A STUDY ON RED BLOOD CELL COUNT, DISTRIBUTION WIDTH AND NEUTROPHIL/ LYMPHOCYTE RATIO AS MARKERS OF VASCULAR INFLAMMATION IN THE EARLY DETECTION OF NON PROLIFERATIVE DIABETIC RETINOPATHY

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **"A study on Red Blood Cell count, Distribution Width and Neutrophil/ Lymphocyte Ratio as Markers of Vascular Inflammation in the Early Detection of Non Proliferative Diabetic Retinopathy"** is the original bonafide work done by **Dr.P.Boopathi Rajan,** Post graduate student, Department of Internal Medicine, Stanley Medical College, Chennai under our direct supervision and guidance.

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DECLARATION

I, **Dr.P.Boopathi Rajan,** solemnly affirm and declare that the dissertation titled **"A study on Red Blood Cell count, Distribution Width and Neutrophil/ Lymphocyte Ratio as Markers of Vascular Inflammation in the Early Detection of Non Proliferative Diabetic Retinopathy"** is the bonafide work done by me at the Department of Internal Medicine, Stanley Medical College under the expert guidance and supervision of Professor **Dr.R.Jayanthi, M.D.,** Head of the Department, Department of Internal Medicine, Stanley Medical college. This dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., degree (Branch-I) in Internal Medicine.

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CERTIFICATE BY THE GUIDE

This is to certify that **Dr.P.Boopathi Rajan** Post Graduate Student (MAY 2012 TO APRIL 2015) in the Department of General Medicine STANLEY MEDICAL COLLEGE, Chennai- 600 001, has done this dissertation on "**A study on Red Blood Cell count, Distribution Width and Neutrophil/ Lymphocyte Ratio as Markers of Vascular Inflammation in the Early Detection of Non Proliferative Diabetic Retinopathy"** under my guidance and supervision in partial fulfillment of the regulations laid down by the Tamilnadu Dr. M.G.R. Medical University, Chennai, for M.D. (General Medicine), Degree Examination to be held in April 2015.

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ABBREVIATIONS

DM	-	Diabetes Mellitus
DR	-	Diabetic Retinopathy
NPDR	-	Non-Proliferative Diabetic Retinopathy
PDR	-	Proliferative Diabetic Retinopathy
VEGF	-	Vascular Endothelial Growth Factor
IRMA	:	Intra Retinal Micro Vascular Abnormalities
ROS	-	Reactive Oxygen Species
AGE	-	Advanced Glycation End Products
PK-C	-	Protein Kinase-C
RAAS	-	Renin Angiotensin Aldosterone System
RDW	-	Red Cell Distribution Width
NLR	-	Neutrophil Lymphocyte Ratio
ABG	-	Average Blood Glucose

INDEX

“A STUDY ON RED BLOOD CELL COUNT, DISTRIBUTION WIDTH AND NEUTROPHIL/ LYMPHOCYTE RATIO AS MARKERS OF VASCULAR INFLAMMATION IN THE EARLY DETECTION OF NON PROLIFERATIVE DIABETIC RETINOPATHY”

ABSTRACT

PRIMARY OBJECTIVE

To study the correlation between RDW, RBC Count and Neutrophil/ Lymphoctye Ratio in the early detection of Diabetic Retinopathy.

METHODOLOGY

About 100 Type-II Diabetic patients with / without visual symptoms attending Ophthalmology and diabetology OPDs after satisfying the Inclusion/ Exclusion Criteria will be included in the study. All patients will be subjected to symptom analysis, clinical examination and laboratory investigation. Final results will be analyzed by SPSS statistical software.

RESULTS

Our study was a Cross-sectional study conducted among 100 Type-II diabetic patients between 30-50 years of age.

Two group were assigned according to the severity of Diabetic Retinopathy. One is a mild grade and another is a moderate grade.

NLR, RDW and RBC count were analyzed in this study population. Also HBAIC and blood glucose levels were correlated with them.

Statistical analysis was done with SPSS Software. Results were studied using unpaired t-test, chi-square test and Pearson coefficient Correlation.

CONCLUSION

From our study we conclude that, both NLR and RDW are useful markers of vascular inflammation for

1) Early diagnosis of Non-proliferative diabetic retinopathy.

2) Assessing the severity of non proliferative Diabetic Retinopathy as mild or moderate grades.

3) Also their prediction of uncontrolled glycaemic status since they correlate well with HBA1C and blood glucose levels.

KEY WORDS

Red Blood Cell, Vascular Inflammation, Diabetic Retinopathy.

INTRODUCTION

Diabetes Mellitus is a group of metabolic disorders characterized by hyperglycemia which results from either defect in insulin secretion, insulin action or both.

According to International diabetes Federation, globally 381 million people have Diabetes. The disease affects 62 million Indians which is more than 7.1% of adult population and nearly one million people die due to diabetes ever year.

Blindness is 25 times more common in diabetics than non-diabetics. Diabetic retinopathy ranks the sixth common cause of blindness in India. Prevalence of Diabetic retinopathy in patients with diabetes was recently estimated to be 34.6%.

Ocular fundus examination every year by an ophthalmoscope is the most important clinical assessment in a diabetic patient to detect Diabetic Retinopathy. But accessability and awareness to undergo such an examination is lacking both in the diabetic population and among the medical practitioners.

Recently vascular inflammation is proposed as the basic pathogenic mechanism behind diabetic microvascular

complications. And hence detection of markers for vascular inflammation can help us to diagnose diabetic microangiopathy particularly diabetic retinopathy very early, so that active intervention in that stage would prevent a diabetic patient from becoming blind.

Many pathophysiological disorders have been involved in the development of diabetic retinopathy but the most common are Rheological disorders of Red Blood cells and decreased RBC deformability.

Hence in this study we would like to investigate the association of Red Cell distribution width, Red blood cell count and Neutrophil/ Lymphocyte ratio with mild to moderate non proliferative diabetic retinopathy.

REVIEW OF LITERATURE

INTRODUCTION

Diabetes Mellitus[1] has been defined as a "Group of Metabolic disorders characterized by hyperglycemia resulting from defects in Insulin Secretion, Insulin action or both". The most common type, Type-II Diabetes Mellitus results from a combination of genetic and acquired factors. Prevalence of Type-II Diabetes is increasing globally and has reached epidemic proportion in many countries especially in India.

EPIDEMIOLOGY

According to the International Diabetes Federation[3,4] the total number of adult Type-II Diabetes in the world was estimated as 366 million in 2011 which was projected to increase to 552 million by 2030[(1)]. Among the top 10 countries with the larger number of diabetic adults, five are in Asia. China tops the list with 90 million followed by India with 61.3 million diabetic population. The numbers are estimated to rise to 129.7 million and 101.2 million respectively by 2030.

The most important predictor of morbidity and mortality[2] in Type-II Diabetes is the control of blood sugar levels. And even

with adequate control of blood sugars, patients with Type-II Diabetes with more than 10-15 years duration of disease are more prone to develop both micro and macro vascular complications. Hence regular screening for complications apart from blood sugar control is the most important determinant for morbidity in diabetes mellitus. Among the micro and macro vascular complications, retinopathy is a major cause of morbidity in patients with diabetes. It is a major cause of blindness even in the most industrialized nations. The prevalence of diabetic retinopathy is nearly 50-80% in Type-II Diabetes after 20 years of duration of disease. And the incidence of blindness in Type-II Diabetes is 25 times higher when compared to the general population.

A causal association between glycemic control and the development and progression of microvascular complications in diabetes particularly retinopathy has been suggested from studies in both animals and humans. These associations were confirmed in the prospective Diabetic control and complications Trial (DCCT)[2].

Before going into the pathogenesis and pathophysiology of Diabetic Retinopathy, an overview of vascular complication in Diabetic Type-II is presented here.

Overview of vascular complications in Type-II Diabetes

Many factors play a pivotal role in the initiation and progression of vascular disease in Type-II diabetes. Out of these factors, two are very important.

1) Mitochondrial dysfunction and insulin resistance.

2) Beta-Cell failure.

MITOCHONDRIAL DYSFUNCTION AND INSULIN RESISTANCE

The main culprit for the development of metabolic syndrome is insulin resistance. This is always associated with hyperinsulinaemia[11].

Insulin resistance induces multiple metabolic alterations through various mechanisms.

Factors that contribute to insulin resistance are

1) Genetics

2) Obesity

3) Physical inactivity

4) Advancing age

Metabolic complications that occur commonly in patient with insulin resistance are

1) Atherogenic dyslipidemia

2) Hypertension

3) Glucose intolerance

4) Prothrombolic state

Defect in insulin action[12] is present in many tissues but mainly in

1) Liver

2) Adipose tissue

3) Skeletal muscle

The main defects underlying insulin resistance are

1) Impaired insulin signaling

2) Intracellular defects in glucose metabolism.

Intracellular defects in glucose metabolism are multiple and the major one among them leading to insulin resistance is the role of free fatty acids with underlying mitochondrial dysfunction.

FREE FATTY ACIDS AND MITOCHONDRIAL DYSFUNCTION IN TYPE-II DIABETES

The main source of fuel in skeletal muscles are free fatty acids. More than 96% of ATP[8] production is derived from free fatty acids mainly during exercise. In Type-II Diabetic individuals the oxidation of FFAS in skeletal muscle is impaired secondary to an abnormality in oxidation capacity of the mitochondria.

Insulin function is impaired slowly in insulin resistant individuals. Mainly the suppression of lipolysis is first affected leading to enhanced production and circulation of free fatty acids. The resultant free fatty acid influx into the skeletal muscle raises their intramuscular concentration.

Since the mitochondrial oxidative capacity is already deranged, there will be enhanced production of intramyocellular fatty acid metabolites like DAG, ceramides and other toxic lipid metabolites. These toxic metabolites produce serine kinase pathway activation. The production of serine kinase directly interfere with intracellular insulin signalling. These kinases also produce defects in the multiple intracellular steps involved in glucose metabolism like

1) Glucose transport

2) Glucose phosphorylation

3) Glycogen synthesis

4) Glucose oxidation.

CONTRIBUTION OF EXPERIMENTAL STUDIES[9]

Experimental studies in human skeletal muscle have documented the clear defect in mitochondrial oxidative phosphorylation and electron transport as the cause of insulin resistance by various molecular, biochemical and magnetic Resonance spectroscopic techniques. Impaired ATP synthesis is also documented by nuclear magnetic resonance guided measurement of oxidative phosphorylation. The NMR also shows a 30-40% reduction in the resting metabolic flux through the cycle and oxidative phosphorylation in the lean, normal glucose tolerant, insulin resistant offspring of subjects with Type-II Diabetes.

Subjects with Type-II diabetes also have

1) Reduced exercise tolerance

2) Impaired recovery of intracellular phosphocreatine concentration in skeletal muscle following exercise.

These results indicate clearly that the defect in mitochondrial oxidative phosphorylation contribute significantly to reduced exercise capacity in individuals with insulin resistance.

Normal aging process is also associated with insulin resistance through a reduction in the mitochondrial ATP synthesis rate and increase in the intracellular fat content. Finally all these steps results in a vicious cycle by further decreasing the oxidative phosphorylation flux in the skeletal muscle. So regardless of etiology insulin resistance is directly due to impaired mitochondrial oxidative phosphorylation.

RESULTS

The mitochondrial defects identified are

1) Reduction in the number of mitochondria with normal function of individual mitochondria.

2) Intrinsic defect in the quantitatively normal number of mitochondria.

3) Some combination of the above

These defects can be both inherited or acquired. But this question is unanswered for more than a decade. Normally if the

defect is acquired it can be reversed or prevented while the inherited defect is permanent.

MITOCHONDRIAL DEFECTS IN INSULIN RESISTANCE: CAUSE OR EFFECT

The mitochondrial defect in oxidative phosphorylation described with invivo MRS showed isolated mitochondrial defect could contribute to the increase in intramyocellular free fatty acid metabolite levels observed in obesity and Type-II diabetes and can contribute to insulin resistance. If the increase in intramyocellular fat content in insulin resistant individuals led to an increase in fat oxidation and production of reactive oxygen species and other toxic reactive metabolites, then the decrease in oxidative pathway for phosporylation leads to mitochondria function down regulation and amelioration of the production of toxic metabolites.

Over expression of PGC-1 alpha gene in the skeletal muscle of mice enhances mitochondrial activity, expression of proteins involved in mitochondrial fatty oxidation and also insulin stimulated glucose disposal and uptake in skeletal muscle.

Activation of sirtuin-1 with reseveratrol in mice led to an increased oxidative phosphorylation in mitochondria and also protected the mice from diet induced obesity and insulin resistance.

Down regulation of mitochondrial function in myotubules with the help of oligomycin or ethidium bromide increased the mitochondrial fat content and also led to impairement of insulin signaling and reduced glucose disposal. But the treatment of the muscle cells with Azide, an inhibitor of mitochondrial complex-II led to increased basal glucose uptake without affecting the insulin stimulated glucose uptake in myotubules.

Down regulation of electron transport chain activity in mice by knocking down initiating factors led to increased insulin resistance and protection from fat induced insulin resistance.

Hence to conclude from the above studies

1) Defective oxidative phosphorylation in mitochondria is the culprit for the development of impaired insulin action in various insulin resistant states including Type-II diabetes, obesity and normal aging process.

2) Excessive FFA supply result in increased intramyocellar fat content in insulin sensitive tissues which in a backdrop of impaired fatty acid oxidation led to the accumulation of toxic lipid metabolites leading to insulin resistance.

BETA CELL FAILURE IN TYPE-II DIABETES

Even though insulin resistance is the major contributor for disease progression in diabetes, it is the decline of beta cell function that determines the rate of the disease progression.

The established support for the above said statement can be obtained from the (UKPDS) United Kingdom prospective diabetes study.

RESULTS FROM UKPDS

A 50% reduction of beta cell function (assessed by HOMA-IR) was present in newly diagnosed patients enrolled in the study at the initial period. But after a 10 years follow up, not much change was observed in the insulin resistance irrespective of treatment. The level of beta cell function followed a linear decline.

RESULTS FROM BELFACT STUDY

According to the belfact diabetes study, subjects developing diabetes have a near 60% reduction of beta cell function at the initial stage itself. Thereafter beta cell failure follows 2 phases.

Phase-A

It precedes overt diabetes and it is further characterized by a slow but constant decline in beta cell function of around 2% per year.

Phase-B

It occurs after the development of overt hyperglycemia and is characterized by an accelerated decline in beta cell function of around 18% per year.

Hence initial alteration in beta cell function reflects intrinsic defects and the accelerated beta cell decline is the consequence of glucotoxicity and lipotoxicity. So a vicious cycle develops after the disease gets manifested.

MECHANISM UNDERLYING BETA CELL FAILURE

Reduction in beta cell mass is associated with increased apoptosis and increased expression of caspase 3 & 8. These caspases are mediators of apoptosis. The reduction in Beta cell function and mass is virtually apparent at the time of diagnosis of impaired glucose tolerance indicating that there is preexisting intrinsic beta cell defect.

Several genetic variants have been identified in the pathogenesis of beta cell decline. OF these 2 are important

1) Genetic variants of transcription factor _alpha

2) Single nucleotide polymorphisms

So a genetic predisposition is always associated with initial beta cell defect which when subjected to increased demand in a case of insulin resistance and obesity leads to overt beta cell failure, development of glucose intolerance and progressive worsening of glycemic control. The 2 main factors which underlie beta cell failure apart from genetic predisposition are

1) Glucotoxicity

2) Lipotoxicity

GLUCOTOXICITY

Persistently high-blood glucose concentration impairs insulin sensitivity and also beta cell function. This phenomenon is known as glucotoxicity. This is usually a reflection of oxidative stress secondary to generation of mitochondrial ROS. Excessive mitochondrial ROS is as a result of enhanced glucose metabolism.

Major markers of oxidative stress are

1) Nitrotyrosine

2) 8-hydroxy- 2- deoxyguanosine

There is an inverse relationship of these markers to glucose stimulated insulin release. Even intermittently elevated glucose

level impair glucose stimulated insulin secretion and also they activate apoptosis and bring about alteration in mitochondrial morphology and density together with increased intracellular nitrotyrosine content. Hence glucose fluctuation in response to meal in prediabetic patients can cause loss of functioning beta cell mass.

Persistant and long standing hyperglycemia results in chronic stimulation of beta cell and increased insulin synthesis. This can lead to stress of endoplasmic reticulum. Normally the endoplasmic reticulum is responsible for the production, modification and delivery of proteins to their target sites. Hence under condition of ER stress these process get impaired.

Usually ER stress is offset by enhancement of its folding capacity via modulation of foldases followed by chaperones. Also there will be downregualtion of the biosynthesis load and increased clearance of unfolded proteins. These processes usually initiate apoptosis.

LIPOTOXICITY

Obesity is the central component of metabolic syndrome and it is always accompanied by dyslipidemia and elevated inflammatory adipocytokines and leptin. These cytokines affect

insulin sensitivity and can also initiate apoptosis. Apoptosis in turn stimulates the inmate immune system through mobilization of T cells leading to auto immune mediated destruction of beta cells and their loss of function.

PATHOPHYSIOLOGICAL EFFECTS OF FFAS

1) Long term elevation of FFAS inhibit beta cell function and also lead to accumulation of toxic lipid metabolites. This process is classically known as Lipotoxicity.

2) Chronic exposure to increased FFA levels attenuates the glucose- stimulated insulin secretion.

3) FFAs can stimulate apoptosis of beta cells via activation of caspases secondary to enhanced ceramide formation within the beta cells.

4) FFAs can induce down regulation of Akt- Phosphorylation leading to defective insulin signaling and also initiation of apoptosis.

5) FFAs also induce the expression of iNOS leading to enhanced production of Nitric oxide resulting in defective insulin signaling.

6) FFAs can also stimulate increased production of reactive oxygen species and this can result in oxidant damage of the beta cell mass.

AMYLOID

Islet amyloid polypeptide is a normal beta cell secretary product and in Type-II diabetes there will be increased deposition of IAPP diffusely throughout the pancreatic islet leading to progressive reduction in beta cell mass, function and glucose intolerance.

PATHOGENESIS OF DIABETIC RETINOPATHY

The mechanism by which retinopathy occurs in diabetes are multifactorial. The risk factors are also plenty.

Risk factors for the development of diabetic retinopathy[2,4,5,6,7].

DURATION OF DIABETES

It is the best predictor of diabetic retinopathy. Studies have reported that after 20 years of diabetes, nearly 99% of patients with Type-I[1] and 60% of Type-II have some degree of Diabetic Retinopathy.

AGE

Diabetic Retinopathy is more frequent in the order onset group.

HYPERGLYCEMIA

Numerous studies have shown that, an average reduction of 1-1.5% in HbA_1C can cut down the risk of retinopathy by 40%, progression to vision threatening retinopathy by 25%, need for laser therapy by 25% and blindness by 15%.

SYSTEMIC HYPERTENSION

It is a major contributor for the development of retinopathy in the setting of uncontrolled diabetes mellitus. Numerous studies have shown that an increase in systolic blood pressure of 10 mmHg increase the risk of early diabetic retinopathy by 10% and proliferative diabetic retinopathy by 15%.

RENAL DISEASE

Nephropathy is a fore runner of diabetic retinopathy and nearly 35% of patients with retinopathy have elevated renal parameters and proteinuria.

PREGNANCY[7]

Risk of developing retinopathy is around 10% during pregnancy for a women with diabetes and it is further complicated by the presence of hypertension and if so there will be progressive disease. Some people will have regression of retinopathy after delivery.

