V. Abdominal obesity, visceralfat and type 2 diabetes- "Asian Indian phenotype. In: MohanV , Rao GHR, editors. *Type 2 diabetes in South Asians:Epidemiology, risk factors and prevention*. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2006p. 138-52

41. Yajnik CS, Fall CH, Coyaji KJ, Hirve SS, Rao S, BarkerDJ,*et al*. Neonatal anthropometry: the thin-fat Indian baby.The Pune Maternal Nutrition Study. *Int J Obes Relat MetabDisord* 2003; *27*: 173-80.

42. V. Radha , V. Mohan.Genetic predisposition to type 2 diabetes among Asian Indians.Indian J Med Res 125, March 2007, pp 259-274.

43. American Diabetes Association. Standards of medical care in diabetes—2012. *Diabetes Care.* 2012;35(Supp1):*S12.*

44. S. E. Kahn. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia (2003) 46:3–19.

45. Kohei KAKU. Pathophysiology of Type 2 Diabetes and Its Treatment Policy.JMAJ 53(2010) 1 : 41–46.

46. Sonmez B,Bozkurt B,Atmaca A , et al .Effect of glycemic control on refractive changes in diabetic patients with hyperglycemia.Cornea 2005; 24:531-37.

47. Feitosa-Santana C, Paramei GV,Nishi Met al.Colour vision impairment in type 2 diabetes assessed by D-15 test and the Cambridge Colour Test .Ophthal Physio Opt 2010;30:717-23.

48. Cavallerano JD.A review of non-retinal ocular complications of diabetes mellitus. J Am Optom Assoc 1990: 61:533-43.

49. Trick GL,Trick LR,Kilo C.Visual field defects in patients with insulin-dependent and non-insulin dependent diabetes. Ophthalmology 1990; 97:475-82.

50. Rush JA: Extraocular muscle palsies indiabetes mellitus. *Int Ophthalmol Clin* 24:155–159, 1984

51. Patel SV, et al: Diabetes and hypertension in isolated sixth nerve palsy: a population- based study. *Ophthalmology* 112:760–763, 2005.

52. Skarbez K, Priestley Y,Huepf M, Koevary SB. Comprehensive review of diabetes on ocular health. Expert Rev Ophthalmol 2010; 5:557-77.

53. Tabatabay CA, et al: Reduced number of hemidesmosomes in the corneal epithelium of diabetics with proliferative vitreoretinopathy.*Graefes Arch Clin Exp Ophthalmol* 226:389–392, 1988.

54. Bernth-Petersen P, Bach E: Epidemiologic aspects of cataract surgery. III. Frequencies of diabetes and glaucoma in a cataract population. *Acta Ophthalmol(Copenh)* 61:406–416, 1983.

55. Wilson MR, et al: A case-control study of risk factors in open angle glaucoma. *Arch Ophthalmol* 105:1066–1071, 1987

56. Schertzer RM, Wang D, Bartholomew LR. Diabetes mellitus and glaucoma. Int Ophthalmol Clin. 1998 Spring;38(2):69-87.

57. Jeganat SE, Wang JJ, Wong TY. Ocular Associations of Diabetes Other Than Diabetic Retinopathy DIABETES CARE 2008;31:1905-1012.

58. Machan CM, Hrynchak PK, Irving EL. Age-related cataract is associated with type 2 diabetes and statin use. Optom Vis Sci. 2012 Aug;89(8):1165-71.

59. Appen RE, Chandra SR, Klein R, Myers FL.Diabetic papillopathy. Am J Ophthalmol.1980 Aug;90(2):203-9.

60. Bandello F, Menchini F: Diabetic papillopathyas a risk factor for progression of diabetic retinopathy. *Retina* 2004;4:183–184.

61. Hayreh SS, Zahoruk RM: Anterior ischemic optic neuropathy. In juvenile diabetics. *Ophthalmologica* 1981(4);198:13–28.

62. Lee MS, Grossman D, Arnold AC, Sloan FA.Incidence of nonarteritic anterior ischemic optic neuropathy: increased risk among diabetic patients. Ophthalmology. 2011 May;118(5):959-63.

63. Yau, JW; Rogers, SL; Kawasaki, R; Lamoureux, EL; Kowalski, JW; Bek, T; Chen, SJ et al. Global Prevalence and Major Risk Factors of Diabetic Retinopathy Diabetes Care. Mar 2012; 35(3): 556–564.

64**.** Rema M, Premkumar S, Anitha B, Deepa R, Pradeepa R, Mohan V.Prevalence of diabetic retinopathy in urban India: The Chennai Urban Rural Epidemiology Study (CURES) Eye Study, I. Invest Ophthalmol Vis Sci 2005; 46 : 2328-33.

65. Rajiv R, Rani PK, Sudhir R, et al. Prevalence of diabetic retinopathy in India Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study report 2. Ophthalmology 2009;116:311–18.

66. Raman R,Ganesan S, Pal SS, et al.Prevalence and risk factors for diabetic retinopathy in rural India. Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetic Study III (SN-DREAMS III), report no 2. BMJ Open Diabetes Research and Care 2014;2:000005. doi:10.1136/ bmjdrc-2013-000005.

67. Klein R, Klein BEK, Moss SE ,et al: The Winconsin Epidemiologic study of diabetic retinopathy,III: Prevalence and risk of diabetic retinopathy when the age of diagnosis is 30 or more years. Arch Ophthalmol 1984;102:527-532

68. The Diabetic Control and Complication Trail Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin dependent diabetes mellitus. N.Eng J Med 1993;329:977-86.

69. UK Prospective Diabetic Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureasor insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes(UKPDS 33).Lancet 1998;352:837-53.

70. Klein R, Klein BEK,Moss SE,et al. Relationship of hyperglycemia to the long term incidence and progression of diabetic retinopathy. Arch Intern Med 1994;154:2169-78.

71. KleinR,Klein BE, Moss SE. The Winconsin Epidemiologic study of diabetic retinopathy, XVI. The relationship of C-peptide to the incidence and progression of diabetic retinopathy.Diabetes 1995;44:796-801.

72. Leibowitz HM, Krueger DE, Maunder LR, Milton RC, Kini MM, Kahn HA, Nickerson RJ, Pool J, Colton TL, Ganley JP, Loewenstein JI, Dawber TR. The Framingham Eye Study monograph: An ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and

visual acuity in a general population of 2631 adults, 1973-1975. Surv Ophthalmol. 1980;24(Suppl):335-61

73. Ballard D, Melton L, Dwyer M, et al. Risk factors for diabetic retinopathy: a population-based study in Rochester, Minnesota.Diabetes Care. 1986;9:334–342.

74. Klein R, Moss SE, Klein BE.Is gross proteinuria a risk factor for the incidence of proliferative diabetic retinopathy? Ophthalmology. 1993 Aug;100(8):1140-6.

75. Padmaja K Rani, Rajiv Raman, Aditi Gupta, Swakshyar S Pal,Vaitheeswaran Kulothungan, and Tarun Sharma.Albuminuria and Diabetic Retinopathy in Type 2 Diabetes Mellitus Sankara Nethralaya Diabetic Retinopathy Epidemiology And Molecular Genetic Study(SN-DREAMS, report 12) Diabetol Metab Syndr. 2011; 3: 9.

76. Davis MD,Fisher MR,Gangnon RE et al. Risk factors for high risk proliferative diabetic retinopathy and severe visual loss. Early Treatment Diabetic Retinopathy Study Report#18.Invest Ophthalmol Vis sci1998;39:233-52.

77. Qiao Q[1], Keinänen-Kiukaanniemi S, Läärä E.The relationship between hemoglobin levels and diabetic retinopathy. J Clin Epidemiol. 1997 Feb;50(2):153-8.