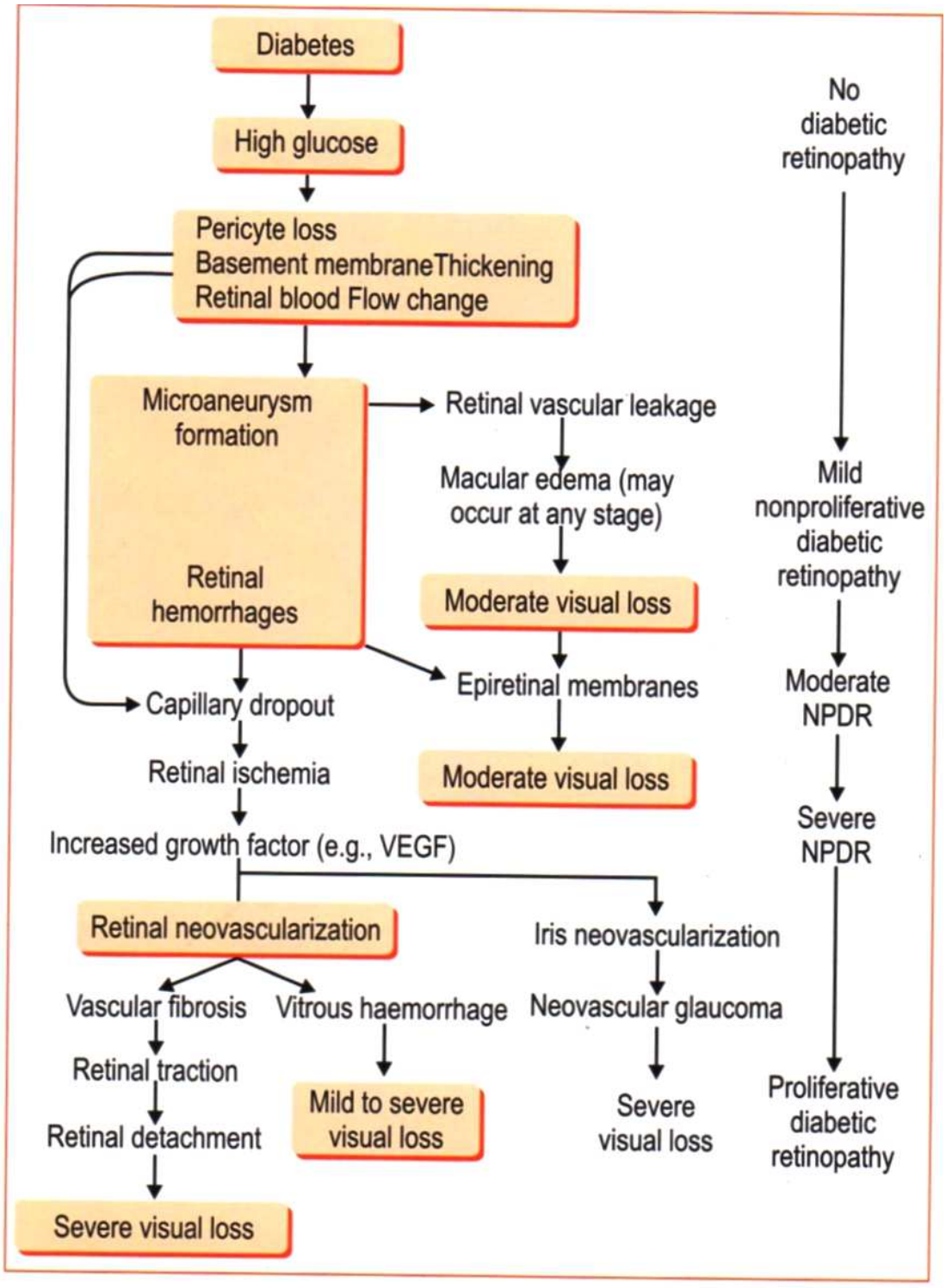
ETHNICITY[5,6]

Prevalence of diabetic retinopathy is reported to be higher in African Americans, Hispanics and South Asians than in white people.

OTHER MINOR RISK FACTORS

Many other minor risk factors also contribute to the development and progression of diabetic retinopathy like smoking, consumption of alcohol, body mass index, physical activity, cataract surgery, dyslipidemia and so on …

PATHOPHYSIOLOGY[10]



The mechanism by which glycemic control leads to vascular disease is not clearly understood. There is a loss of retinal pericytes and endothelial cells of microvasculature which starts at an early stage in diabetes and when coupled with the thickening of basement membrane heralds the onset of diabetic retinopathy. Death of the pericytes in the retina and cells of the microvasculature along with the impairment of basement membrane function leads to the formation of microaneurysms, increased vascular permeability and increased activity of vasoproliferative substances.

VASCULAR ALTERATIONS IN DIABETIC RETINOPATHY

Impairment of Retinal Blood Vessel Autoregulation[14,15]

Normally the retinal capillaries are lined by pericytes and endothelial cells. Endothelial cell layer is a single one lying on a basement membrane and they are linked by tight junctions constituting the inner blood retinal barrier. Hence endothelial cell layer damage leads to enhanced vascular permeability.

The pericytes[16,18] are present on the endothelial cells and they envelop the capillaries. Their main function is contractile and hence they regulate the retinal capillary perfusion. Pericyte damage leads to abnormal autoregulation of blood flow in retina and weakening of the capillary walls leading to a saccular outpouching called

microaneurysm and they are the earliest sign of diabetic retinopathy[6,8,9,16].

Weakening of capillary walls leads to the formation of microaneurysm[22] and their rupture leads to intraretinal hemorrhage. In fundus fluorescein angiography microaneurysms will be hyperfluorescent and intraretinal haemorrhages will be hypofluorescent.

Further abnormal autoregulation leads to increased vascular permeability leading to increased deposition of extracellular matrix component called hard exudates resulting in basement membrane thickening of the capillaries in retina and also can cause retinal edema. Ultimately these process can lead to loss of vision.

Progressive retinal vascular disease results in hypoxia secondary to retinal capillary closure and the resultant infarction of the nerve fibre layer leads to the formation of what so called cotton wool spots. Also these will be concomitant obstruction in the axoplasmic flow.

Abnormalities in the venous system of the retina also occurs leading to the formation of loops, beading and dilatation. These changes signify retinal ischaemia.

NEOVASCULARISATION[28]

Development of new vessels in retina (Neovascularisation) occurs in the advanced stages of DR.

Retinal ischemia described earlier is the potent stimulator of new vessel proliferation through the activation of angiogenic factors particularly VEGF.

New vessel formation within the retinal tissue or endothelial proliferation within the pre-existing vessels is represented by the term (IRMA) intraretinal microvascular abnormalities.

Major biochemical mechanisms that modulate the pathogenesis of DR operate mainly by exerting their effects on cellular metabolism, cell signalling and expression of growth factors.

MAJOR BIOCHEMICAL PATHWAYS UNDERLYING DR PATHOGENESIS [6,8,9,16]

Aldose Reductase

Chronic hyperglycemia causes enhanced production of sorbitol from glucose by the enzyme aldose reductase. Accumulation of sorbitol intracellularly causes osmotic damage to the retinal endothelial cells and also to the pericytes.

Protein Kinase C

Activation of PR-C occurs secondary to uncontrolled blood sugar levels resulting in enhanced expression of matrix proteins and vaso active mediators.

Both matrix proteins and vasoactive mediators cause thickening of basement membrane and enhanced vascular permeability

ADVANCED GLYCATION END PRODUCTS (AGE)

Glycosylation of serum and tissue proteins occurs in the setting of chronic hyperglycemia resulting in the formation of AGEs.

AGE products increase vascular permeability, stimulate cell proliferation and also they can promote the influx of mononuclear cells leading to inflammation.

Net result of increased circulating AGEs lead to loss of pericytes, damage to endothelial cells and also formation of microaneurysm.

OXIDATIVE STRESS

Chronic hyperglycemia leads to increased production of (ROS) Reactive Oxygen Species.

Increased ROS promotes progression of DR mainly by 4 mechanisms.

- ❖ Activation of protein kinace-C
- ❖ Activation of Polyol Pathway
- ❖ Increased production of VEGF
- ❖ Increased Formation of AGEs

VEGF

Retinal ischaemia and concurrent hypoxia stimulates the production of VEGF.

VEGF causes both angiogenesis and increased capillary permeability.

RENIN ANGIOTENSIN SYSTEM: (RAAS)

Upregulation of local RAAS in retina occurs in chronic hyperglycemia and increased angiotensin II stimulates VEGF expression.

Erythropoietin

Retinal ischaemia enhances the production of erythropoietin which promotes angiogenesis independent of VEGF.

Carbonic Anhydrase

Extracellular carbonic anhydrase levels increases in DR resulting in elevation of PH leading to increased vascular permeability.

Growth Hormone (GH) and Insulin like growth factors

GH and IGF levels are elevated in retinal hypoxia and they modulate the function of endothelial precursor cells leading to angiogenesis.

Further IGF causes disruption of blood retinal barrier and causes retinal edema by increasing vascular permeability.

Inflammation

Finally the latest updation in the pathogenesis of DR is the proposal of vascular inflammation as the main culprit.

Retinal endothelial cells and neural cells in response to inflammation causes increased production of VEGF and also recruitment of inflammatory mediators.

These mediators enhance vascular permeability, neurodegeneration and neovascularization.

Classification of Diabetic Retinopathy[61]

Diabetic Retinopathy is divided into two main forms

1. Non proliferative DR

2. Proliferative DR

Classification of DR is mainly based on the presence of (IRMA) intra retinal microvascular abnormalities and neovascularization. Various classification systems are available but used in current scenario were three of them.

ETDRS CLASSIFICATION SYSTEM[20,21]

Disease severity level	Findings observable upon dilated ophthalmoscopy
Mild NPDR	Presence of at least one microaneurysm
Moderate NPDR	Presence of haemorrhage / microaneurysm, cotton wool spots (CWS), venous beading (VB) and IRMA but less than that of severe NPDR
Severe NPDR (4-2-1)	Haemorrhages and microaneurysms in 4 retinal quadrants VB in at least 2 retinal quadrants IRMA in at least 1 retinal quadrant
Early PDR	New vessels. Criteria not met for high-risk PDR
High-risk PDR	Neovascularization on or within one disc diameter of the optic disc (NVD), with or without vitreous or preretinal haemorrhage; or Neovascularization elsewhere (NVE) and vitreous and/or preretinal haemorrhage
Advanced PDR	Posterior fundus obscured by preretinal or vitreous haemorrhage; or Center of macula detached

The gold standard for assessing DR is the airlie house classification system. This involves the grading of seven 30^0 stereoscopic images of the retina comparing with standard

photographs. Recently this system is modified and updated as “The early treatment of diabetic retinopathy study” (ETDRs) Classification.

INTERNATIONAL CLASSIFICATION SYSTEM[34,62]

Developed by the American Academy of ophthalmology (AAO) to improve the communication between primary care physicians and ophthalmologists.

Proposed disease severity level	Clinical findings (on dilated ophthalmoscopy)
No apparent retinopathy	No abnormalities
Mild NPDR	Microaneurysms only
Moderate NPDR	More than just microaneurysms but less than severe NPDR
Severe NPDR	**Any of the following** >20 intraretinal haemorrhages in 4 retinal quadrants; Definite VB in >2 retinal quadrants; or Prominent IRMA in > 1 retinal quadrant
PDR	**One or more of the following** Neovascularization; Vitreous haemorrhage; or Preretinal haemorrhage

CLINICAL FEATURES

Diabetic Retinopathy in its earlier stages is entirely asymptomatic and it is very prudent to screen for the signs of retinopathy in every diabetic patient regularly. The changes seen in Diabetic Retinopathy are divided into two important categories.

1) Non proliferative diabetic retinopathy (NPDR)

2) Proliferative Diabetic Retinopathy (PDR).

NON PROLIFERATIVE DIABETIC RETINOPATHY

It is characterized by five important clinical findings

1) Retinal capillary microaneurysms

2) Haemorrhages

3) Hard exudates and retinal edema

4) Cotton wool spots

5) Venous beading and IRMA

MICRO ANEURYSMS

Saccular outpouchings of the capillary wall in the retina along with hypercellularity constitute microaneurysms. On ophthalmoscopic examination they will be identified as Red

coloured dots. But usually the best identification technique for microaneurysms is fundus fluorescein angiography. Micro aneurysms are the earliest detected fundus abnormality in Type-2 Diabetic patients.

RETINAL HAEMORRHAGES

Retinal haemorrhages are divided into two types.

1) Dot-Blot Haemorrhages

2) Retinal nerve fibre layer haemorrhages

Dot-blot haemorrhages

These are punctate haemorrhages within the retina intra retinally and they usually arise from the capillaries at their venous terminal. They appear as red dots compactly damaged within the middle layers of retina.

Small dot-blot haemorrhages appears like microaneurysms on ophthalmoscopic examination and the best differentiating investigation is fundus fluorescein angiography.

Retina nerve fibre layer haemorrhages

These are otherwise known as flame shaped haemorrhages. Their alignment to the retinal nerve fibre layer will be more or less horizontal. They originate from the precapillary arterioles located

superficially within the retinal nerve fibre layer. These flame shaped haemorrhages actually indicate the presence of systemic hypertension or co-added venous obstruction.

Hard Exudates and retinal edema

Leakage of lipoproteins from the tight junctions of endothelium in the retinal capillaries give rise to lipid deposits what are known as hard exudates. They are yellowish deposits situated intraretinally in a circinate pattern around micro aneurysms.

Breakdown of the retinal blood barrier leads to increased capillary permeability resulting in retinal edema. This edema is always accompanied by hard exudates and usually they tend to occur in the macular region.

Cotton Wool Spots

Cotton wool spots are soft, yellowish, superficial fluffy lesions and they are otherwise known as soft exudates. They tend to occur in the areas of localized capillary ischemia. As a result of ischaemia, the axoplasmic transport within the nerve fibre layer gets obstructed focally leading to the accumulation of neuronal debris. These neuronal debris clinically resemble cotton wool spots

and they tend to obscure the underlying blood vessels in the region of the nerve fiber layer.

VENOUS BEADING AND IRMA

Saccular outpouching of venous walls are called venous beading. They tend to occur with increased frequency in the areas of capillary non- perfusion. They also manifest as venous loops in these areas.

Intra retinal microvascular abnormalities or IRMA are capillary dilatations and they usually function as collateral channels in the areas of capillary non-perfusion.

Both IRMA and venous beading are signs of enhanced retinal ischaemia and they usually reflect the severity of capillary hypoperfusion.

PROLIFERATIVE DIABETIC RETINOPATHY

Retinal neovascularization is the hallmark of proliferative diabetic retinopathy. About 50 % of patients with non proliferative diabetic retinopathy of severe grade progress to proliferative stage almost within a year. Almost one fourth of their retina should be non perfused before the development of proliferative diabetic retinopathy.

The stage of proliferative diabetic retinopathy is characterized by 2 findings:

- ❖ Neo vascularization.
- ❖ Vitreous / pre – retinal hemorrhages.

NEO-VASCULARISATION

It is defined as the appearance of multiple new fine vessels arising either from the disc, retina or iris. It is the hallmark of Proliferative Diabetic Retinopathy. These new vessels are classified as NeoVascularisation of the Disc or NeoVascularisation Elsewhere according to their Origin.

NEO-VASCULARIZATION OF THE DISC (NVD)[52]

New vessels originating either from the disc or within one disc diameter from the optic nerve are known as Neo Vascularization of the Disc (NVD).

Neo-vascularization Elsewhere (NVE):

New vessels originating more than one disc diameter from the optic nerve are known as Neo Vascularization Elsewhere (NVE).

SIGNIFICANCE OF NVD AND NVE[58]

The blood vessels in NVD and NVE are not like normal retinal vessels. They are very fragile and more prone to rupture

easily causing leakage into the vitreous leading to formation of vitreous or pre- retinal hemorrhages. As the new vessel mature, the fibrous component becomes more prominent. The collagen scaffold contracts and elevates the underlying retina leading to tractional retinal detachment.

VITREOUS OR PRE – RETINAL HAEMORRHAGES (PRH):

Pre – retinal hemorrhage occur in the space between internal limiting membrane of retina and posterior hyaloid face. These hemorrhages are classically boat shaped.

SIGNIFICANCE OF PRH

Small pre-retinal hemorrhages can cause floaters but when they are found more extensive, sudden and painless loss of vision can occur.

THE STAGE OF ADVANCED DIABETIC EYE DISEASE:

Retinal detachment, rubeosis iridis and secondary glaucoma complete the picture of advanced **End – stage diabetic retinopathy.**

Two types of retinal detachment occur

- Those due to traction called as Non – Rhegmatogenous Retinal Detachment.

- ❖ Those due to the formation of new holes in the retina where ischaemia is prominent called as Rhegmatogenous Retinal Detachment. The holes are formed in the ischaemic areas by extensive tissue break down.

DIAGNOSIS OF DIABETIC RETINOPATHY[50]

The diagnosis of both Non – proliferative and Proliferative diabetic retinopathy includes a detailed medical history and a thorough ophthalmic examination.

HISTORY

Proper consideration should be given to

1) Duration of diabetes.
2) Ocular History: Trauma / Surgery for refractive error correction / laser treatment / ocular injections
3) Glycaemic control in the past (HbA1c)
4) Medications.
5) Other comorbid conditions (renal diseases, obesity, Systemic hypertension, dyslipidemia) etc.

SYMPTOMS

The patient's quality of vision should be investigated to elicit symptoms like.

1) Blurred, distorted or fluctuating vision.

2) Double vision.

3) Night blindness.

4) Floaters or flashes.

OPHTHALMIC EXAMINATION[53]

- ❖ Initial examination.
- ❖ Ancillary test.

INITIAL OPHTHALMIC EXAMINATION

Includes the assessment of visual acuity, Gonioscopy and slit lamp microscopy.

(i) Visual acuity

Visual acuity is assessed after pupillary dilatation. Before dilating the pupil, it is always prudent to recognize rubeosis iridis or neovascularization of iris. Pupillary dilatation is achieved by administration of either 0.5% to 1% Tropicamide or 2.5% Phenylephrine.

(ii) Gonioscopy

In the presence of rubeosis iridis or elevation of intra ocular pressure, gonioscopy is performed to rule out neo vascularization of the anterior chamber angle.

(iii) Slit –lamp biomicroscopy

Retinopathy changes in the mid peripheral retina and posterior pole is best assessed by Slit lamp bio-microscopy. This can be done with the help of accessory lenses and when this examination is accompanied with a contact lens, visualization of peripheral retina will be of superior quality.

ANCILLARY TEST

(i) Color Fundus Photography (CFP)

This is a very useful investigation in documenting the progression of retinopathy and also the response to treatment. Hence, this test is recommended in all clinical research studies in diabetic retinopathy. The main disadvantage of this test is that, it is not useful in the early stages of diabetic retinopathy with minimal fundal changes.

(ii) Optical Coherence Tomography (OCT)

Quantification of retinal thickness, identification and monitoring of macular edema, vitreo macular traction in selected patients with Diabetic Macular Edema can be done by Optical Coherence Tomography.

- OCT is useful for imaging the retina, subretinal space and vitreo-retinal interface with the help of high resolution (10 micron) imaging.

- OCT guided measurement of thickness of the retina is not precise sometimes and it correlates poorly with visual acuity.

(iii) Fundus Fluorescein Angiography (FFA)

- FFA is the gold standard investigation for the diagnosis of both non proliferative and proliferative DR.

- FFA can identify macular capillary non–perfusion, capillary leakage and their sources leading to macular edema as possible explanation for visual loss.

- FFA can identify even subtle neo vascularization before other investigations.

- FFA can be used as a mean of evaluating the source of unexplained visual loss.

- FFA is always useful for documentation of the retinal lesions before laser pan – photo coagulation and also for follow up.

SCREENING RECOMMENDATIONS[52]

Screening is the best possible way to prevent and reduce the progression of disease in diabetic retinopathy. Several methods have been proposed for screening of diabetic retinopathy.

CLINICAL METHODS

- Direct opthalmoscopy.
- Indirect optholmoscopy.
- Slit lamp biomicroscopy.

PHOTOGRAPHIC METHODS

- Mydriatic
- Non Mydriatic
- Polaroid Cameras
- Digital Imaging

Out of all investigations, the gold standard is seven field stereoscopic colour fundus photography. In all other investigations a large proportion of sight threatening retinopathy can be missed.

LIMITATIONS OF THE AVAILABLE SCREENING TESTS

1) All screening test could be done only with the help of a peson with expertise in ophthalmology.

2) Most of the screening tests are incomplete and some changes can be missed on examination.

3) Seven field stereo colour fundus photography and fundus fluorescein angiography which are considered as gold standard in the detection of DR are available only in specialized centres in ophthalmology / tertiary care centres so that mass screening of diabetic patients at risk could not be done.

Hence in the search for a very simple tool in identifying diabetic retinopathy at the earlier stages without cumbersome procedures and at affordable cost, so that every diabetic patient can get benefitted from such an investigation either by mass screening programmes or regular screening at a local centre, the author here tries to elucidate the importance of Red cell distribution width, Red blood cell count and Neutrophil/ Lymphocyte ratio as the markers of vascular inflammation in the early diagnosis of Non proliferative DR.

The author stresses here the imporotance of Red cell indices like.

(1) RBC Count

(2) Red Cell distribution Width

and also the (3) Neutrophil- Lymphocyte ratio in the early diagnosis of non proliferative diabetic retinopathy. Hence the author wants to brief the salient physiological, biochemical and pathological aspects of these indices in their contribution towards the diagnosis of NPDR.

STRUCTURE OF THE RED CELL

Mature RBCs are unique among the cells of human tissues, in that they normally lack nuclei and cytoplasmic structures such as Lysosomes, endoplasmic reticulum and mitochondria. Hence they cannot carry out protein synthesis, unable to undergo mitosis and mitochondrial oxidative reactions. RBCs are biconcave discs of 7-8µm in diameter, but their shape changes to a parachute- like configuration in the capillaries whose diameter, is less than that of RBCs in the biconcave disc form. The membrane of red cell is elastic and so they resume biconcave shape. Once they re-enter the large blood vessels, loss of flexibility or elasticity leads to membrane damage and change in shape leading to diminished life span.

ORGANIZATION OF THE ERYTHROCYTE MEMBRANE

The membrane and the cytoskeleton of the RBC are collectively known as stroma. The membrane is highly deformable

and non-expansile structure. Its integrity is firmly maintained by the attachment of its inner surface to a lattice like structure of specilized cytoskeletal proteins which support the membrane and also decides the shape of the RBCs.

LIPIDS

Phosphatidyl Choline

Phosphatidyl Ethanolamine

Phosphatidyl Serine

Sphingomyelin

These lipid molecules account for most of the phospholipids. The aminophospolipids lie in the inner cytoplasmic monolayer and the choline phosphatides lie in the outer monolayer.

Membrane fluidity is taken care of by cholesterol making it more viscous when compared to pure phospholipid membranes.

PROTEINS

1) Transmembrane proteins

2) Cytoskeletal proteins

Transmembrane proteins

The two predominant transmembrane proteins age (AE-1) anion exchanger and glycophorin -A (GPA).

FUNCTIONS OF AE-1

- It encases the channels through which facilitated transport of glucose and anions take place.