78. Cruickshanks KJ,Moses SE,Klein R,Klein BEK. Physical activity and proliferative retinopathy in people diagnosed with diabetes before age of 30 years.Diabetes Care 1991;14:119-26.

79. Leske CM,Wu S,Hennis A,the Barbados Study Group.Hyperglycemia,blood pressure,and the 9 years incidence of diabetic retinopathy. Ophthalmology 2005;112:799-805.

80. Mayer-Davis EJ,Bell RA,Reboussin BA,et al, Antioxidant nutrient intake and diabetic retinopathy.The San Luis Valley Diabetes Study.Ophthalmology 1998;105:2264-70

81. Moss SE,Klein R,Klein BEK. Association of cigarette smoking with diabetic retinopathy.Diabetes Care1991;14:119-26.

82. Moss SE,Klein R,Klein BEK.The association of alcohol consumption with the incidence and progression of diabetic retinopathy.Ophthalmology 1994;101:1962-68.

83. Dandona L,Dandona R,Naduvilath TJ,et al. Population based assessment of diabetic retinopathy in an urban population in Southern India.Br J Ophthalmol 1999;83:937-40.

84. Abhary, S.; Burdon, K.P.; Laurie, K.J.; Thorpe, S.; Landers, J.;Goold, L.; Lake, S.; Petrovsky, N.; Craig, J.E. Aldose reductase gene polymorphisms and diabetic retinopathy susceptibility. *Diabetes Care*, 2010, 33(8), 1834-6.

85. Rand LI,Krolewski AS,Aiello LM,et al.Multiple factors in the prediction of risk of proliferative diabetic retinopathy.N Eng J Med 1985;313:1433-8.

86. Simo-Servat O, Hernandez C, Simo R. Genetics in Diabetic Retinopathy: Current Concepts and New Insights. Curr Genomics. 2013;15:289–299.

87. Uthra, S.; Raman, R.; Mukesh, BN.; Rajkumar, SA.; Padmaja,K.R.; Paul, P.G.; Lakshmipathy, P.; Gnanamoorthy, P.; Sharma, T.;McCarty, C.A.; Kumaramanickavel, G. Association of VEGF gene polymorphisms with diabetic retinopathy in a south Indian cohort.*Ophthalmic. Genet.*, 2008, 29(1),11-5.

88. Niu, W.; Qi, Y.; Wu, Z.; Liu, Y.; Zhu, D.; Jin, W. A meta-analysis of receptor for advanced glycation end products gene: four wellevaluated polymorphisms with diabetes mellitus. *Mol. Cell. Endocrinol.*,2012, 358(1), 9-17.

89. Zhao, S.; Li, T.; Zheng, B.; Zheng, Z. Nitric oxide synthase 3 (NOS3) 4b/a, T-786C and G894T polymorphisms in association with diabetic retinopathy susceptibility: a meta-analysis. *Ophthalmic.Genet.*, 2012, 3(4), 200-7.

90. Lu,Y.; Ge, Y.; Hu, Q.; Shi, Y.; Xue, C.; Shi, Y.; Chen, S.; Huang.Z. Association between angiotensin-converting enzyme gene polymorphism and diabetic retinopathy in the Chinese population. *J. Renin Angiotensin Aldosterone Syst.*, 2012, 13(2), 289-95.

91. Stolpe, AV and PT Saag. (1996). Intercellular adhesion molecule - 1. *Journal of Molecular Medicine*. 74: 13-33.

92. Roebuck, KA and A Finnegan. (1999). Regulation of intercellular adhesion molecule - 1 (CD54) gene expression. *Journal of Leukocyte Biology*. 66: 876-88.

93. Niessen, HW, PA Krijnen, CA Visser, CJ Meiher, CE Hack. (2002). Intercellular Adhesion Molecule - 1 in the Heart. *Annals of the New York Academy of Science*. 973: 573-85.