- It interacts with the cytoskeleton by binding to ankyrin, hence its mutation may lead to RBC membranopathies and structural defects interfering with the rheological properties of the RBCs.

FUNCTIONS OF GPA

- It is enriched with large amounts of sialic acid which contribute to the negative charge of the outer surface of the red cell at physiological PH.

CYSTOSKELETAL PROTEINS

- Spectrin
- Actin
- Ankyrin
- Adducin
- Protein 4.1, 4.9
- Tropomyosin
- Tropomodulin

The most important constituent of the cytoskeleton is spectrin. They are intertwined and linked together by other proteins forming a lattice like network which is attached to the internal surface of the membrane. Because of this resilient structure red cells resume their biconcave disc forms after their distortion forces have been removed.

RELATIONSHIP BETWEEN RBC COUNT, ULTRASTRUCTURAL MEMBRANE ALTERATIONS AND MICROVASCULAR COMPLICATIONS[59]

- Chronic hyperglycemia causes non enzymatic glycosylation of RBC membrane proteins. This decreases the negative surface electric charge so that there will be accelerated aging of RBCs.

- Normally the negative charge leads to firm adhesion between surfaces causing electrostatic repulsion between the erythrocytes resulting in diminished aggregation. This also results in low-shear rate viscosity and yield stress of blood.

- Hence the net result of reduction in negative surface charge increases microviscosity, aggregation and adhesiveness of RBCs. The decrease of surface charge leads to the collinear decrease of membrane deformability.

- The velocity of RBC movement comes to a stand still secondary to the reduction in the movement of RBCs through the capillary segments as a result of reduction on the net surface charge.

- Mature and aged RBCs show more aggregability, deformability and increased mechanical fragility.

- Haemoglobin molecules of some aging RBCs get aggregated and attaches to the inside of the cell membrane leading to the reduction in membrane flexibility. This greatly influences the oxygenation of Hb.

- Reudced surface negative charges directly causes changes in the properties of the basement membrane of retina. These changes causes breakdown of the retinal- blood barrier leading to increased permeability of the capillaries resulting in exudation of proteins into the superficial and deep layers of the retina.

- Normally phospholipid symmetry is maintained in the inner layer of the plasma membrane by increased concentrations of phophatidyl serine which contain a negative charge. This symmetry is distorted secondary to excessive oxidative stress

within the cell leading to externalization of the serine moiety. Hence this asymmetry and externalization renders the RBC surface as thrombogenic and these cells are removed by macrophages in circulation by way of phagocytosis.

- The environment of abnormal RBCs, Phosphatidyl serine asymmetry and recruited leucocytes leads to enhanced coagulation cascade.

- Anaemia causes tissue hypoxia leading to expression of growth factors from the already compromised kidney resulting in mitogenic and fibrogenic effects.

- The decrease in RBC count may contribute to the microvascular complications by a reduced haemoglobin level also.

- Reduced RBC count in the case of normocytic normochromic picture actually signals the damage that has occurred to the renal tubular interstitium and may herald the onset of diabetic nephropathy.

RED CELL DISTIRBUTION WIDTH

Red cell distribution width is an index of variation in RBC size or RBC volume. Normally red cell size variation is known as anisocytosis and RDW is a measure of extent of anisocytosis.

Most automated instruments produce a quantitative assessment of the variation in red cell volume indicated by RDW which corresponds to the microscopic analysis of the degree of anisocytosis. The RDW derived from pulse height analysis can be expressed either as (SD) standard deviation in fl or as the percent of coefficient of variation (CV) of the measurements of red cell volume.

NORMAL REFERENCE RANGES OF RDW

RDW – SD : 39-46 fl

RDW-CV : 12-14% in adults

RDW-SD

It is a measurement of width of RBC size distribution histogram and it is measured by calculating the width at the 20% height level of the RBC size distribution histogram. Hence RDW-SD is not influenced by the average RBC size (ie) mean corpuscular volume.

RDW-CV

It is calculated from standard deviation and MCV by the formula.

RDW-CV (%) = 1 SD of RBC volume / Mcv x 100%

Since RDW- CV is obtained mathematically from MCV it is affected by changes in average size of RBCs.

SIGNIFICANCE OF ELEVATED RDW

- Early diagnosis of nutritional deficiency (d/t) iron, B12 and folic acid.
- Differentiation of iron deficiency anaemia from thalassemia.
- Differentiation of megaloblastic anaemia from other causes of macrocytosis.
- Identification of Red cell fragmentation, agglutination and dimorphic red cells in peripheral smear examination.

RED CELL DISTRIBUTION WIDTH IN NON-PROLIFERATIVE DR

- Increased RDW leads to reduced RBC deformability. This results in the impairment of blood flow through the microcirculation.
- Elevated RDW is associated wih increased vascular inflammation and reduced level of anti oxidants. Hence RDW is considered as a global marker of oxidative stress and chronic inflammation. Inflammation influences deformability and half life of erythrocytes,

affects erythropoiesis, promotes anisocytosis and hence leads to elevation of RDW levels.

- ❖ Increased RDW also reflect reduced negative surface electric charges which through already explained detailed mechanisms causes changes of non-proliferative DR.

NEUTROPHIL- LYMPHOCYTE RATIO

Normally there are 4000-11000 WBCS/ micro litre in the human blood. Of these granulocytes are the most numerous. Young granulocytes have horse shoe shaped nuclei that become multilobed as the cells grow older. Most of them contain neutrophilic granules.

NEUTROPHILS

Neutrophils have cytoplasmic granules that contain biologically active substances involved in inflammatory reactions. The average half- life of a neutrophil in the circulation is 6 hours. They are attracted to the endothelial surface by selectins and they roll along it. Neutrophil adhesion molecules of the integrin family helps them to get bound to selectins. They insinuate themselves through the walls of the capillaries by a process known as diapedesis. Many of those that leave the circulation enter the GI tract and are lost from the body.

Neutrophilic granules contain various proteases and in addition they also contain enzymes such as NADPH oxidase, catalase and myeloperoxidases. NADPH oxidase is associated with a sharp increase in oxygen intake and metabolism in the neutrophil, what we call as the “Respiratory burst” and this reaction generates plenty of free O-radicals. The myeloperoxidase catalyses the conversion of Halides and cyanides to their corresponding acid forms. These acids inturn are potent oxidants by themselves.

In addition to myeloperoxidase and NADPH oxidase neutrophil granules also contain an elastase and two metalloproteinases.

The total body neutrophils can be divided into circulating pool (CGP) and marginating granulocyte pool. In these two pools, the cells are equal size and they are in constant equilibrium. MGP represents the neutrophils involved in adhesion and rolling along the endothelial cells in post capillary venules and they are not found in blood obtained by venepuncture. So the neutrophil content actually represents about half of the total no of neutrophils in the vascular compartment.

LYMPHOCYTES

Lymphocytes are motile non phagocytic cells. There are many subpopulations of lymphocytes which interact with each other and with cells of the monocyte macrophage system. They help in maintaining both humoral and cell mediated immunity. Proliferating lymphocytes are enriched with enhanced levels of enzyme n-terminal deoxyribonucleic acid transferase. It is found in immature lymphoid cells in the bone marrow and thymocytes, but not in mature lymphocytes. Adenosine de aminase is present in large amounts in T-lymphocytes and it is necessary for their immune function.

INFLAMMATION

Inflammation is naturally a protective mechanism against invasion of microbes and toxins. The inflammatory response consists of 2 main components- a vascular reaction and a cellular reaction. Both the reactions are mediated by chemical factors that are derived from plasma proteins or cells produced as a result of inflammatory response.

Chronic inflammation is of prolonged duration in which active inflammation, tissue destruction and repair are proceeding simultaneously. Atheroscleorosis and vascular disease are chronic

inflammatory processes of the arterial wall induced partly by endogenous toxic plasma lipid components.

Morphological features of chronic inflammation

- Mononuclear cell infiltration
- Tissue destruction
- Healing by connective tissue replacement
- New blood vessel formation by elaboration of vascular endothelial growth factor and other angiogenic factors.
- Fibrosis

Most of these elements of chronic inflammation are found in the pathogenesis of both non proliferative and proliferative diabetic retinopathy.

NLR IN SUBCLINICAL INFLAMMATION

High Neutrophil lymphocyte ratio is a marker of subclinical inflammation in many disease states of the vascular system. NLR reflects the systemic inflammatory response that accompanies chronic disease but might also be influenced by systemic infections, atherosclerosis, hypertension, chronic renal disease and diabetes.

Subclinical vascular inflammation measured by derived NLR is linked with traditional risk factors of chronic diseases such as smoking, obesity, hypertension and elevated levels of triglycerides.

MECHANISMS

1) Endothelial dysfunction secondary to cellular response of blood components heralds the onset of inflammation. Endothelial dysfunction leads to impaired production of nitric oxide and prostacylins. This leads to the depletion of anti-atherogenic, antithrombotic and vasodilator properties of the vascular endothelium.

2) Diabetes Mellitus has been reported to the associated with acute phase response. In type-2 diabetes sialic acid, alpha-1 acid glycoprotein, c-reactive protein, amyloid and interleukin-6 are increased. Also in parallel leukocyte count is elevated significantly than other markers indicating ongoing subclinical vascular inflammation.

   The normal d-NLR is < 2.0 in control population.

AIMS AND OBJECTIVES

The aim and objective of the study is

- To evaluate the usefulness of “Red Cell Distribution width, Red Blood Cell Count and Neutrophil / Lymphocyte ratio as potential markers of vascular inflammation in the early detection of non proliferative diabetic retinopathy”.

MATERIALS AND METHODS

This is a cross sectional study and was conducted after Ethical Committee Clearance.

The study composed of a total number of 100 subjects, all of them were Type-II diabetic patients enrolled into the study as cases. These subjects were from among the Type-II DM individuals attending the Diabetology and Ophthalmology Outpatient Clinic in Stanley Medical College Hospital, Chennai.

Inclusion Criteria

Cases Type-II Diabetic patients between 30-50 years diagnosed to have Mild to Moderate Non Proliferative diabetic retinopathy (Micro Aneurysm, Hard Exudate, Haemorrhage, Phlebopathy)

Exclusion Criteria

1) Type-II Diabetic Patients with Proliferative Diabetic Retinopathy, Micro/Macro Albuminuria, Established Diabetic Nephropathy, Chronic Kidney disease of any etiology.

2) Type-II Diabetic patients with iron deficiency anaemia/ megaloblastic anaemia/ or recent blood loss.

3) Type-II Diabetic patients with any evidence of sepsis / infectious disease in prior 4 weeks.

4) Type-II Diabetic patients with any form of arthritis on NSAIDS/ Active GI Ulcer.

5) Type-II Diabetic Patients with Hypertension.

SAMPLE COLLECTION

Blood Samples

A random sample was collected in the morning hours during the outpatient clinic time from the anticubital vein of the study subjects. The blood samples were analysed on the same day within 4 hours of collection. The biochemical parameters relevant to the study were analysed by the following methodologies.

Step-I: Estimation of RDW, RBC Count and NLR

CBC WITH DIFFERENTIAL

Test Method

Sysmex XN and XS Systems:

- ***WBC:*** Flow cytometry
- ***RBC:*** Impedance counting
- ***Platelet Count:*** Impedance counting

- ***Platelet F Count:*** Flow cytometry fluorescence count (performed when appropriate)

- ***HGB:*** Converted to SLS-hemoglobin and read photometrically.

- ***MCV:*** The average volume of individual erythrocytes derived from the RBC histogram.

- ***RDW:*** The size distribution spread of the erythrocytes population derived from the RBC histogram.

- ***MPV:*** The average volume of individual platelets derived from the PLT histogram.

- ***HCT:*** Measured as a ratio of the total RBC volume to whole blood using cumulative pulse height detection.

- ***MCH, MCHC:*** Calculated parameters. MCH: Weight of HGB in the average RBC. MCH = HGB ÷ RBC x 10 MCHC: Average weight of HGB in a measured dilution. MCHC = HGB ÷ HCT x 100

- ***WBC Differential:*** The instrument makes 3 measurements (volume, conductivity and scatter) as each cell passes through the flow cell. The low frequency impedance measurement defines cell volume. The high frequency conductivity

measurement indicates the internal conductivity. The light scatter measurement indicates the structure and shape. An algorithm is applied to determine different cell populations.

- Abs Neutrophils, Abs Lymphocytes, Abs Monocytes, Abs Eosinophils, Abs Basophils: Calculated Parameters [Total WBC x (%Diff Analyte Result/100)] = Absolute Diff Analyte Count

Reference Values

RBC count Male	4.5 to 5.9 m/cumm
RBC count Female	3.8 to 5.2 m/cumm
Haemoglobin Male	13 to 18 g/dl
Haemoglobin Female	11.5 to 16.5g/dl
PCV Male	42 to 50%
PCV Female	36 to 45%
MCV	80 to 100 fl
MCH	27 to 31 pg
MCHC	32 to 36 g/dl

RDW-CV	11.6 to 14 %
Total WBC Count	4000 to 11000 cells/ cumm

Differential Count

Neutrophils	40 to 75%
Lymphocyte	20 to 45%
Eosinophils	1 to 6%
Monocyte	2 to 10%
Basophils	0 to 1%
Platelets Count	1.5 to 4.5 Lakhs / cumm

MEASUREMENT OF HBA1C

Technique

Cation exchange high performance liquid chromatography.

System

The Biorad D10 haemoglobin Testing System.

Procedure

The Bio Rad D-10 Haemoglobin Testing System is the newly introduced fully automated analyzer based on Cation Exchange HPLC. The dual kit reorder pack contains whole blood primer,

calibrator 1 and 2, calibrator diluent, wash reagent, elusion buffer 1 and 2 and analytical cartridge. The manufacturer's instructions were followed for the quality control and calibration. This Technique requires no predilution or manual handling of patient's samples. The samples are directly introduced in their primay tubes following calibrators and control samples. The instrument draws sample directly from the EDTA vacutainer and all processing of the sample is performed internally. Samples are automatically mixed, diluted and injected into the cartridge. The analyzer delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where haemoglobins are separated on the bases of their ionic interactions.

The separated haemoglobins are passed through flow cell of the filter photometer where changes in the absorbance at 415nm are measured. The run time is approximately 3 minutes per sample with a throughput of 20 samples per hour. A sample report and a chromatogram are generated for each sample.

HB1AC

Method : Bio Rad HPLC

Normal : 4 to 6%

Good Control : 6 to 7%

Fair Control : 7 to 8%

Poor Control : > 8%

ESTIMATION OF AVERAGE BLOOD GLUCOSE

Method

Nathan et al.

Equation

eAG = (28.7 X A1C) - 46.7
eAG (estimated average glucose)

Reference

Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Translating the A1C assay into estimated average glucose values. Diabetes Care 2008; 31 (8): 1473-8.

ESTIMATION OF URINE PROTEIN TO CREATININE RATIO

Technique

Random Urine Collection

Urine Creatinine in mg

Urine Protein in mg

Calculation of Urine Protein mg to Urine Creatinine mg ratio

Interpretation

Normal ratio < 0.2gms protein / gm creatinine

Reference

Ruggenenti (1998) BMJ 316: 504.

(2002) AM J Kidney Di 39: S1

ESTIMATION OF UREA

Technique

Urea (UV- GLDH) SLR – Assay

Principle

The test is performed as a 2 point kinetic assay in which the initial rate of the reaction is linear for a limited period of time.

Urea in the sample is hydrolyzed by urease to ammonia and carbondioxide. The second reaction, catalyzed by the glutamate dehydrogenase converts ammonia and alpha-ketoglutarate to glutamate and water. Two moles of NADH are oxidized for each mole of urea present.

The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample.

Sample Collection

Serum is collected by standard procedure. Heparin is recommended as anticoagulation.

Calculation

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times (50 \text{ Standard Concentration})$$

= mg/dl urea in the sample.

Reference Range 15-39mg/dl

ESTIMATION OF CREATININE

Technique

Liquixx Creatinine (Modified Jaffe's Method)

Principle

Creatinine reacts with alkaline picrate to produce orange-yellow colour (The Jaffe's Reaction). Specificity of assay has been improved by the introduction of an initial rate method.

The absorbance of the orange-yellow colour formed is directly proportional to creatinine concentration and is measured photometrically at 500-520nm.

Sample

Serum

Calculation

$$\frac{\Delta \text{ A of Test}}{\Delta \text{ A of Standard}} \times \text{Concentration of Standard (mg/dl)} = \text{Creatinine mg/dl}$$

Reference Range

Males 0.7 to 1.4mg/dl

Females 0.6 to 1.2mg/dl

FUNDUS PHOTOGRAPHY

Fundus photography (also called fundography) is the creation of a photograph of the interior surface of the eye, including the retina, optic disc, macula, and posterior pole (i.e. thefundus).

Fundus photography is used by optometrists, ophthalmologists, and trained medical professionals for monitoring progression of a disease, diagnosis of a disease (combined with retinal angiography), or in screening programs and epidemiology.

Compared to ophthalmoscopy, fundus photography generally needs a considerably larger instrument, but has the advantage of availing the image to be examined by a specialist at another location and/or time, as well as providing photo documentation for future reference. Modern fundus photographs generally recreate

considerably larger areas of the fundus than what can be seen at any one time with handheld ophthalmoscopes.

PROCEDURE

Fundus photography is performed by post mydriatic test. Seven field stereo colour fundus photographic pictures of the patients were taken. The presence of microaneurysms, retinal haemorrhages (Dot and blot, retinal nerve fibre layer haemorrhages) hard exudates, cotton wool spots, venous beading and IRMA were identified. Presence of proliferative stage identified by neovascularization are excluded from the study. Grading of nonproliferative retinopathy into mild moderate and severe was done. Severe grade patients were excluded from the study.

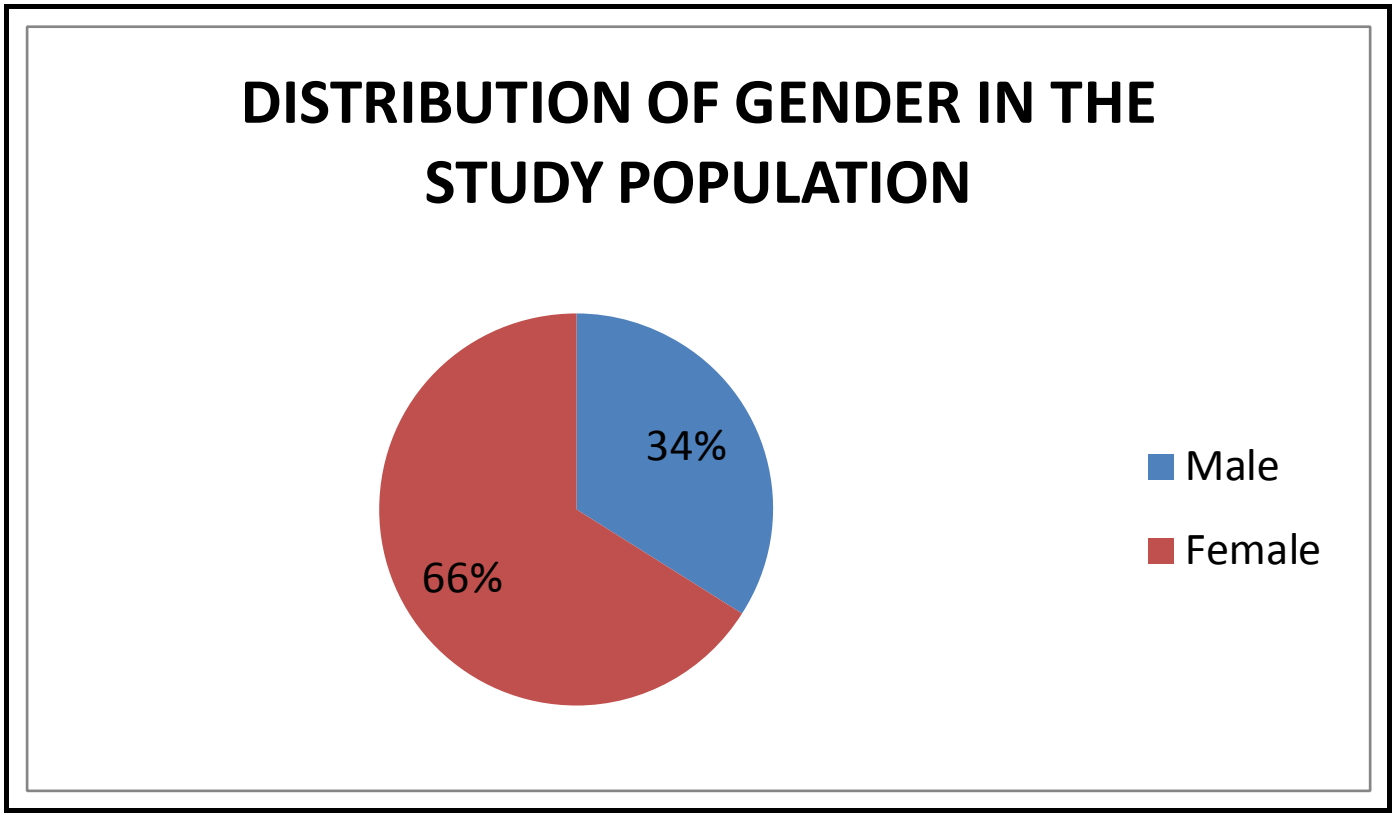
STATISTICAL ANALYSIS

Our study was a Cross-sectional study conducted among 100 Type-II diabetic patients between 30-50 years of age.