94. Janeway, CA, P Travers, M Walport, MJ Shlomchik. *Immunobiology 5*. New York: Garland Publishing, 2001.

95. Schleimer, RP and BS Bochner. (1998). The role of adhesion molecules in allergic inflammation and their suitability as targets of antiallergenic therapy. *Clinical Experimental Allergy*. 28: 15-23

96. Nachman, Michael W. (2001). "Single nucleotide polymorphisms and recombination rate in humans". *Trends in genetics* **17** (9): 481–485.

97. Vinita K, Sripriya S, Prathiba K, et al. ICAM-1 K469E polymorphism is a genetic determinant for the clinical risk factors of T2D subjects with retinopathy in Indians: a population-based case–control study. BMJ Open 2012;0:e001036

98. Petrovic MG, Osredkar J, Saraga-Babić M, Petrovic D.K469E polymorphism of the intracellular adhesion molecule 1 gene is associated with proliferative diabetic retinopathy in Caucasians with type 2 diabetes.Clin Experiment Ophthalmol. 2008 Jul;36(5):468-72.

99. Liu L, Yu Q, Wang H, Zhang SX, Huang C, Chen X.Association of intercellular adhesion molecule 1 polymorphisms with retinopathy in Chinese patients with Type 2 diabetes.Diabet Med. 2006 Jun;23(6):643-8.

100. Balasubbu S, Sundaresan P, Rajendran A, et al. Association analysis of nine candidate gene polymorphisms in Indian patients with type 2 diabetic retinopathy. BMC Med Genet 2010;11:158.

101. Kamiuchi K, Hasegawa G, Obayashi H, et al. Intercellular adhesion molecule-1(ICAM-1) polymorphism is associated with diabetic retinopathy in type 2 diabetes mellitus.Diabetes Med 2002;19: 371–6.

102. Stephen J Ryan. Diabetic retinopathy.Retina. Vol2.5th edition.2013. Elsevier. 907 - 1000.

103. Chandra Mohan K..Retinal Vascular Disorders.1st edition .2005.Academa. 19-38.

104. Kanski J. Diabetic Retinopathy.Clinical Ophthalmology – A Sytematic Approach.7th Edition.2011: 534-550.

105. The ETDRS Research Group. Early photocoagulation for diabetic retinopathy. Report No.9. Ophthalmology 1991; 98:766-785.

106. Diabetic Retinopathy.Peferrred Practice Pttern.Americn Academy of Ophthalmology 2004.

107. Early Treatment Diabetic Retinopathy Study Research Group. Early Treatment Diabetic Retinopathy Study design and baseline patient characteristics. ETDRS report number 7. Ophthalmology 1991;98:742.

108. Raman R, Ganesan S, Pal SS, et al.Prevalence and risk factors for diabetic retinopathy in rural India. Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetic Study III (SN-DREAMS III), report no 2. BMJ Open Diabetes Research and Care2014;2:000005.

109. Jayalakshmi.V, Satya Narayana.K , Sravanthi Koora , Ivvala ,Anand Shaker. The Evaluation of Serum Fasting Blood Sugar and Lipid Profileincluding Apo A and Apo B in Diabetic Retinopathy Subjects.” Indian Journal of Basic & Applied Medical Research; March 2012: 2(1) P:94-102

The genotype phenotype correlation of ICAM1 K469E gene polymorphism and severity of retinopathy in patients with Type 2 Diabetes Mellitus

STUDY PROFORMA

1) AGE :
2) SEX :
3) DURATION OF DIABETES :
4) COMORBID CONDITIONS :
5) DURATION OF DIABETES :
6) LOCAL EXAMINATION OF EYE :

FINDINGS	RIGHT EYE	LEFT EYE
LIDS		
CONJUNCTIVA		
CORNEA		
ANTERIOR CHAMBER		
IRIS		
PUPIL		
LENS		
VISION		
TENSION		
OCULAR MOVEMENTS		
FUNDUS		