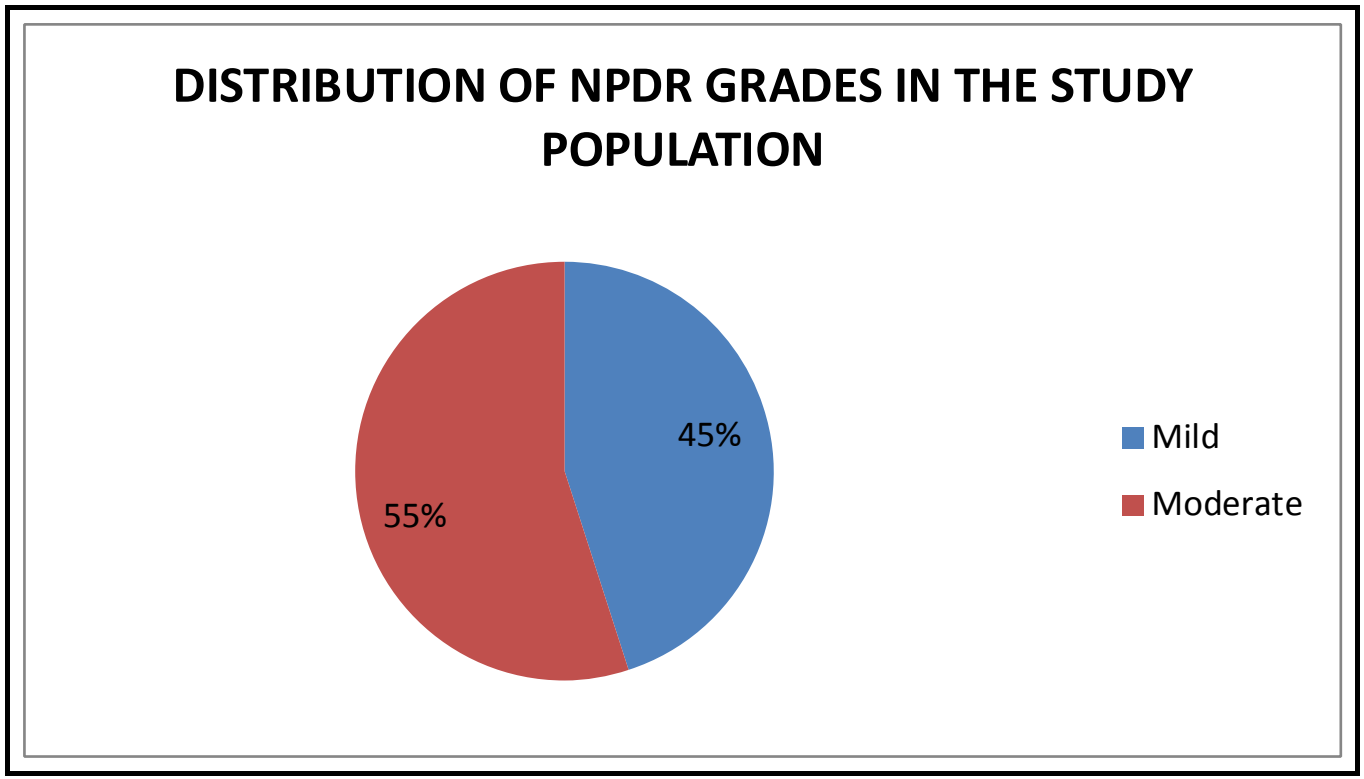
Two group were assigned according to the severity of Diabetic Retinopathy. One is a mild grade and another is a moderate grade.

NIR, RDW and RBC count were analyzed in this study population. Also HBAIC and blood glucose levels were correlated with them.

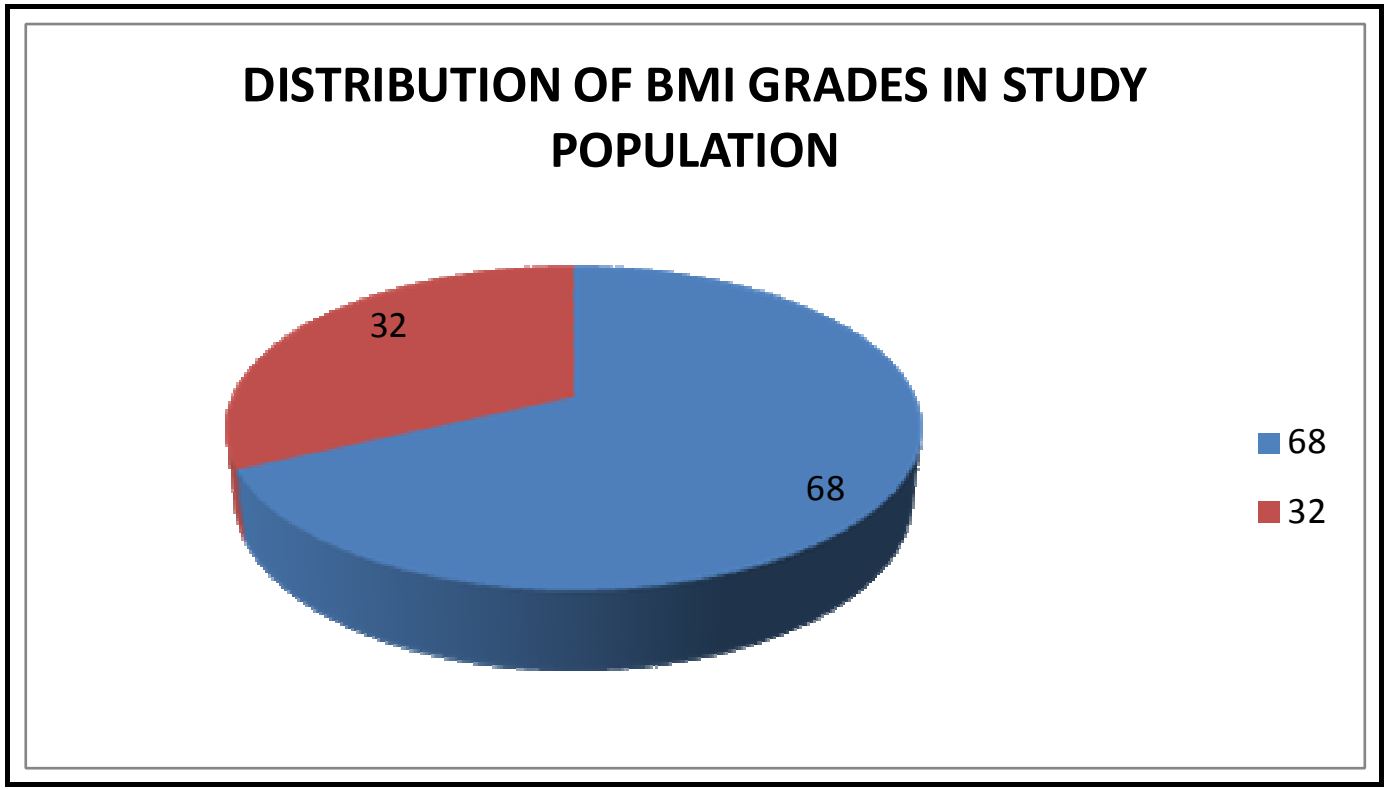
Statistical analysis was done with SPSS Software. Results were studied using unpaired t-test, chisquare test and Pearson coefficient Correlation.



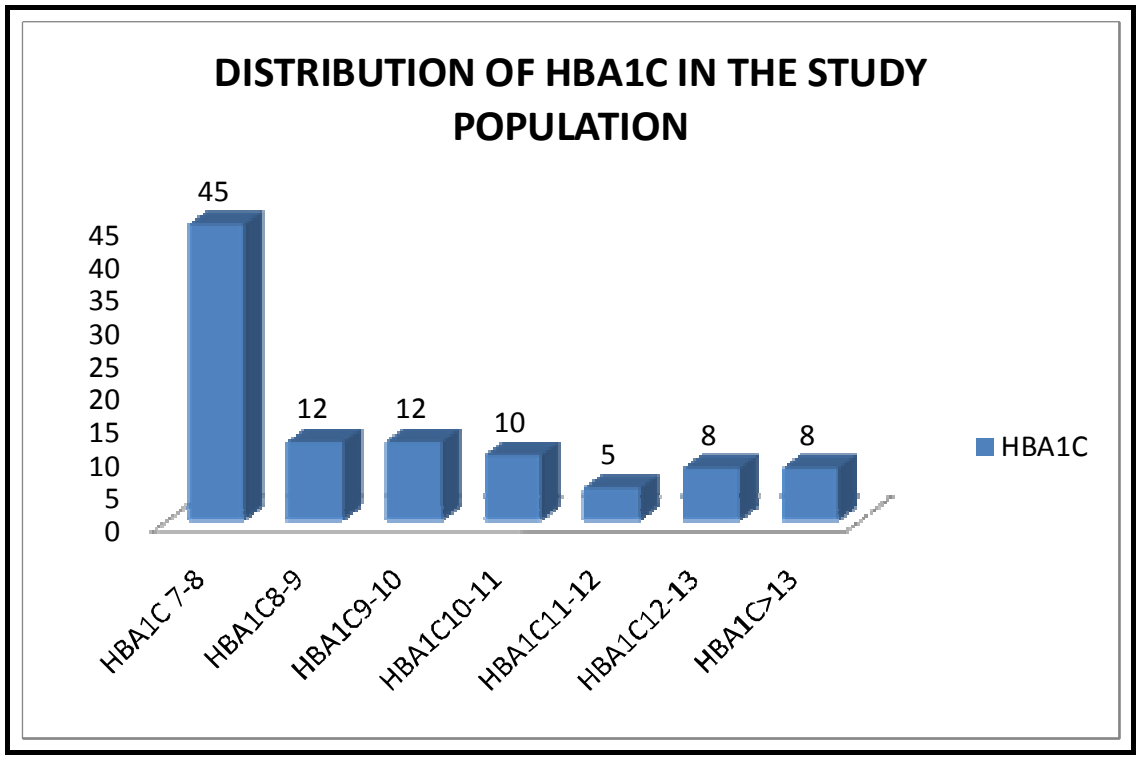
This diagram illustrates distribution of males and females among the study population. Female population comprises most of the study subjects with mild or moderate diabetic retinopathy.



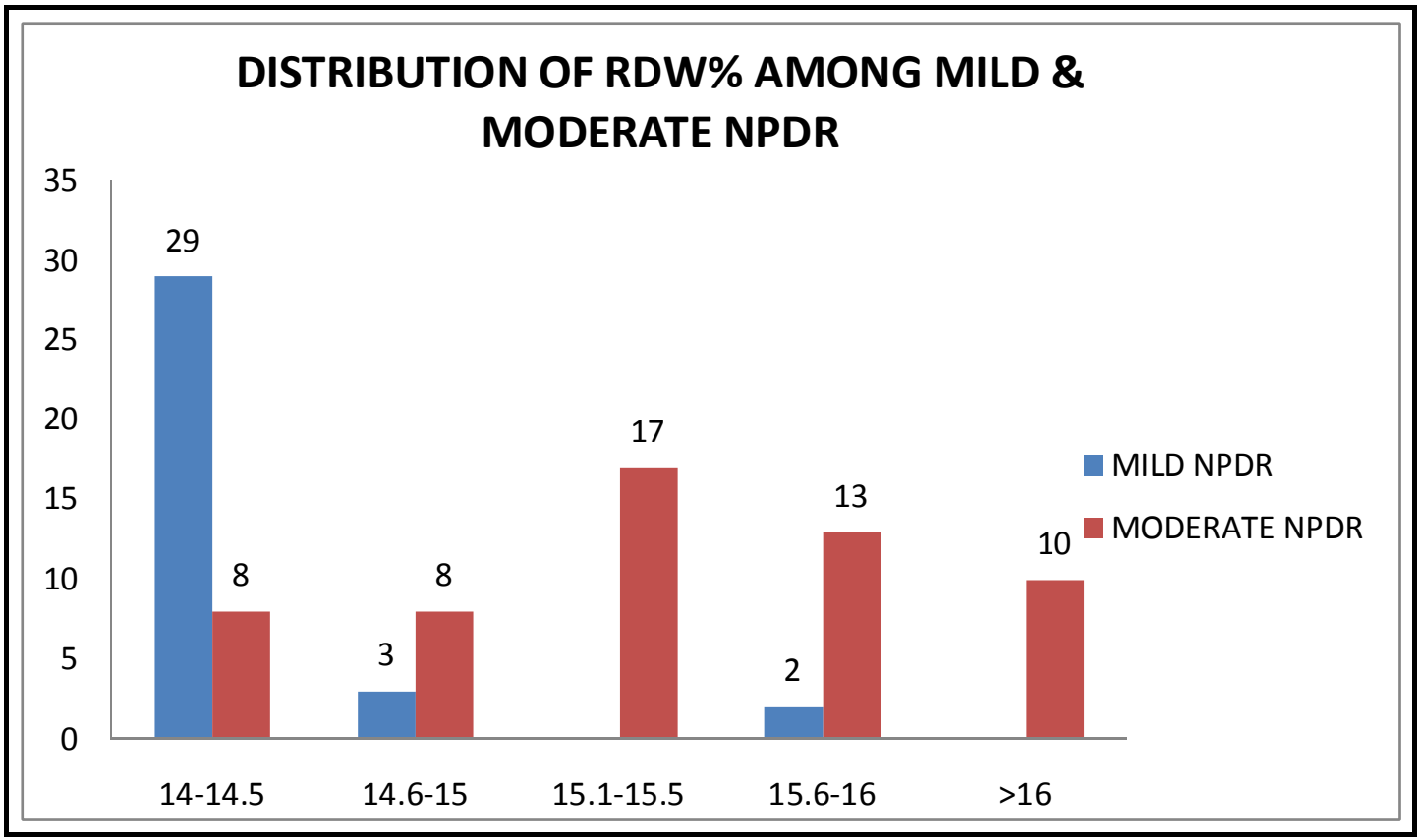
This diagram illustrates the distribution of mild and moderate non proliferative diabetic retinopathy among the study population. Moderate non proliferative diabetic retinopathy constitutes 55% of the study group.



This diagram illustrates the distribution of body mass index among the study population. About 68% of the study subjects were the distributed in the over weight group. Only 32% of the patients constitute subjects with normal BMI.

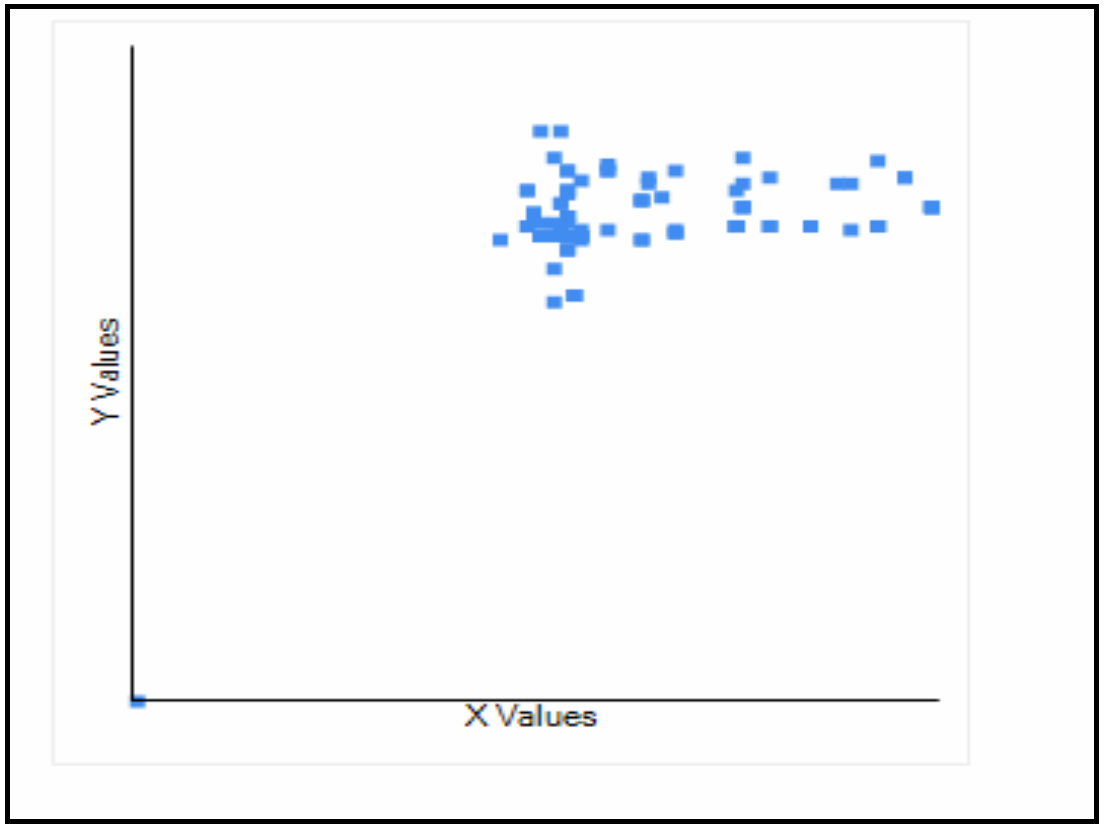


This diagram illustrates the distribution of percentage of HBA1C among the study population. About 45% of the study population has HBA1C between 7% to 8%. Remaining group constitute poorly controlled glycemic status reflected by HBA1C more than 8%.



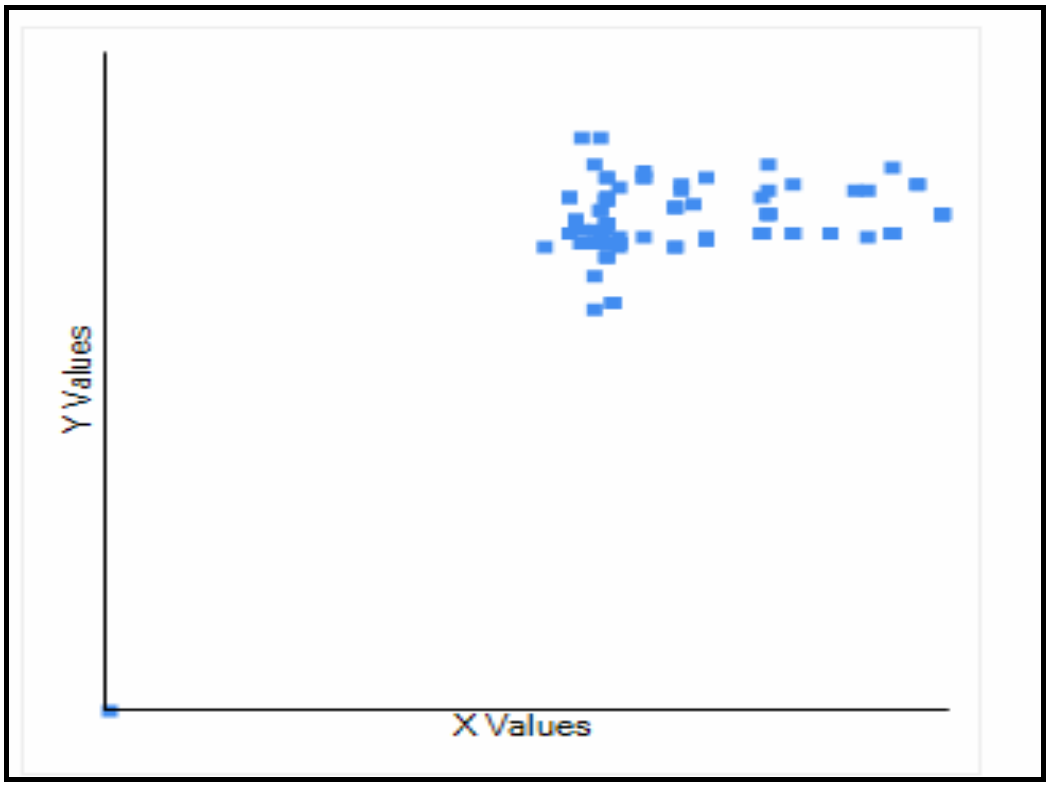
This diagram illustrates the distribution of Red Cell distribution with among subjects with mild and moderate non proliferative diabetic retinopathy. RDW is significantly increased in the moderate NPDR group.

PEARSON COEFFICIENT CORRELATION - COMPARING BMI AND HBA1C



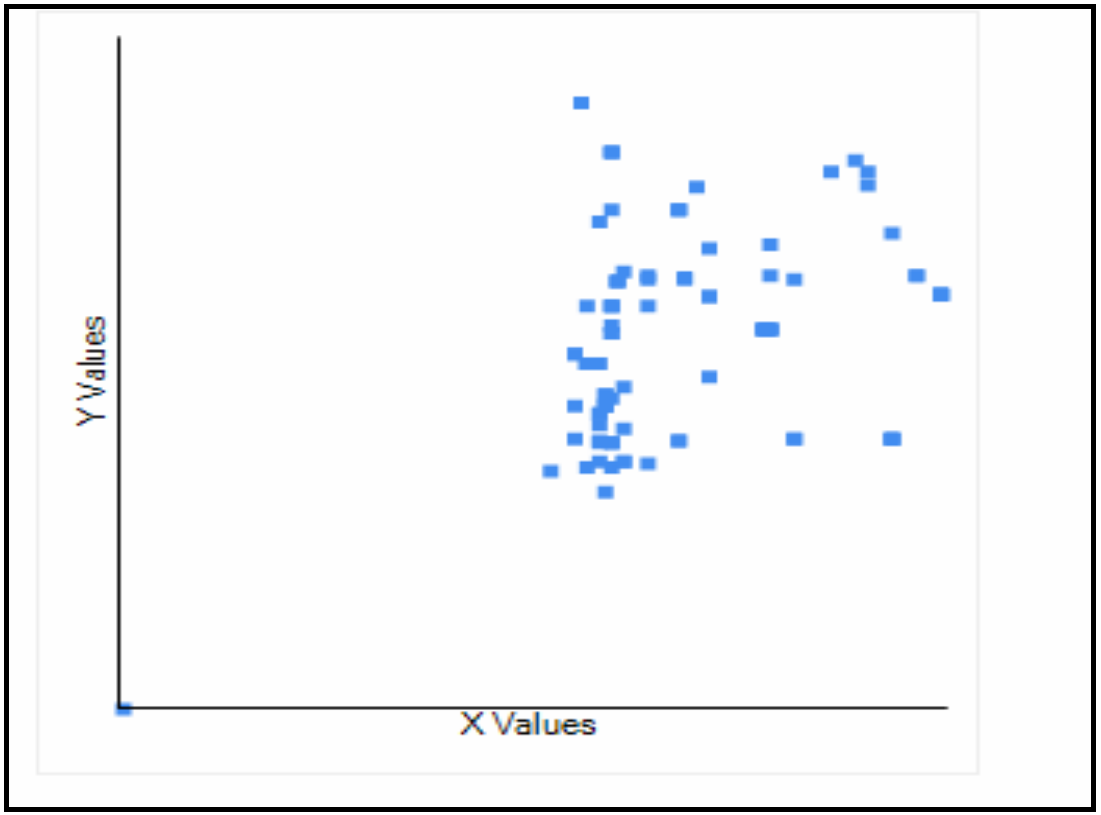
This diagram illustrates the pearson coefficient correlation between BMI and HBA1C

PEARSON COEFFICIENT CORRELATION - RDW AND ABG



This diagram illustrates the pearson coefficient correlation between RDW and ABG

PEARSON COEFFICIENT CORRELATION - RDW AND HBA1C

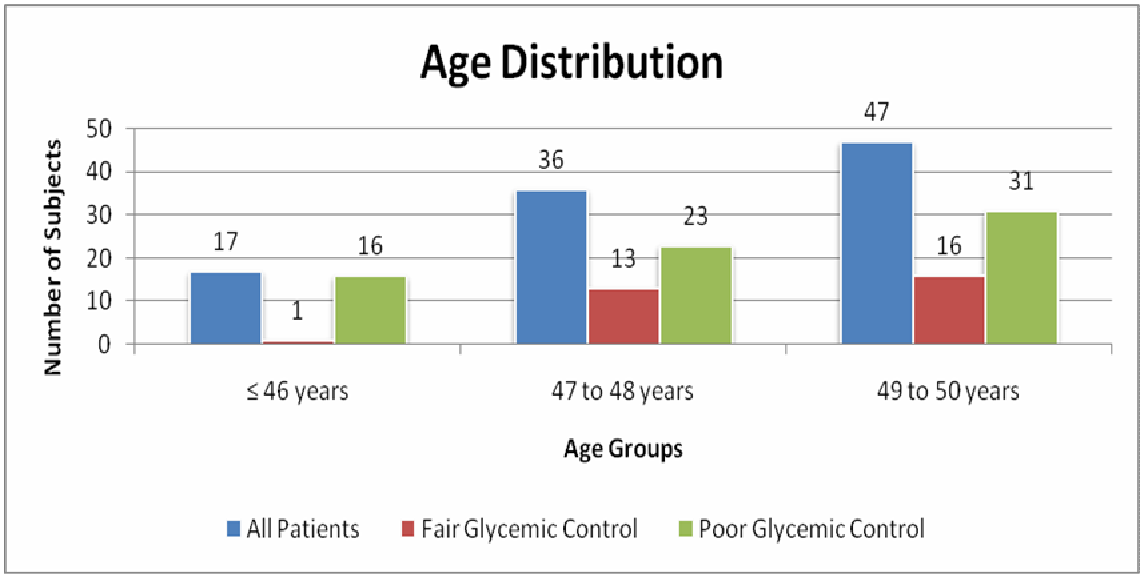


This diagram illustrates the pearson coefficient correlation between RDW and HBA1C

AGE

Age Distribution	All Patients	%	Mild NPDR	%	Moderate NPDR	%
≤ 46 years	17	17.00	4	9.30	13	22.81
47 to 48 years	36	36.00	12	27.91	24	42.11
49 to 50 years	47	47.00	27	62.79	20	35.09
Total	100	100	43	100	57	100

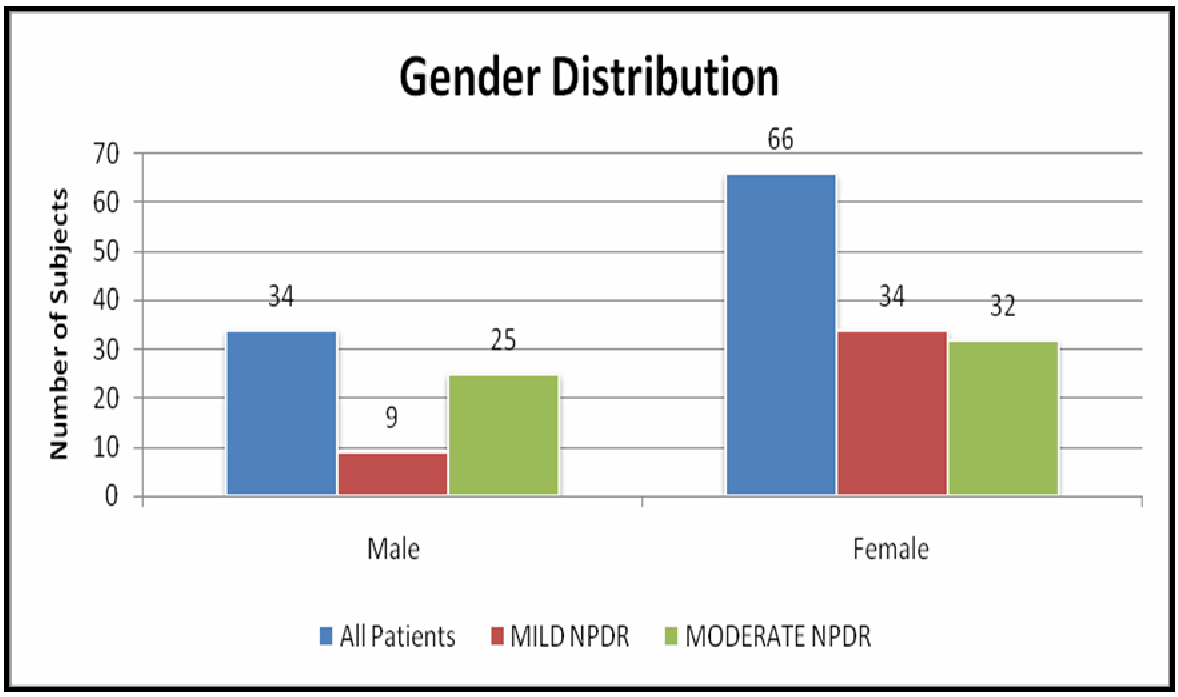
Age Distribution	Mild NPDR	Moderate NPDR
N	43	57
MEAN	48.84	48.00
SD	1.27	1.43
P value Unpaired t test	0.2603	



By conventional criteria the association between the study groups and age is considered to be not statistically significant since $p > 0.05$.

GENDER

Gender Distribution	All Patients	%	Mild NPDR	%	Moderate NPDR	%
Male	34	34.00	9	20.93	25	43.86
Female	66	66.00	34	79.07	32	56.14
Total	100	100	43	100	57	100
Chi-square value		5.74				
Degrees of freedom		1				
P value Chi Squared Test		0.1700				



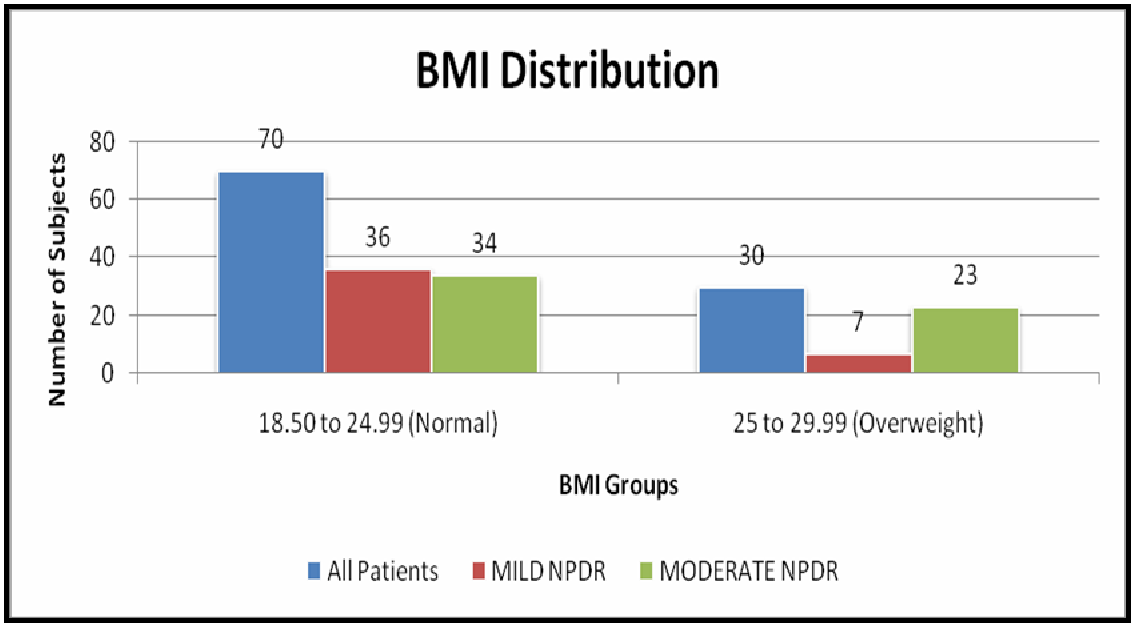
By conventional criteria the association between the study groups and gender is considered to be not statistically significant since $p > 0.05$.

Since age and gender is not statistically significant, it means that there is no difference between the groups. In other words the groups contain subjects with the same basic demographic characteristics.

BMI

BMI Distribution	All Patients	%	Mild NPDR	%	Moderate NPDR	%
18.50 to 24.99 (Normal)	70	70.00	36	83.72	34	59.65
25 to 29.99 (Overweight)	30	30.00	7	16.28	23	40.35
≥ 30 (Obese)	0	0.00	0	0.00	0	0.00
Total	100	100	43	100	57	100

BMI Distribution	Mild NPDR	Moderate NPDR
N	43	57
MEAN	22.75	24.55
SD	1.87	2.86
P value Unpaired t test	0.0002	



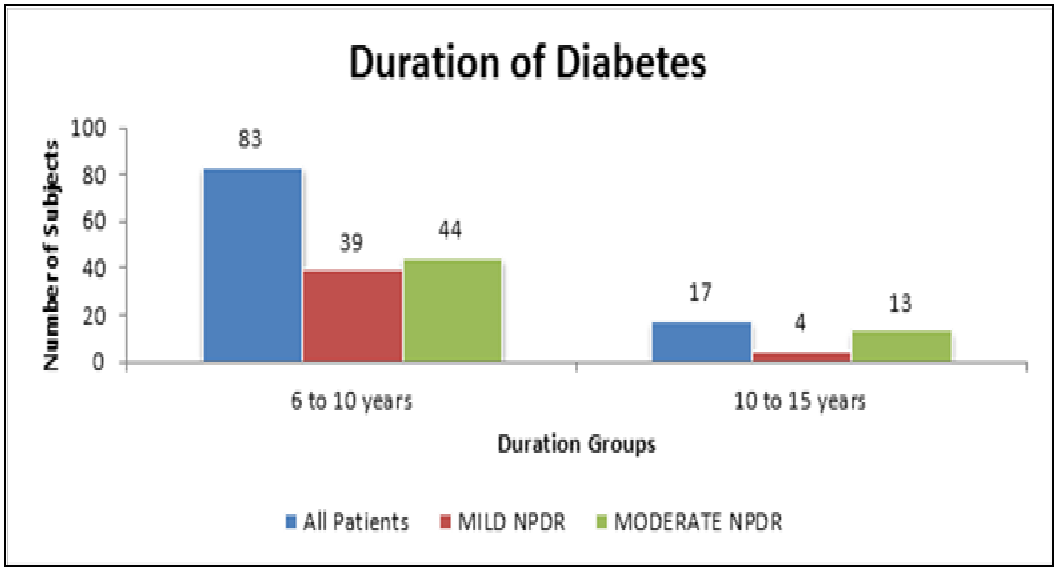
By conventional criteria the association between the study groups and BMI among study subjects is considered to be statistically significant since $p < 0.05$.

We conclude that there is meaningfully real increase in risk of developing moderate NDPR in persons with increased BMI among our study subjects. In short progression of NDPR increases significantly with higher BMI.

DURATION OF DIABETES

Duration of Diabetes	All Patients	%	Mild NPDR	%	Moderate NPDR	%
≤ 5 years	0	0.00	0	0.00	0	0.00
6 to 10 years	83	83.00	39	90.70	44	77.19
10 to 15 years	17	17.00	4	9.30	13	22.81
Total	100	100	43	100	57	100

Duration of Diabetes	Mild NPDR	Moderate NPDR
N	43	57
MEAN	9.01	9.18
SD	1.08	1.59
P value Unpaired t test	0.5408	



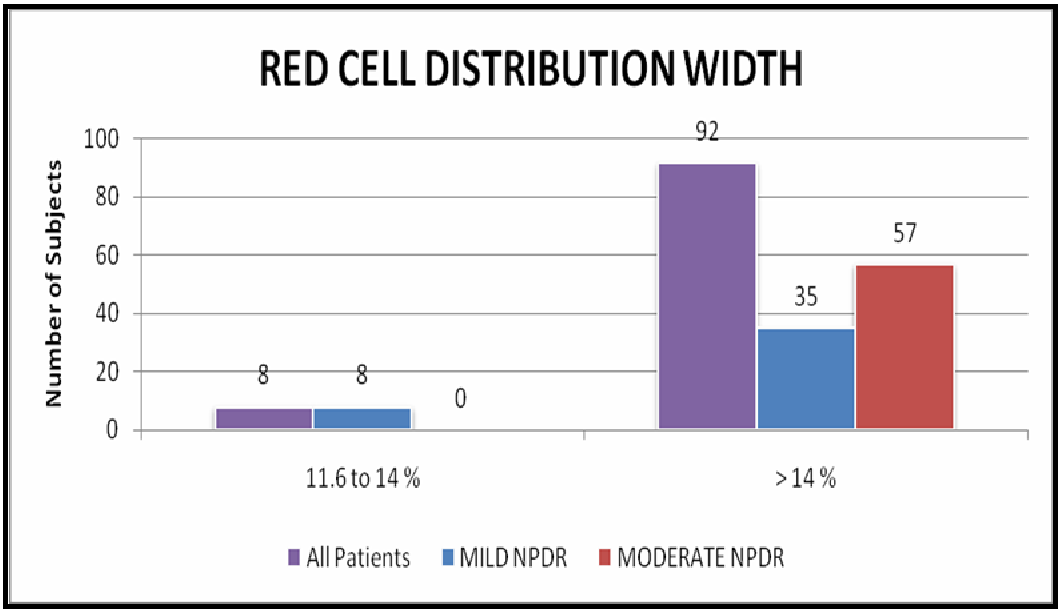
By conventional criteria the association between the study groups and duration of diabetes among study subjects is considered to be statistically significant since $p < 0.05$.

We conclude that there is meaningfully real increase in risk of developing moderate NDPR in persons with increased duration of diabetes among our study subjects. In short progression of NDPR increases significantly with higher duration of diabetes.

RED CELL DISTRIBUTION WIDTH

Red Cell Distribution Width	All patients	%	Mild NPDR	%	Moderate NPDR	%
< 11.6 %	0	0.00	0	0.00	0	0.00
11.6 to 14 %	8	8.00	8	18.60	0	0.00
> 14 %	92	92.00	35	81.40	57	100.00
Total	100	100	43	100	57	100

Red Cell Distribution Width	Mild NPDR	Moderate NPDR
N	43	57
MEAN	14.14	15.44
SD	0.70	0.72
P value Unpaired t test	0.0000	



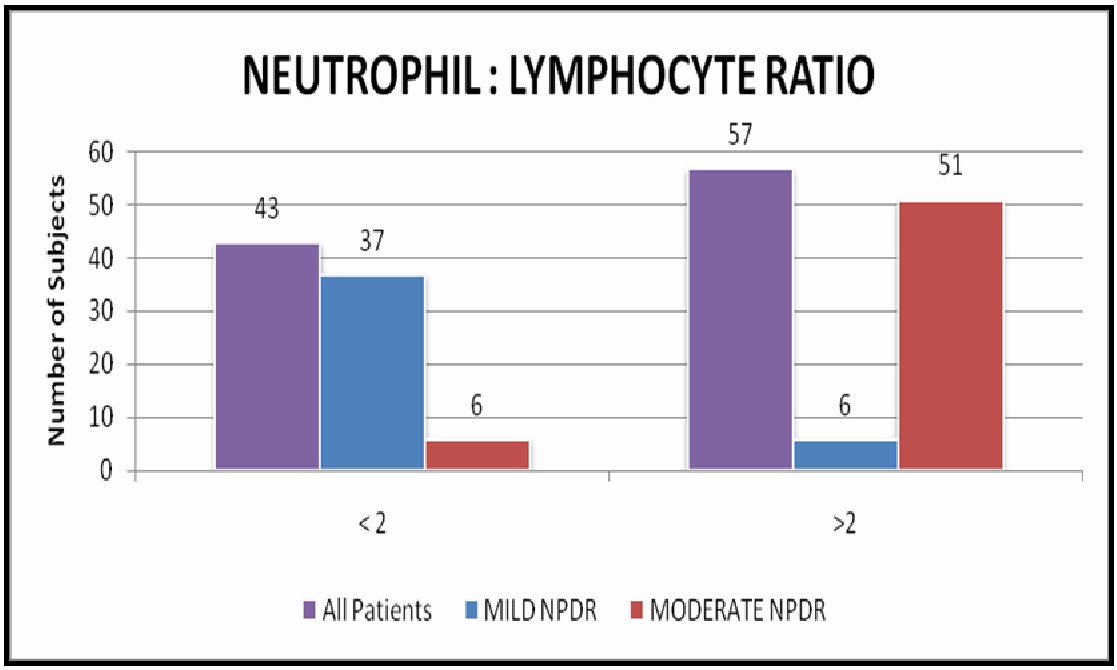
By conventional criteria the association between the study groups and red cell distribution width among study subjects is considered to be statistically significant since $p < 0.05$.

We conclude that there is meaningfully real increase in risk of developing moderate NDPR in persons with increased and red cell distribution width levels among our study subjects. In short the value of and red cell distribution width shows significantly increasing trend as severity of diabetic retinopathy increases.

NEUTROPHIL: LYMPHOCYTE RATIO

Neutrophil : Lymphocyte ratio	All Patients	%	Mild NPDR	%	Moderate NPDR	%
< 2	43	43.00	37	86.05	6	10.53
>2	57	57.00	6	13.95	51	89.47
Total	100	100	43	100	57	100

Neutrophil : Lymphocyte ratio	Mild NPDR	Moderate NPDR
N	43	57
MEAN	1.62	2.27
SD	0.34	0.44
P value Unpaired t test	0.0000	



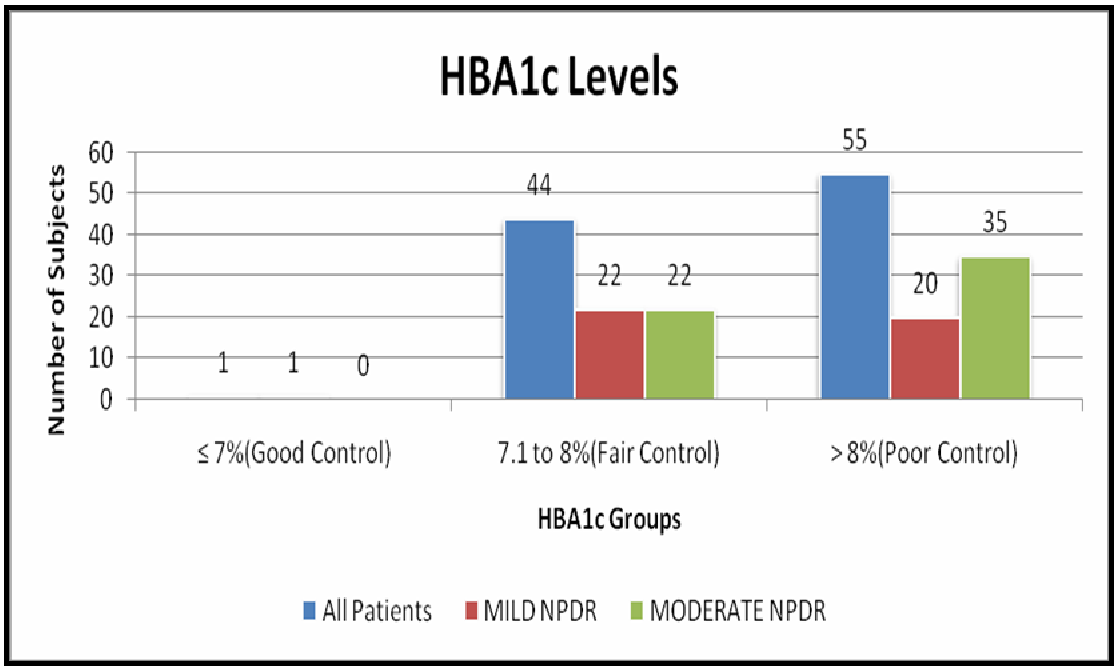
By conventional criteria the association between the study groups and neutrophil lymphocyte ratio among study subjects is considered to be statistically significant since $p < 0.05$.