7) GRADING OF DIABETIC RETINOPATHY BY ETDRS CLASSIFICATION:
8) RESULT OF PCR RFLP FOR ICAM-1 K469E POLYMORPHISM:
9) FASTING BLOOD SUGAR:
10) HBAIC LEVELS:

MASTER CHART

S. NO	AGE	SEX	DIABETES DURATOION	CO-MORBIDITIES	EYE COMORBIDITIES	IOP	INSULIN EQUIREMENT	GRADES OF DIABETIC RETINOPATHY	FBS	HBA1c	ICAM GENE POLYMORPHISM
1	63	M	8	NIL	N	14.6	O	MILD NPDR	218	9.88	WILD HOMOZYGOUS (A)
2	63	F	20	NIL	N	17.3	O	MILD NPDR	244	6.72	WILD HOMOZYGOUS (A)
3	65	M	5	NIL	N	17.3	O	MILD NPDR	131	12.57	HETEROZYGOUS (G/A)
4	55	M	15	SEPSIS	N	17.3	O	MILD NPDR	166	10.1	WILD HOMOZYGOUS (A)
5	66	F	12	SHT	IMC	14.6	O	MILD NPDR	142	8.02	HETEROZYGOUS (G/A)
6	60	M	6	SHT,CKD,ULCER,	IMC	17.3	I	MILD NPDR	167	10.94	MUTANT (G)
7	50	M	5	NIL	ELC	17.3	I	MILD NPDR	120	6.2	MUTANT (G)
8	45	F	5	NIL	N	14.6	O	MILD NPDR	138	10.89	HETEROZYGOUS (G/A)
9	75	M	5	SHT/CKD	SI	17.3	O	MILD NPDR	148	7.04	HETEROZYGOUS (G/A)
10	57	F	6	NIL	N	12.2	O	MILD NPDR	139	6.77	HETEROZYGOUS (G/A)
11	54	M	5	NIL	N	17.3	O	MILD NPDR	145	6.95	HETEROZYGOUS (G/A)
12	68	M	25	HT	N	17.3	O	MILD NPDR	130	6.5	HETEROZYGOUS (G/A)
13	51	M	5	NIL	ELC	17.3	O	MILD NPDR +CSME	110	9.69	WILD HOMOZYGOUS (A)
14	64	M	35	NIL	HETRO.IRIS	14.6	O	MILD NPDR	118	6.14	WILD HOMOZYGOUS (A)
15	67	M	10	NIL	LE-PTOSIS	17.3	O	MILD NPDR	223	9.29	HETEROZYGOUS (G/A)
16	69	M	18	SHT	IMC	17.3	O	MILD NPDR	178	7.94	HETEROZYGOUS (G/A)
17	65	F	15	SHT	NAD	17.3	O	MILD NPDR	156	10.43	HETEROZYGOUS (G/A)
18	60	F	20	SHT	IMC	17.3	O	MILD NPDR	247	10.01	WILD HOMOZYGOUS (A)
19	66	M	25	CVA+SHT	IMC	17.3	I	MILD NPDR	91	5.33	HETEROZYGOUS (G/A)
20	63	M	8	SHT+IHD	IMC	14.6	O	MILD NPDR	136	6.68	HETEROZYGOUS (G/A)
21	65	M	5	NIL	PSPH	17.3	O	MILD NPDR	73	4.7	WILD HOMOZYGOUS (A)
22	61	M	5	NIL	IMC	17.3	I	MILD NPDR	158	7.34	HETEROZYGOUS (G/A)
23	46	M	5	NIL	IMC	17.3	I	MILD NPDR	101	5.63	HETEROZYGOUS (G/A)
24	59	M	5	NIL	IMC	17.3	O	MILD NPDR	273	8.14	WILD HOMOZYGOUS (A)
25	49	M	5	NIL	ELC	17.3	O	MILD NPDR	246	11.76	MUTANT (G)
26	54	M	10	NIL	N	12.2	O	M NPDR	91	5.33	HETEROZYGOUS (G/A)
27	49	M	5	CVA	N	14.6	O	M.NPDR	213	8.9	HETEROZYGOUS (G/A)
28	68	M	25	Pulmonary nodule	N	17.3	O	M.NPDR	137	6.71	HETEROZYGOUS (G/A)