We conclude that there is meaningfully real increase in risk of developing moderate NDPR in persons with increased neutrophil lymphocyte ratio levels among our study subjects. In short the value of neutrophil lymphocyte ratio shows significantly increasing trend as severity of diabetic retinopathy increases.

HBA1C

HBA1c Levels	All Patients	%	Mild NPDR	%	Moderate NPDR	%
≤ 7% (Good Control)	1	1.00	1	2.33	0	0.00
7.1 to 8% (Fair Control)	44	44.00	22	51.16	22	38.60
> 8% (Poor Control)	55	55.00	20	46.51	35	61.40
Total	100	100	43	100	57	100

HBA1c Levels	Mild NPDR	Moderate NPDR
N	43	57
MEAN	8.69	9.72
SD	1.62	2.00
P value Unpaired t test	0.0052	



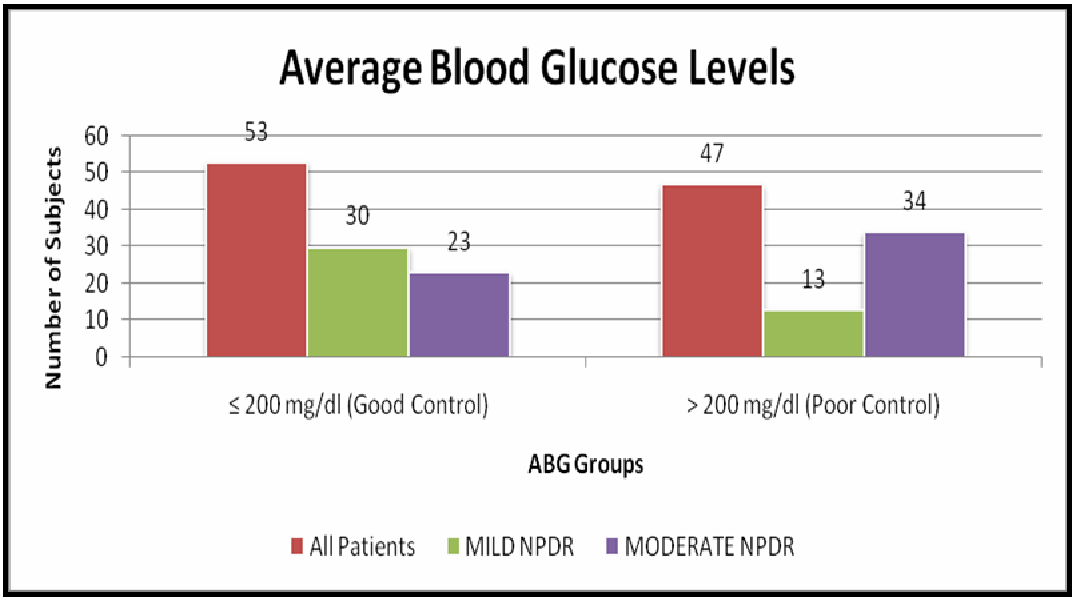
By conventional criteria the association between the study groups and Blood HBA1c levels among study subjects is considered to be statistically significant since $p < 0.05$.

We conclude that there is meaningfully real increase in risk of developing moderate NDPR in persons with increased HBA1c levels among our study subjects. In short the value of glycosylated haemoglobin shows significantly increasing trend as severity of diabetic retinopathy increases.

ABG

Average Blood Glucose Levels	All Patients	%	Mild NPDR	%	Moderate NPDR	%
≤ 200 mg/dl (Good Control)	53	53.00	30	69.77	23	40.35
> 200 mg/dl (Poor Control)	47	47.00	13	30.23	34	59.65
Total	100	100	43	100	57	100

Average Blood Glucose Levels	Mild NPDR	Moderate NPDR
N	43	57
MEAN	202.59	232.24
SD	46.38	57.29
P value Unpaired t test	0.0052	



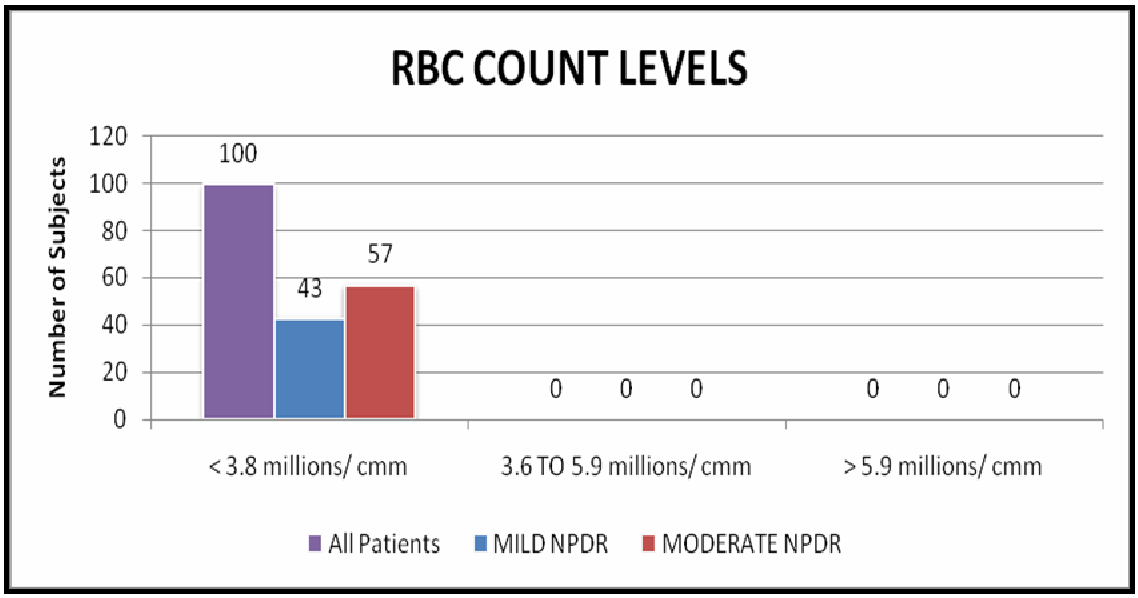
By conventional criteria the association between the study groups and average blood glucose levels among study subjects is considered to be statistically significant since $p < 0.05$.

We conclude that there is meaningfully real increase in risk of developing moderate NDPR in persons with increased average blood glucose levels among our study subjects. In short the value of average blood glucose shows significantly increasing trend as severity of diabetic retinopathy increases.

RBC COUNT

RBC Count	All Patients	%	Mild NPDR	%	Moderate NPDR	%
< 3.8 million/cumm	0	0.00	0	0.00	0	0.00
3.6 To 5.9 million/cumm	100	100.00	43	100.00	57	100.00
Total	100	100	43	100	57	100

RBC Count	Mild NPDR	Moderate NPDR
N	43	57
MEAN	4.65	4.80
SD	0.29	0.30
P value Unpaired t test	0.0143	

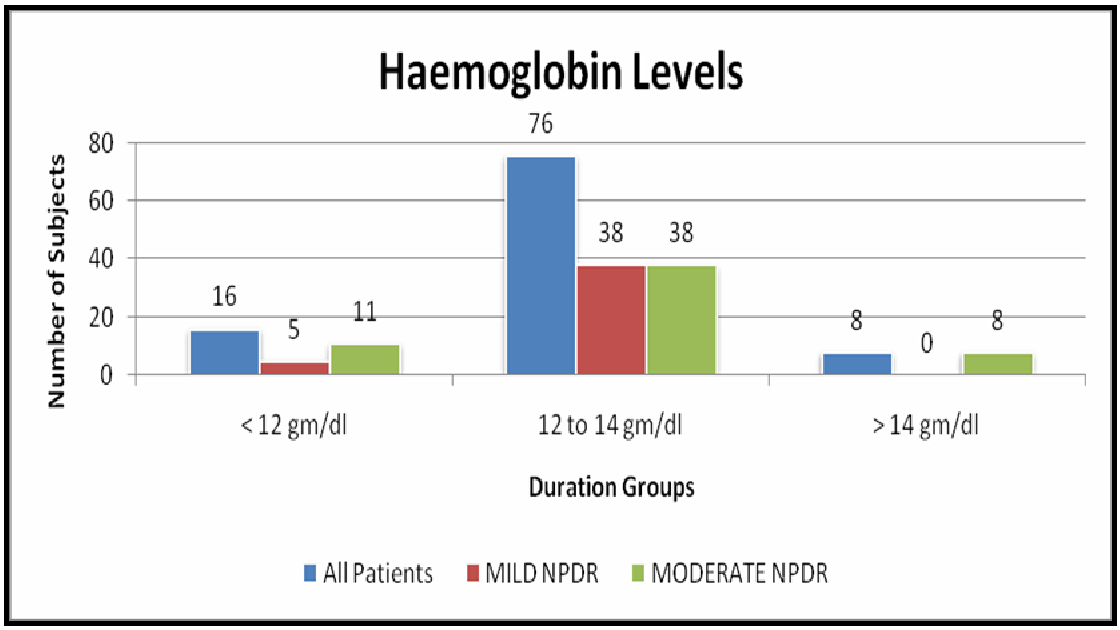


The RBC count levels in mild NDPR group is meaningfully less than moderate NDPR group by 1.12 times with a mean difference of 0.55 million/cu mm. This difference is true and significant and has not occurred by chance.

HAEMOGLOBIN

Haemoglobin Levels	All Patients	%	Mild NPDR	%	Moderate NPDR	%
< 12 gm/dl	16	16.00	5	11.63	11	19.30
12 to 14 gm/dl	76	76.00	38	88.37	38	66.67
> 14 gm/dl	8	8.00	0	0.00	8	14.04
Total	100	100	43	100	57	100

Haemoglobin Levels	Mild NPDR	Moderate NPDR
N	43	57
MEAN	13.00	12.94
SD	0.64	0.92
P value Unpaired t test	0.7203	

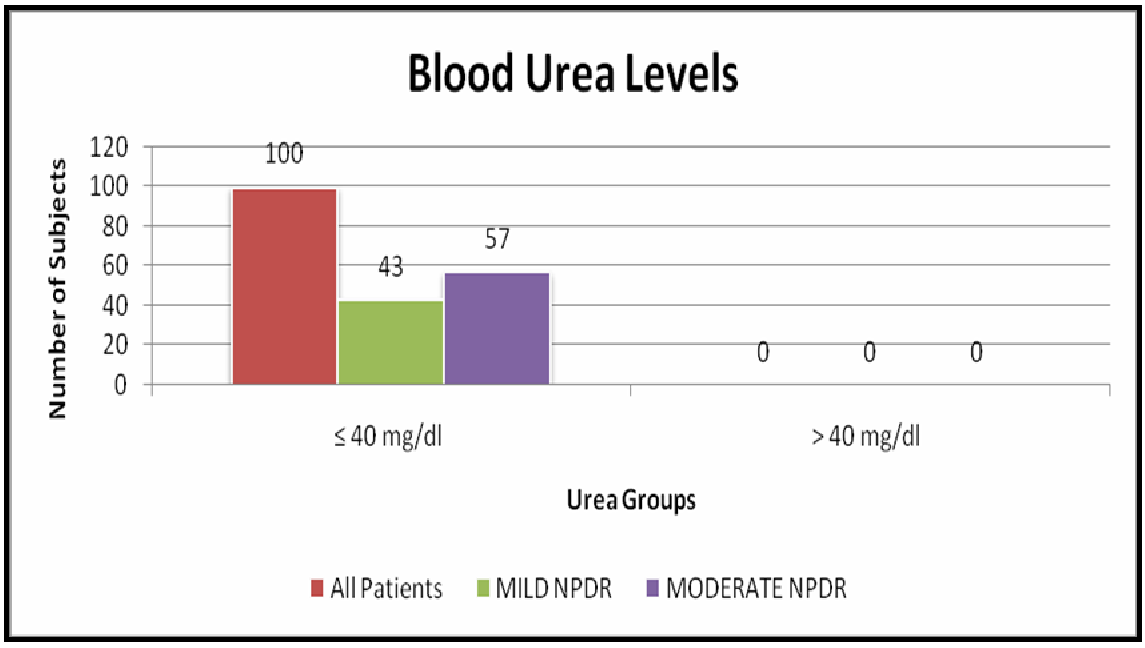


By conventional criteria the association between the study groups and Haemoglobin status is considered to be not statistically significant since $p > 0.05$.

UREA

Blood Urea Levels	All Patients	%	Mild NPDR	%	Moderate NPDR	%
≤ 40 mg/dl	100	100.00	43	100.00	57	100.00
> 40 mg/dl	0	0.00	0	0.00	0	0.00
Total	100	100	43	100	57	100

Blood Urea Levels	Mild NPDR	Moderate NPDR
N	43	57
MEAN	26.65	27.72
SD	3.52	3.24
P value Unpaired t test	0.1236	

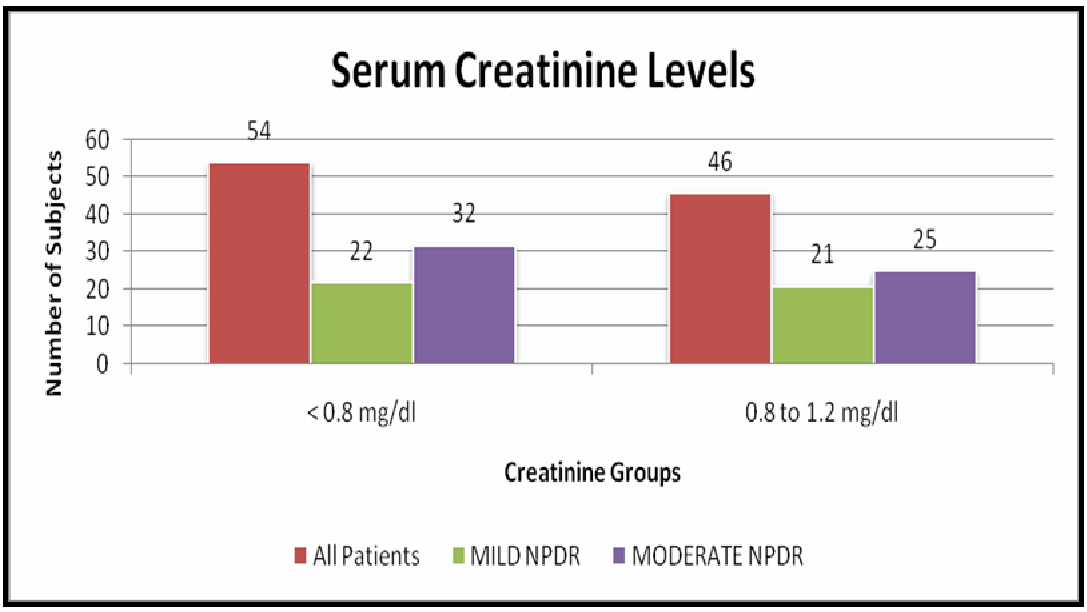


By conventional criteria the association between the study groups and blood urea status is considered to be not statistically significant since $p > 0.05$.

CREATININE

Serum Creatinine Levels	All Patients	%	Mild NPDR	%	Moderate NPDR	%
< 0.8 mg/dl	54	54.00	22	51.16	32	56.14
0.8 to 1.2 mg/dl	46	46.00	21	48.84	25	43.86
Total	100	100	43	100	57	100

Serum Creatinine Levels	Mild NPDR	Moderate NPDR
N	43	57
MEAN	0.66	0.65
SD	0.18	0.15
P value Unpaired t test	0.8206	

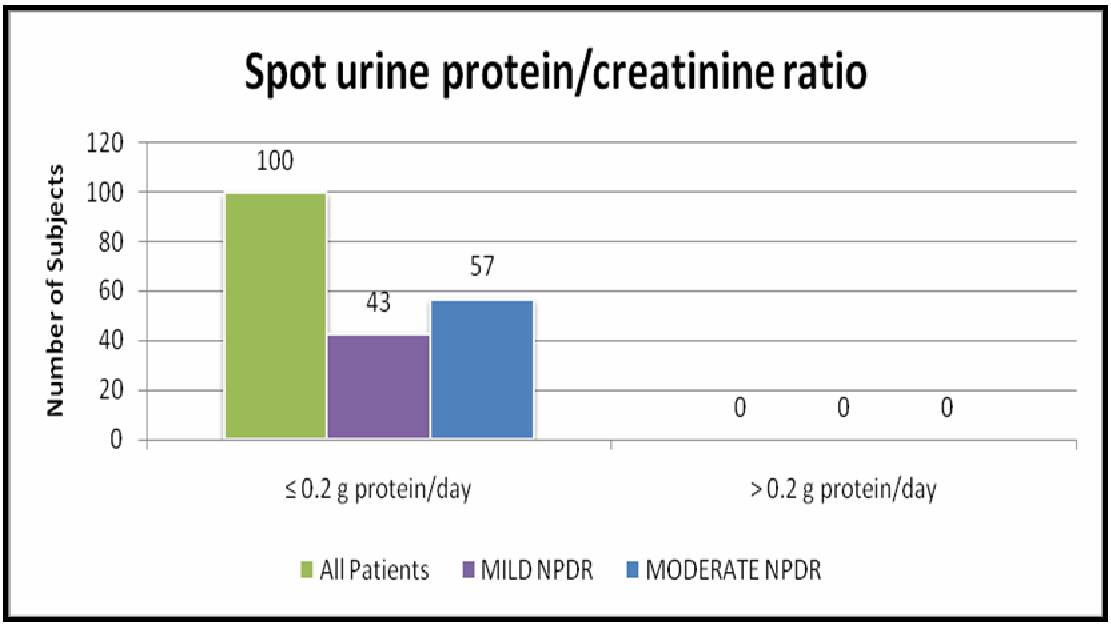


By conventional criteria the association between the study groups and serum creatinine status is considered to be not statistically significant since $p > 0.05$.

U.SPOT PCR

U.SPOT PCR Levels	All Patients	%	Mild NPDR	%	Moderate NPDR	%
≤ 0.2 g prote in/day	100	100.00	43	100.00	57	100.00
> 0.2 g prote in/day	0	0.00	0	0.00	0	0.00
Total	100	100	43	100	57	100

U.SPOT PCR Levels	Mild NPDR	Moderate NPDR
N	43	57
MEAN	0.12	0.12
SD	0.03	0.03
P value Unpaired t test	0.9951	



By conventional criteria the association between the study groups and U Spot PCR is considered to be not statistically significant since $p > 0.05$.

DISCUSSION

Diabetic Retinopathy is a major cause of blindness among diabetic patients. It is the sixth common cause of blindness in India. Prevalence of Diabetic retinopathy in patients with diabetes was recently estimated to be 34.6%.

Ocular fundus examination every year by an ophthalmoscope is the most important clinical assessment in a diabetic patient to detect diabetic retinopathy. But accessibility and awareness to undergo such an examination is lacking both in the diabetic population and among the medical practitioners.

Many pathophysiological disorders and mechanisms have been proposed for the development and progression of diabetic retinopathy but the most common are Rheological disorders[2] of red blood cells and RBC deformability.

Recently vascular inflammation in proposed as the basic pathogenic mechanism behind microvascular complications and hence detection of markers for vascular inflammation[4] can help us to diagnose diabetic microangiopathy particularly diabetic

retinopathy very early, so that active intervention in that stage would prevent a diabetic patient from becoming blind.

Since rheological disorders of RBCS play a pivotal sole in the pathogenesis of diabetic retinopathy, red cell distribution width[4] (a measure of variation in size and volume of RBCS) can be a useful marker for the detection of Diabetic Retinopathy.

Also the basis for such rheological disorders of RBCS leading to the development of NPDR is recently proposed as vascular inflammation, we tried to prove the markers of vascular inflammation also gets elevated in parallel to RDW during the progression of disease in NPDR. There are many number of inflammatory markers available, but Neutrophil– lymphocyte ratio[5] appears to be more significant.

Numerous studies[5] are available in literature for the correlation of NLR and vascular inflammation. Hence NLR in the early detection of NPDR appears to be the best predictor of progression of disease in DR.

In our study, (Cross sectionally designed observational study) we selected 100 Type-II diabetic patients with features of mild or moderate stages of diabetic retinopathy. No excluded severe NPDR

and proliferate DR because numerous studies have shown that a degree of erythropoietin resistance and albuminuria heralds the onset of proliferative DR. Also we excluded anaemia[3] from the study because of its interference with RDW and sometimes clinically undetectable erythropoietin resistance from early CKD can present leading to anaemia.

In our study, we found significant elevation of RDW[6] among subjects with mild and moderate NPDR. The elevation was significant in the moderates NPDR group. Statistically p value was found to be highly significant. Pearson coefficient correlation was found to be 0.465 when compared with HBA1C.