S. NO	AGE	SEX	DIABETES DURATOION	CO-MORBIDITIES	EYE COMORBIDITIES	IOP	INSULIN EQUIREMENT	GRADES OF DIABETIC RETINOPATHY	FBS	HBA1c	ICAM GENE POLYMORPHISM
29	59	M	17	SHT,RF	PSPH	17.3	I+O	M.NPDR	120	6.2	HETEROZYGOUS (G/A)
30	57	M	8	SHT	IMC	12.2	I+O	M.NPDR	260	9.58	HETEROZYGOUS (G/A)
31	59	M	5	RF	NAD	17.3	I+O	M.NPDR	223	9.29	HETEROZYGOUS (G/A)
32	63	M	5	CAD	ELC	12.2	O	M.NPDR ,CSME	151	7.13	HETEROZYGOUS (G/A)
33	52	F	5	NIL	NAD	17.3	O	M.NPDR	122	6.26	HETEROZYGOUS (G/A)
34	55	M	6	CVA	IMC	17.3	I+O	M.NPDR	123	6.29	HETEROZYGOUS (G/A)
35	50	M	5	DLD CIRRHOSIS	NAD	17.3	O	M.NPDR	114	6.02	HETEROZYGOUS (G/A)
36	59	M	8	SHT	IMC	17.3	O	M.NPDR	91	7	HETEROZYGOUS (G/A)
37	66	M	25	NAD	NAD	17.3	I	M.NPDR	161	6.8	HETEROZYGOUS (G/A)
38	57	M	10	UPPER LID-PAPILLOM	ELC	17.3	O	M.NPDR	121	6.23	HETEROZYGOUS (G/A)
39	54	M	7	LVD,COPD,IHD	IMC	17.3	O	M.NPDR	117	5.81	HETEROZYGOUS (G/A)
40	61	M	5	NAD	NAD	17.3	O	M.NPDR	240	9.8	MUTANT (G)
41	48	M	6	NAD	IMC	17.3	I	M.NPDR	156	7.28	MUTANT (G)
42	56	f	7	Nad	IMC	17.3	O	M.NPDR	130	13.4	MUTANT (G)
43	72	M	38	SHT	NAD	17.3	O	M.NPDR,GR 2 HRT	120	7.82	MUTANT (G)
44	80	M	15	HEMATOMA SCALP	IMC	17.3	O	M.NPDR	104	9.8	MUTANT (G)
45	52	M	10	SHT,PN	PSPH,IMC	17.3	O	M.NPDR	347	13.01	MUTANT (G)
46	52	M	5	NAD	IMC	17.3	O	M.NPDR	217	10.17	MUTANT (G)
47	52	M	5	NAD	IMC	17.3	O	M.NPDR	175	7.85	MUTANT (G)
48	52	M	8	NIL	ELC	17.3	O	M.NPDR	158	8.32	WILD HOMOZYGOUS (A)
49	60	F	20	SHT,CM,CKD	IMC ,CDC	17.3	I	M.NPDR	150	7.1	WILD HOMOZYGOUS (A)
50	63	M	15	SHT,CEREBELLAR INF/	IMC	17.3	O	M.NPDR	246	9.98	WILD HOMOZYGOUS (A)
51	48	M	8	Nil	NAD	17.3	O	S NPDR	281	11.03	HETEROZYGOUS (G/A)
52	58	M	10	NIL	NAD	17.3	O	S NPDR +CSME	131	6.53	HETEROZYGOUS (G/A)
53	73	M	20	NIL	IMC	10.4	O	S.NPDR+CSME	170	7.7	HETEROZYGOUS (G/A)
54	58	F	20	NIL	PSPH	17.3	I	S.NPDR+CSME	150	9.1	HETEROZYGOUS (G/A)
55	53	F	8	SHT	IMC	17.3	I+O	S.NPDR+CSME	190	8.3	HETEROZYGOUS (G/A)
56	54	F	10	NIL	IMC	17.3	I+O	S.NPDR+CSME	258	10.34	HETEROZYGOUS (G/A)
57	62	M	15	NIL	IMC	17.3	O	S.NPDR	155	7.25	HETEROZYGOUS (G/A)