In the moderate NPDR group RDW elevation also parallels the HBA1C and average blood glucose values. All these subjects belonging to the moderate NPDR group had uncontrolled blood glucose levels and elevated HBA1C levels.

Neutrophil Lymphocyte Ratio[5] was also found to be significantly elevated among our subjects particularly in moderate NPDR group. Statistically p value was found to be very significant. These subjects also had elevation of HBA1C and blood glucose levels in parallel with the elevation of NLR. Pearson coefficient

correlation in comparing the NLR with HBA1C values was found to be significant.

RBC count is not significantly decreased in any of the subjects of our group. There is also no significant correlation with RDW and NLR.

All subject had normal urea and creatinine levels without any evidence of albuminuria.

Haemoglobin values were of normal levels and had no significant correlation with any of the parameter in our study.

HBA1C was elevated particularly among the moderate NPDR group and had significant correlation with the severity of disease and also duration of diabetes.

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SUMMARY AND CONCLUSION

SUMMARY

A study an establishing the role of RDW, NLR and RBC count as markers for the early detection of mild and moderate NPDR was conducted.

A total of 100 subjects meeting the inclusion and exclusion criteria were selected.

After a thorough clinical examination, fundus examination and blood sampling was done.

All results were analyzed by SPSS software and derivation were done by unpaired t test, chi-square test and Pearson coefficient correlation.

After analysis we found that both mild and moderate NPDR group of patients had significant elevation of RDW and NLR.

Hence RDW and NLR can be potential markers for early detection of mild and moderate NPDR.

CONCLUSION

Diabetic Retinopathy is a major concern among diabetic patients because failure to detect it early and failure to prevent the progression of disease leads to blindness in 90% of diseased population. Only expertise in fundus photography could be able to detect NPDR and PDR in diabetic patients.

Taking into account the huge diabetic population and the scarce availability of such experts, it will be difficult to screen & diagnose the entire diabetic population which is enlarging it an exponential manner yearly. Hence in the search for markers to defect early DR even at the primary care physician level, we took the opportunity to correlate RDW and NLR with different grades of NIDR.

From our study we conclude that, both NLR and RDW are useful markers of vascular inflammation for

1) Early diagnosis of Non-proliferative diabetic retinopathy.

2) Assessing the severity of non protiferative Diabetic Retinopathy as mild or moderate grades.

3) Also their prediction of uncontrolled glycaemic status since they correlate well with HBA1C and blood glucose levels.

LIMITATIONS OF OUR STUDY

Our study correlated RDW and NLR with vascular inflammation in Diabetic patients. Further diagnostic indices like Doppler study of carotid and peripheral vessels for identifying carotid intima medial thickness sand peripheral atherosclerotic vascular disease could have been done for correlation. But many factors like time and economy led to a limitation of evaluation of certain factors in our study.

SCOPE FOR FURTHER STUDY

FUTURE PROSPECTS OF THE STUDY

RDW and NLR are now both physiologically and pathologically established diagnostic indices of vascular disease particularly in diabetic population. Hence apart from their ability in diagnosing NPDR both can be used for certain other scenarios like

1) RDW and NLR can be used to diagnose diabetic macrovascular disease.

2) RDW and NLR can be used to diagnose and monitor diabetic foot complications.

3) RDW and NLR can be used to define the glycemic status apart from HBA1C levels.

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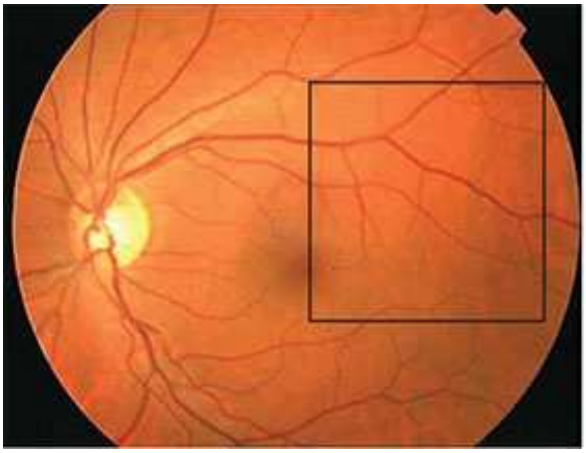
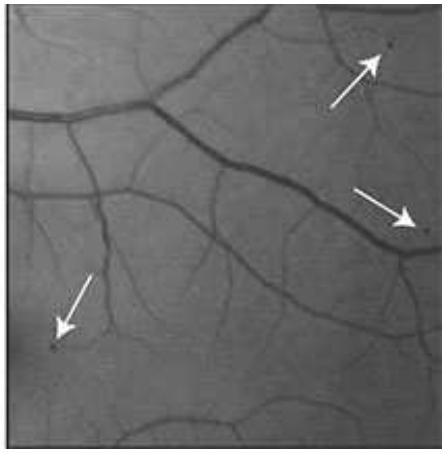
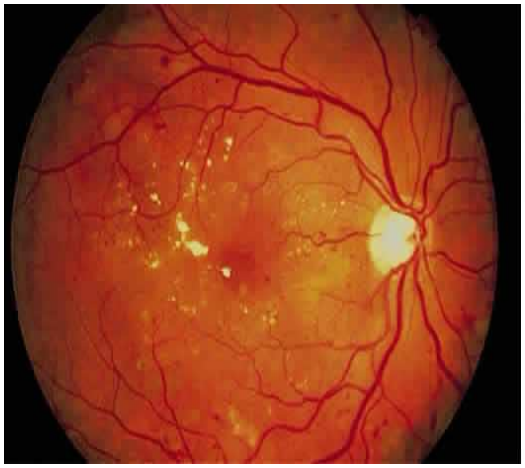
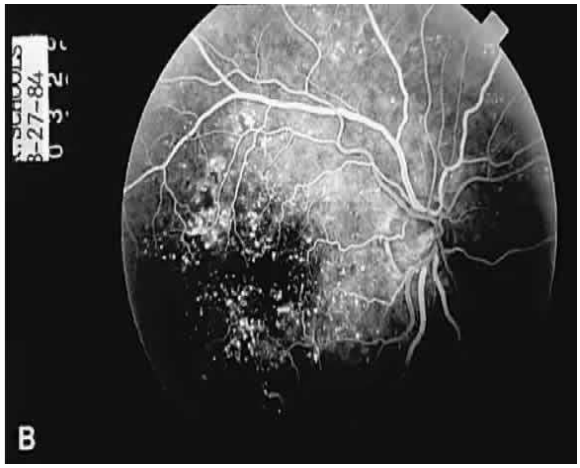
MICROANEURYSMS (HYPERFLUORESCENT DOTS), DOT BLOT HAEMORRHAGES (DARK SPOTS)



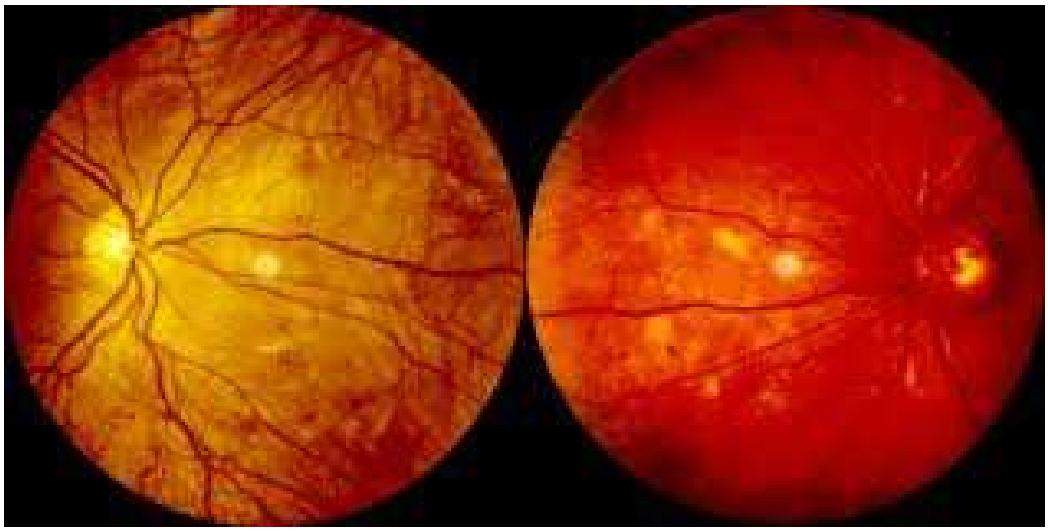


MACULAR EDEMA WITH HARD EXUDATES

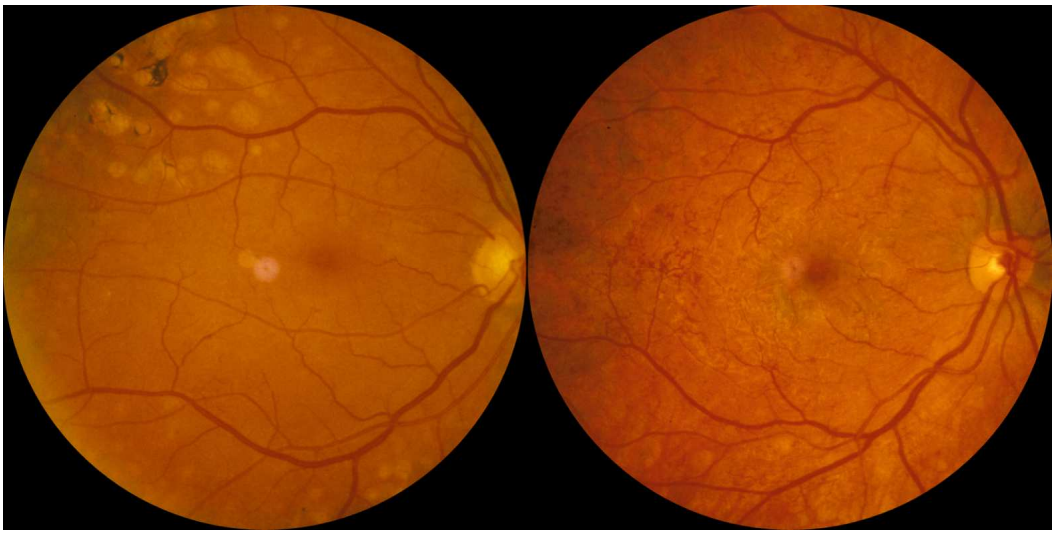




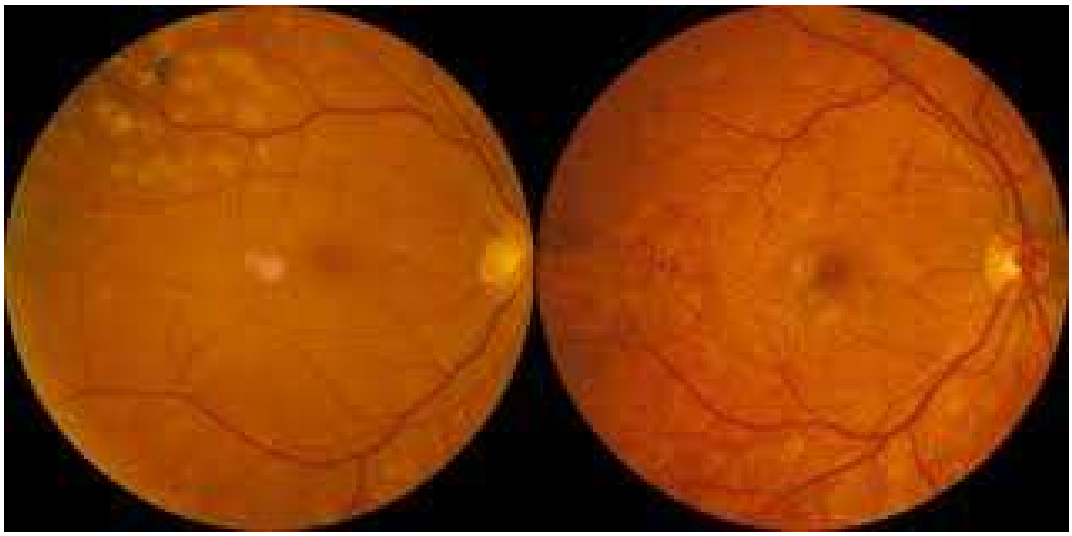
FUNDUS IMAGES OF COTTON WOOL SPOTS



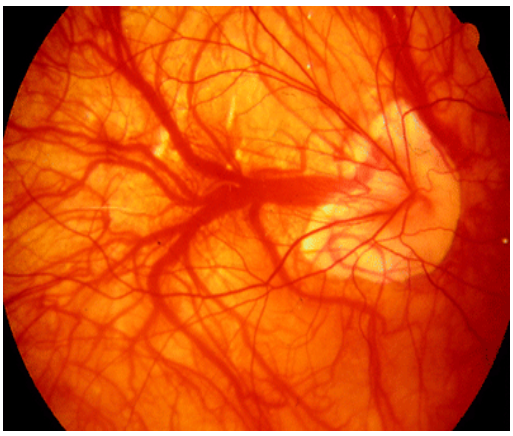
FUNDUS IMAGES OF VENOUS BEADING

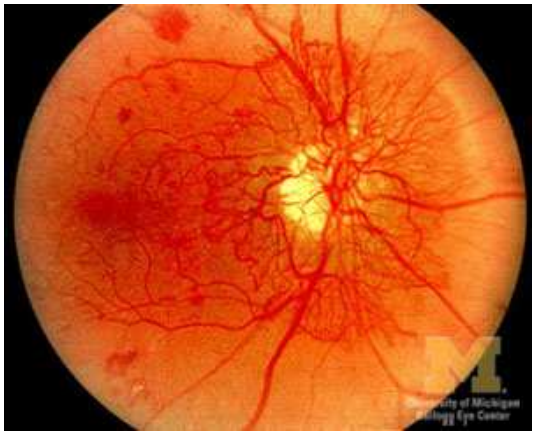


FUNDUS IMAGES OF IRMA

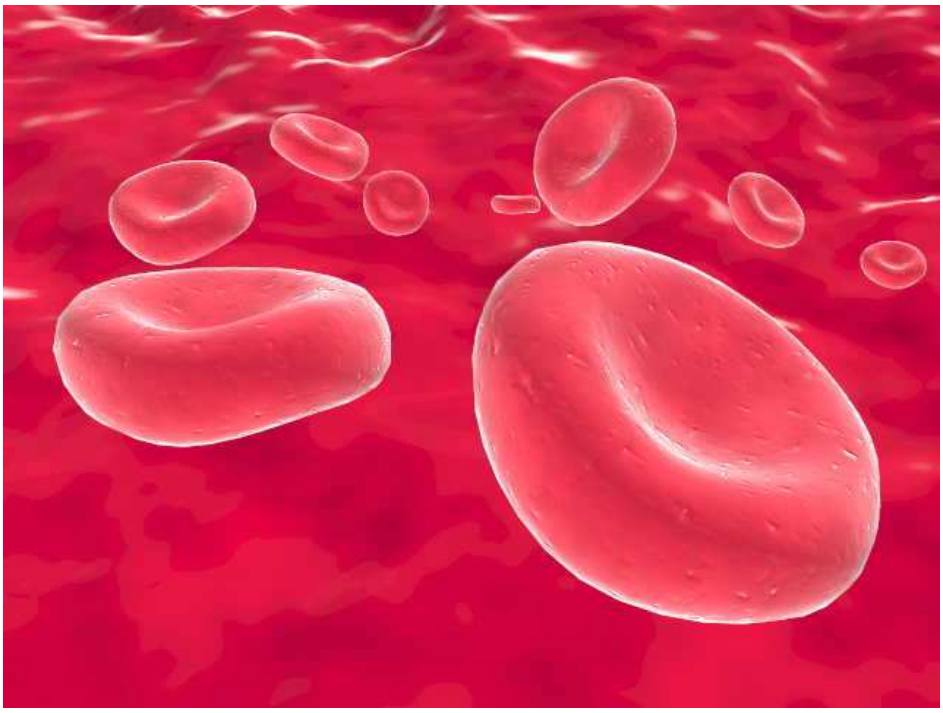


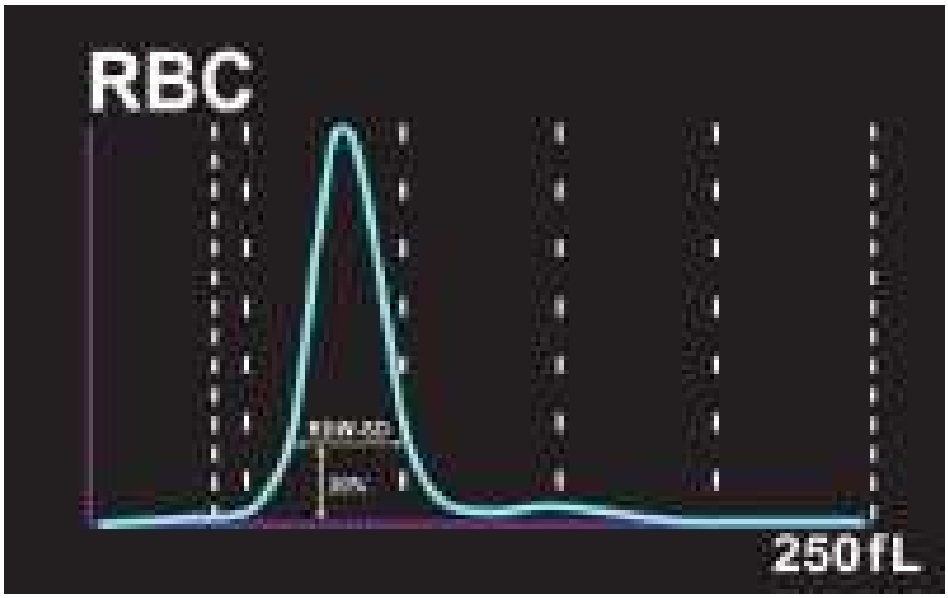
FUNDUS IMAGES OF NEOVASCULARIZATION



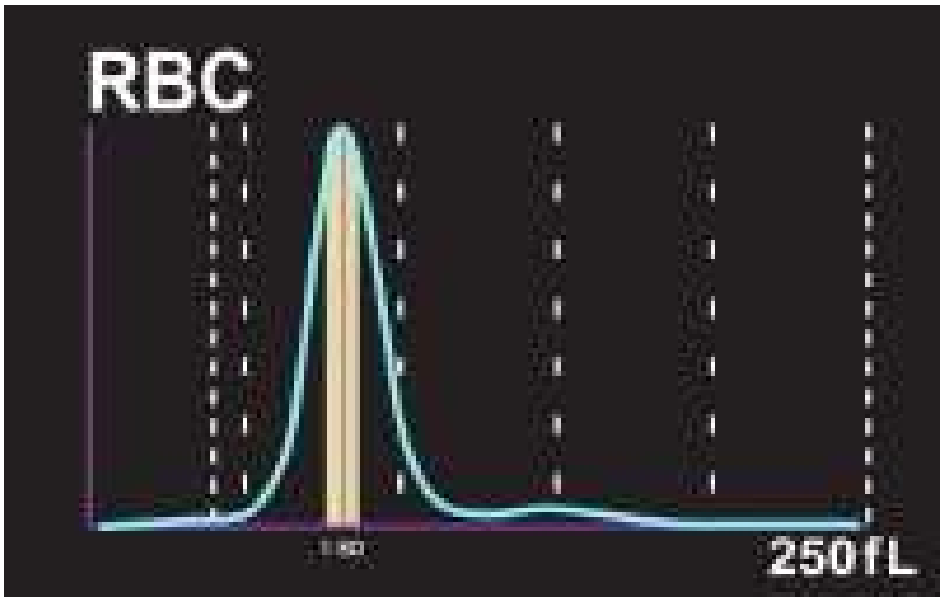


RED BLOOD CELL - 3D IMAGE



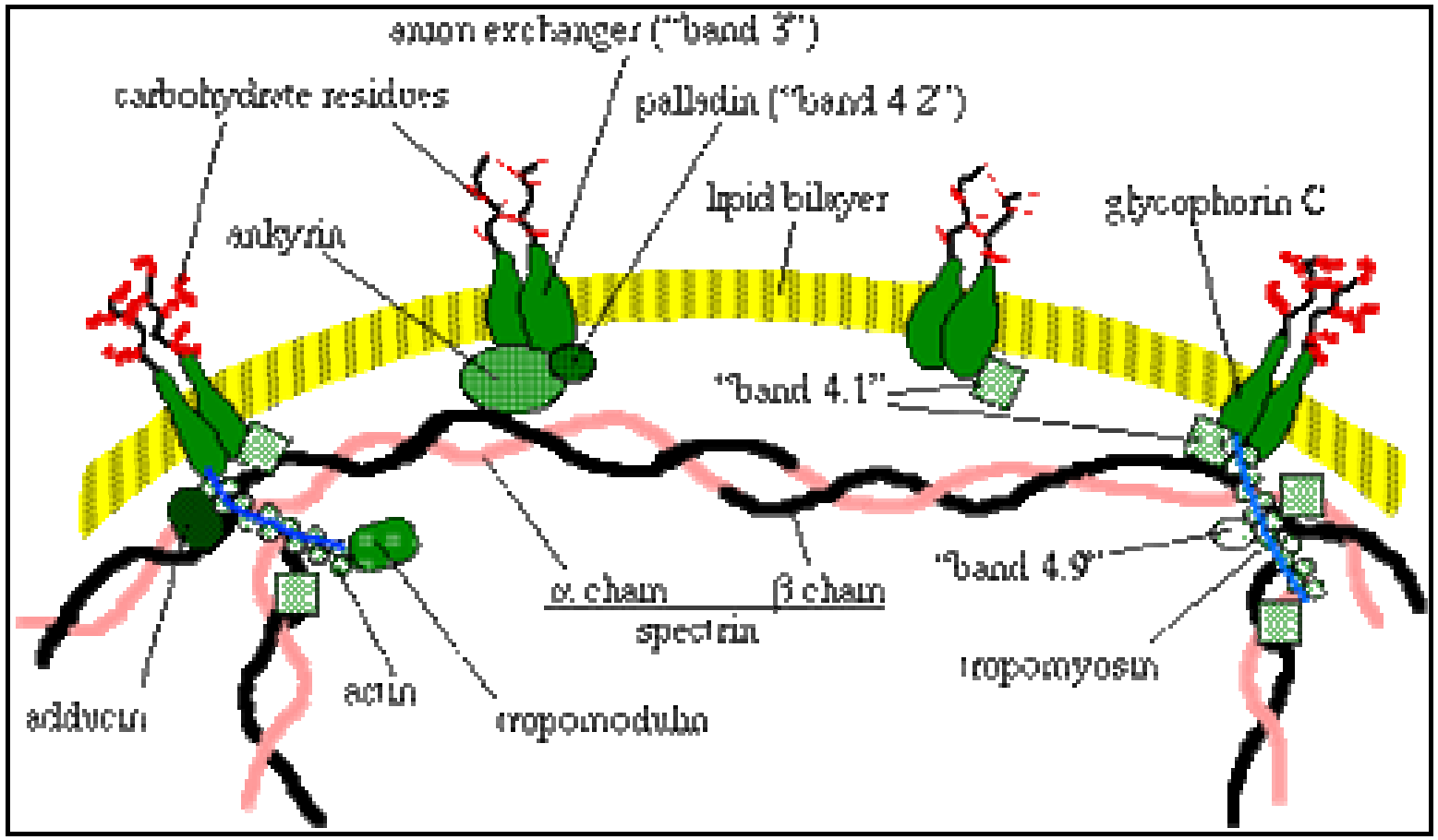


Determination of RDW-SD measurement.
In this example, RDW-SD is 38.2 fL.