S. NO	AGE	SEX	DIABETES DURATOION	CO-MORBIDITIES	EYE COMORBIDITIES	IOP	INSULIN EQUIREMENT	GRADES OF DIABETIC RETINOPATHY	FBS	HBA1c	ICAM GENE POLYMORPHISM
58	60	M	22	SHT	ELC	17.3	O	S.NPDR	128	9.6	HETEROZYGOUS (G/A)
59	61	F	20	SHT	ELC	17.3	I+O	S.NPDR	112	5.96	HETEROZYGOUS (G/A)
60	62	M	18	SHT	NC	14.6	I	S.NPDR	118	6.14	HETEROZYGOUS (G/A)
61	61	M	17	POST CABG	IMC	14.6	O	S.NPDR+PRH+CSME	114	6.02	HETEROZYGOUS (G/A)
62	69	F	10	DM,HT,RF	ALT.EXOTROPIA	14.6	I	S.NPDR +CSME+GR.3 HRT	134	6.32	HETEROZYGOUS (G/A)
63	65	M	5	NAD	NAD	17.3	O	S.NPDR	144	9.3	HETEROZYGOUS (G/A) (G/A)
64	58	F	15	NAD	IMC	17.3	O	S.NPDR	105	8.72	MUTANT (G)
65	67	M	5	NSHT	ELC	17.3	O	S.NPDR	100	10.6	MUTANT (G)
66	62	M	18	NAD	IMC	17.3	I+O	S.NPDR	313	11.69	MUTANT (G)
67	60	M	14	NAD	IMC	17.3	I	S.NPDR	165	7.25	WILD HOMOZYGOUS (A)
68	75	M	5	NAD	IMC	17.3	O	V.S.NPDR+PDR	118	5.84	WILD HOMOZYGOUS (A)
69	57	M	6	SHT	NAD	17.3	O	S.NPDR	115	7.91	WILD HOMOZYGOUS (A)
70	57	M	17	NAD	IMC	17.3	O	S.NPDR	239	6.7	WILD HOMOZYGOUS (A)
71	50	F	10	NIL	IMC	17.3	O	S. NPDR.	233	9.59	WILD HOMOZYGOUS (A)
72	62	F	20	Renal failure	PSPH	17.3	O	S.NPDR	119	6.17	WILD HOMOZYGOUS (A)
73	65	F	15	NIL	IMC	17.3	I+O	S.NPDR	120	6.2	WILD HOMOZYGOUS (A)
74	49	M	8	SHT	IMC	17.3	I+O	S.NPDR	259	10.37	WILD HOMOZYGOUS (A)
75	63	F	15	SHT	PSPH	17.3	I	S.NPDR	168	7.64	WILD HOMOZYGOUS (A)
76	62	M	13	NIL	NAD	17.3	O	PDR	321	12.23	HETEROZYGOUS (G/A)
77	65	F	20	HEMIPLEGIA	IMC,RD	17.3	I+O	PDR	180	7.7	HETEROZYGOUS (G/A)
78	62	M	15	SHT	IMC	17.3	O	PDR	179	7.97	HETEROZYGOUS (G/A)
79	55	M	10	nil	IMC	17.3	O	PDR	290	11.3	HETEROZYGOUS (G/A)
80	50	M	6	SHT +CKD	PSPH	17.3	O+A	PDR	388	10.83	HETEROZYGOUS (G/A)
81	59	M	5	SHT	PSPH	14.6	I+O	HR PDR	149	7.07	HETEROZYGOUS (G/A)
82	65	F	10	NIL	RE-RAPD	17.3	O	PDR+VH	190	6.54	HETEROZYGOUS (G/A)
83	58	F	10	NAD	ELC	17.3	I	PDR+VH	139	6.77	HETEROZYGOUS (G/A)
84	65	M	11	NAD	PSPH,IMC	17.3	O	PDR	130	6.7	HETEROZYGOUS (G/A)
85	54	M	5	CORTICAL BLINDNEES	ELC	17.3	O	PDR	190	8.3	HETEROZYGOUS (G/A)
86	59	M	5	SHT X 5Y	RAPD WITH PCIOL	17.3	O	POST PRP STABLE PDR	144	6.92	HETEROZYGOUS (G/A)

S. NO	AGE	SEX	DIABETES DURATOION	CO-MORBIDITIES	EYE COMORBIDITIES	IOP	INSULIN EQUIREMENT	GRADES OF DIABETIC RETINOPATHY	FBS	HBA1c	ICAM GENE POLYMORPHISM
87	61	M	20	GR2PHARYNGEAL VA	IMC	17.3	O	PDR	248	10.04	HETEROZYGOUS (G/A)
88	50	M	9	SHT X 5 Y	IMC,N.PTERY	17.3	O	PDR	264	10.52	HETEROZYGOUS (G/A)
89	59	M	5	NIL	R-RAPD	17.3	O	PDR	141	11.78	HETEROZYGOUS (G/A)
90	53	M	10	NAD	NAD	17.3	O	PDR	117	9.1	HETEROZYGOUS (G/A)
91	58	M	10	NAD	IMC,PXF,AST.HY	17.3	O	PDR	97	5.51	MUTANT (G)
92	66	F	13	NAD	NAD	20.6	O	PDR	342	11.38	MUTANT (G)
93	65	M	17	SHT X 2Y,LUMBAR SP	NAD	17.3	O	PDR	180	10.86	MUTANT (G)
94	52	M	7	SHT-7Y,CKD,HD	PSL,PSPH	17.3	O+AVS INJ	PDR	83	5.09	MUTANT (G)
95	64	M	15	SHT,GERD	NAD	17.3	I+O	PDR	110	7.8	MUTANT (G)
96	62	M	15	SHT-7,GLUTEAL ABSC	NAD	17.3	O	PDR	168	9.2	MUTANT (G)
97	52	M	5	SHT-2	IMC	17.3	I	PDR	156	7.28	WILD HOMOZYGOUS (A)
98	54	M	5	SHT,CAD	IMC	17.3	I+O	PDR	121	6.23	WILD HOMOZYGOUS (A)
99	53	M	7	PVD	IMC	17.3	O	PDR	111	5.93	WILD HOMOZYGOUS (A)
100	51	M	5	Gangrene leg	ELC	17.3	O	PDR	156	7.28	WILD HOMOZYGOUS (A)

O	-	Oral hypo glycemics
I	-	Insulin
N	-	Normal
IMC	-	Immature Cataract
PXF	-	Pseudoexfoliation
SHT	-	systemic Hypertension
CAD	-	Coronary Artery Disease
PVD	-	Peripheral Vascular Disease
NPDR	-	Non Proliferative Diabetic Retinopathy
PDR	-	Proliferative Diabetic Retinopathy
RAPD	-	Relative Afferant Pupillary Defect
PCIOL	-	Posterior Capsular Intraocularlens