Calculation of RDW-CV measurement, which is derived from 1SD divided by MCV times 100%. In this example, RDW-CV is 12.8%.

ORGANIZATION OF THE ERYTHROCYTE MEMBRANE



PROFORMA

Name: Age: Sex:

Address: Ph.No:

Occupation: Income: Religion:

Duration of Diabetes:

Preventing Complaints: Pertinent to Vision

PAST MEDICAL HISTORY

Hypertension/ IHD/ Stroke/ Trauma/ Recent Surgeries

Drug Intake:

Family History: DM/HT/IHD

Personal History: Diet, Veg/ Non Veg

Smoking: Average No. of beedies/ Cigarates/ Day / Year

Alcohol: Quantity consumed/ day x Duration in Years

GENERAL EXAMINATION

Built and Nourishment

Height in cm: Weight in Kgs: BMI in Kg/m2:

Pallor/ cyanosis/ Clubbing/ Edema/ Jaundice/ Lymphadenopathy

VITAL PARAMETERS

Pulse: BP:

SYSTEMIC EXAMINATION

CVS: RS: P/A: CNS:

FUNDUS EXAMINATION

Micro Aneurysms

Hard Exudates

Haemorrhages

Phlebopathies

INVESTIGATIONS

Blood

Hb%
PCV
RBC Count
RDW
WBC Count
Differential Count: N: L: E: M:
MCV: MCH: MCHC
PLT Count: PDW:
ESR:
ABG: HBA1C:
Urea: Creatinine:
Urine Spot PCR

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : A Study on red Cell distribution width, red Blood Cell count and neutrophil/lymphocyte ratio as potential markers of vascular inflammation in the early detection of micro-vascular complications particularly diabetic retinopathy in patients with diabetes attending Govt Stanley Hospital, Chennai

Principal Investigator : Dr. P Boopathi Rajan

Designation : PG in MD (General Medicine)

Department : Department of General Medicine
Government Stanley Medical College,
Chennai-01

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 02.07.2014 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

K Vasanta
MEMBER SECRETARY,
IEC, SMC, CHENNAI

GOVT. STANLEY MEDICAL COLLEGE, CHENNAI – 600001

INFORMED CONSENT

A study on Red cell Distribution width, Red Blood Cell count and Neutrophil/ Lymphocyte Ratio as Potential Markers of Vascular Inflammation in the Early Detection of Microvascular Complications Particularly Diabetic Retinopathy in patients with Diabetes Attending Government Stanley Hospital, Chennai.

Place of study: Govt. Stanley Medical College, Chennai

I ..... have been informed about the details of the study in my own language.

- I have completely understood the details of the study.
- I am aware of the possible risks and benefits, while taking part in the study.
- I understand that I can withdraw from the study at any point of time and even then, I can receive the medical treatment as usual.
- I understand that I will not get any money for taking part in the study.
- I will not object if the results of this study are getting published in any medical journal, provided my personal identity is not revealed.
- I know what I am supposed to do by taking part in this study and I assure that I would extend my full cooperation for this study.

Volunteer: Name and address Signature/thumb impression Date	Witness: Name and address Signature/thumb impression Date

Investigator
Signature and date

அரசு ஸ்டான்லி மருத்துவக் கல்லூரி, சென்னை-600 001.

A study on Red cell Distribution width, Red Blood Cell count and Neutrophil/ Lymphocyte Ratio as Potential Markers of Vascular Inflammation in the Early Detection of Microvascular Complications Particularly Diabetic Retinopathy in patients with Diabetes Attending Government Stanley Hospital, Chennai.

நான் இந்த ஆராய்ச்சியின் விவரங்களை முற்றிலும் புரிந்துகொண்டேன்.

ஆய்வில் பங்கு எடுத்தபோது, சாத்தியமான அபாயங்கள் மற்றும் பயன்களை பற்றி நான் அறிந்துள்ளேன்.

நான் எந்தவொரு வேளையிலும் ஆய்வில் இருந்து திரும்ப முடியும், அதன் பின்னர், நான் வழக்கம்போல் மருத்துவ சிகிச்சை பெற முடியும் என்று புரிந்துகொள்கிறேன்.

நான் ஆய்வில் பங்கு எடுத்து பணம் எதையும் பெற முடியாது என்று அறிந்துள்ளேன்.

இந்த ஆய்வின் முடிவுகள் எந்த மெடிக்கல் ஜர்னலில் வெளியிடப்பட இருந்தால் அதை நான் எதிர்க்கவில்லை, என் தனிப்பட்ட அடையாளத்தை வெளிப்படுத்தப்பட்டு இருக்கக் கூடாது.

நான் இந்த ஆய்வில் பங்கெடுப்பதன் மூலம் நான் என்ன செய்யப் போகிறேன் என்று தெரியும். நான் இந்த ஆய்வில் என் முழு ஒத்துழைப்பையும் கொடுப்பேன் என்று உறுதியளிக்கிறேன்.

தன்னார்வளர்
பெயர் மற்றும் முகவரி
கையொப்பம்/ விரல் ரேகை

சாட்சி
பெயர் மற்றும் முகவரி
கையொப்பம்/ விரல் ரேகை

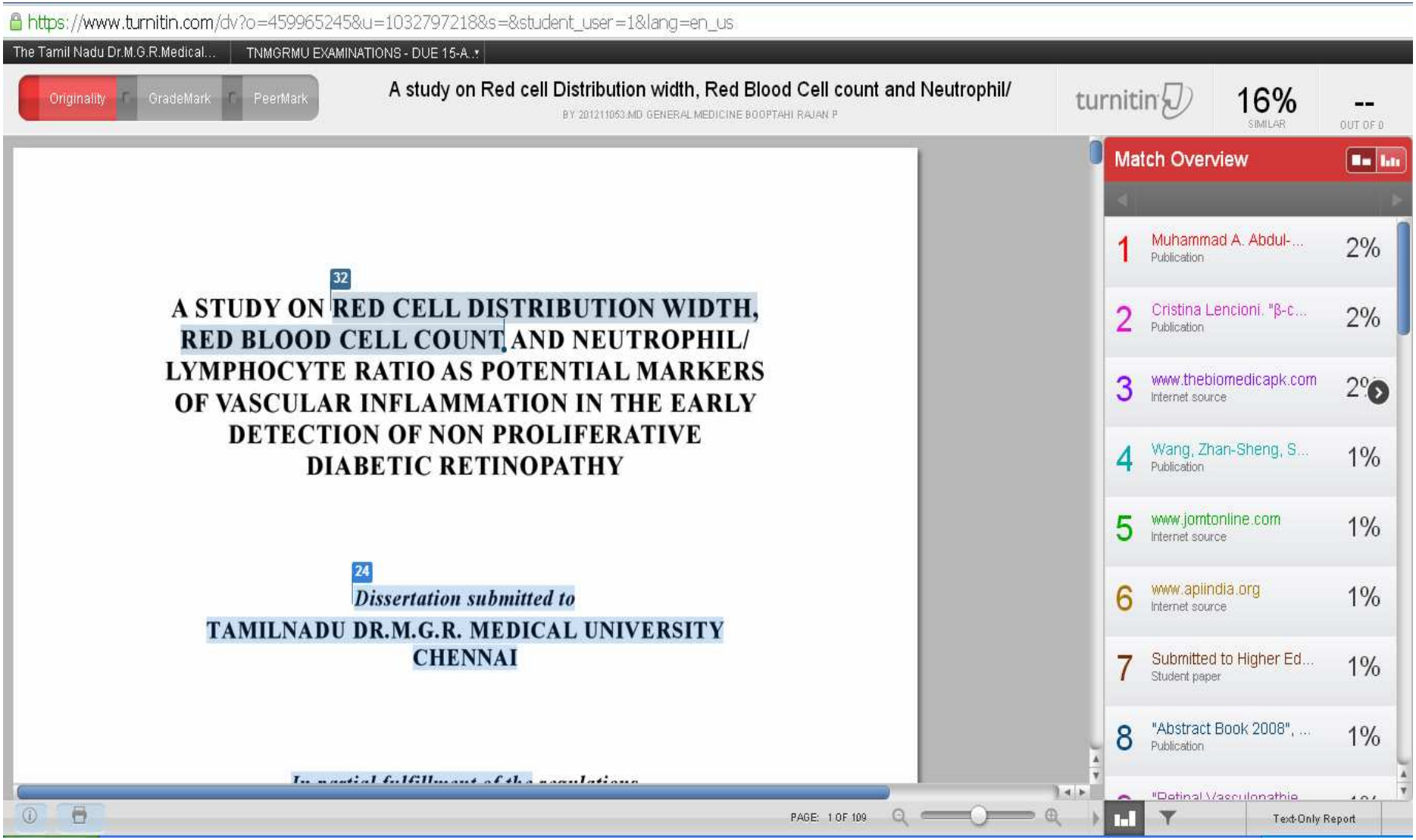
ஆராய்ச்சியாளராக
கையொப்பம் மற்றும் தேதி

SL.NO	Age	SEX	BMI	D-DIABET	HB	HBA1C	ABG	UREA	CREAT	U.SPOT PCR	RBC COUNT	N/L	RDW	NPDR- GR
1	50	F	21.1	8	11.8	7.4	165.68	28	0.8	0.12	4.41	1.85	15.6	MILD
2	50	F	27.3	11	13.5	8.2	188.64	29	0.8	0.11	4.77	1.29	14.2	MILD
3	50	F	26.1	10	13.2	12.6	314.92	27	0.8	0.14	4.88	1.41	14.5	MILD
4	48	F	24	12	11.6	10.6	257.52	27	0.8	0.12	4.84	1.98	15.1	MOD
5	48	M	27.6	10	14.9	7.5	168.55	30	0.6	0.08	5.55	3.16	14.9	MOD
6	46	M	29	11	13.5	13.4	337.88	32	0.8	0.13	5.03	2.16	15.1	MOD
7	48	F	27.2	12	11.8	7.9	180.03	28	0.6	0.09	4.73	1.13	17.4	MOD
8	50	F	24	8	13.7	7.8	177.16	30	0.9	0.12	4.96	1.54	12.2	MILD
9	49	F	23.2	9	11.8	8.1	185.77	29	0.9	0.14	4.03	2.23	12.4	MILD
10	49	F	21.6	8	12.7	7.9	180.03	30	0.6	0.09	4.69	1.58	14.5	MOD
11	50	F	22.3	10	13.9	8	182.9	34	0.6	0.16	5.05	1.39	13.8	MILD
12	48	F	21.9	8	12.9	9.6	228.82	22	0.8	0.08	4.63	2.15	14.3	MILD
13	50	F	21	8	11.9	12.2	303.44	30	0.5	0.09	4.56	2.73	14.4	MOD
14	46	F	23.1	8.5	13.5	9.1	214.47	21	0.6	0.12	4.42	1.4	14.1	MILD
15	48	M	21.3	8	13.2	7.6	171.42	26	0.5	0.11	4.41	1.8	14.2	MILD
16	47	F	22.6	6.5	11.9	10.5	254.65	23	0.6	0.14	4.63	1.98	14.5	MOD
17	46	M	29	11	13.5	13.4	337.88	32	0.8	0.13	5.03	2.16	15.1	MOD
18	50	F	27.1	10	12	8	182.9	28	0.8	0.09	4.42	2.1	15.5	MOD
19	48	F	22	8	12.4	9.1	214.47	26	0.3	0.12	4.71	2.6	15.3	MOD
20	46	M	21.6	8.5	14.1	8	182.9	28	0.6	0.16	5.2	2.9	14.8	MOD
21	48	M	25.1	8	13.2	10.6	257.52	32	0.8	0.14	4.5	2.42	15.8	MOD
22	50	F	21.3	9	12.2	7.8	177.16	26	0.6	0.08	4.71	1.29	13.2	MILD
23	49	F	21	8.5	13.5	7	154.2	25	0.2	0.11	4.96	1.24	14.1	MILD
24	46	F	21.3	8	12.9	7.6	171.42	22	0.6	0.09	4.41	1.8	14.2	MILD
25	46	M	21.6	8.5	14.1	8	182.9	28	0.6	0.16	5.1	2	14.8	MOD
26	50	F	25.1	10	13.9	12.2	303.44	26	0.5	0.09	4.54	2.8	15.8	MOD
27	48	F	27.2	12	12.8	7.6	171.42	28	0.6	0.12	4.73	2.1	17.4	MOD
28	50	F	24	10	13.2	11	269	27	0.8	0.11	4.88	1.41	14.5	MILD
29	46	M	29	8	13.5	13	326.4	32	0.8	0.14	5.03	2.26	16	MOD
30	49	F	21.6	8	12.7	7.9	180.03	30	0.8	0.13	4.68	1.64	15.2	MOD
31	50	F	21	8.5	12	9.4	223.08	28	0.8	0.12	4.54	2.72	15.4	MOD
32	50	M	25.2	12	13.2	11.6	286.22	32	0.8	0.14	4.52	2.8	14.5	MILD
33	49	F	21.1	8	12.7	7.8	177.16	26	0.2	0.09	5.05	1.39	14.2	MILD
34	50	F	24	8.5	13.1	8.6	200.12	24	0.4	0.16	4.54	2.26	16.2	MOD
35	47	F	24.6	6.5	12.3	10.5	254.65	28	0.4	0.08	4.64	1.98	15.6	MOD
36	49	F	23.1	9	12.1	7.8	177.16	30	0.8	0.09	4.94	1.4	14.6	MILD
37	48	M	21.4	8	13.8	8	182.9	26	0.4	0.12	4.42	1.62	14.2	MILD
38	50	M	23.4	10	13.2	8.2	188.64	30	0.8	0.11	4.68	1.46	14.1	MILD
39	48	F	21.2	8	12.6	7.6	171.42	26	0.4	0.14	4.54	1.26	14.6	MILD
40	49	F	22.4	9.5	12.2	7.4	165.68	28	0.5	0.13	4.64	1.41	14.5	MILD
41	48	M	27.6	10	14.9	7.5	168.55	30	0.6	0.09	5.55	3.16	14.9	MOD
42	46	M	29	11	13.5	13.4	337.88	32	0.8	0.12	5.03	2.16	15.1	MOD
43	50	F	22.3	10	13.9	8	182.9	34	0.6	0.11	5.05	1.39	13.8	MILD
44	48	F	21.9	8	12.9	9.6	228.82	22	0.8	0.14	4.63	2.15	14.3	MILD
45	50	F	26.1	10	13.2	12.6	314.92	27	0.8	0.12	4.88	1.41	14.5	MILD
46	48	F	24	12	11.6	10.6	257.52	27	0.8	0.08	4.84	1.98	15.1	MOD
47	49	F	23.2	9	11.8	8.1	185.77	29	0.9	0.13	4.03	2.23	12.4	MILD
48	49	F	21.6	8	12.7	7.9	180.03	30	0.6	0.09	4.69	1.58	14.5	MOD
49	46	F	23.1	8.5	13.5	9.1	214.47	21	0.6	0.12	4.42	1.4	14.1	MILD
50	48	M	21.3	8	13.1	7.6	171.42	26	0.5	0.14	4.41	1.8	14.2	MILD
51	46	F	19.8	8	12.6	8.2	188.64	24	0.6	0.09	4.42	1.68	14.4	MILD
52	49	F	21.2	10	13.1	8	182.9	22	0.4	0.16	4.84	1.96	16.2	MOD
53	50	M	24	11	13.6	10.6	257.52	24	0.8	0.08	4.74	2.26	16.6	MOD
54	50	M	21.8	8	13.2	12.6	314.92	24	0.6	0.09	4.68	2.48	16.5	MOD
55	48	F	22.2	8	12.8	8.6	200.12	22	0.5	0.16	4.65	1.28	14.4	MILD
56	50	M	21.2	10.5	13.6	7.8	177.16	30	0.8	0.13	5.06	1.48	14.2	MILD
57	46	F	24	8	13.2	9.6	228.82	24	0.8	0.12	4.42	2.4	16.2	MOD
58	48	F	22.6	9	12.8	9.2	217.34	22	0.6	0.08	4.63	2.25	16	MOD
59	49	F	21.2	10	13.1	8	182.9	22	0.4	0.06	4.82	1.26	14.6	MILD

SL.NO	Age	SEX	BMI	D-DIABET	HB	HBA1C	ABG	UREA	CREAT	U.SPOT PCR	RBC COUNT	N/L	RDW	NPDR- GR
60	50	M	24	10	13.7	7.8	177.16	30	0.8	0.11	4.96	2.54	16.6	MOD
61	50	F	26.1	10	13.2	12.6	314.92	27	0.8	0.13	4.88	1.41	14.5	MILD
62	48	F	24	12	12.1	10.6	257.52	27	0.8	0.16	4.84	1.98	15.1	MOD
63	48	M	27.6	10	14.9	7.5	168.55	30	0.6	0.12	5.55	3.16	14.9	MOD
64	49	F	23.2	9	11.8	8.1	185.77	29	0.9	0.14	4.03	2.23	12.4	MILD
65	49	F	21.6	8	12.7	7.9	180.03	30	0.6	0.09	4.69	1.58	14.5	MOD
66	50	F	22.3	10	13.9	8	182.9	34	0.6	0.16	5.05	1.39	13.8	MILD
67	48	M	21.3	8	13.1	7.6	171.42	26	0.5	0.08	4.41	1.8	14.2	MILD
68	47	F	22.6	6.5	11.9	10.5	254.65	23	0.6	0.09	4.63	1.98	14.5	MOD
69	46	M	29	11	13.5	13.4	337.88	32	0.8	0.16	5.03	2.16	15.1	MOD
70	48	M	27.6	9	13.8	8.6	200.12	30	0.8	0.13	4.9	2.1	16.4	MOD
71	47	F	21.6	8	12.8	7.4	165.68	22	0.6	0.12	4.69	1.58	14.5	MILD
72	48	F	21.8	8.5	12.1	9.6	228.82	26	0.8	0.08	4.56	1.73	14.4	MILD
73	48	F	22.6	8.5	13.2	7.8	177.16	22	0.6	0.06	4.42	1.8	14.2	MILD
74	48	M	21.2	8	13.2	9.2	217.34	30	0.6	0.09	4.42	2.24	15.8	MOD
75	49	M	22	8	13.7	7.8	177.16	22	0.6	0.12	4.96	1.54	14.2	MILD
76	48	F	27.2	10	12.1	11	269	30	0.6	0.11	4.56	2.24	16	MOD
77	49	M	26	9	13.2	12	297.7	22	0.8	0.14	4.54	2.86	15.8	MOD
78	50	M	22	8	13.1	8.6	200.12	28	0.6	0.12	4.62	2.24	16.2	MOD
79	49	F	24	8.5	11.8	8.2	188.64	22	0.4	0.08	4.68	2.28	15.9	MOD
80	48	F	20.6	8	11.8	8	182.9	20	0.4	0.13	4.84	1.96	16.2	MOD
81	48	M	21	8	13.6	7.6	171.42	26	0.5	0.09	4.4	1.8	14.2	MILD
82	47	F	21.6	6.5	11.9	10.5	254.65	23	0.7	0.12	4.63	1.98	14.5	MOD
83	46	M	29	11	13.5	13.4	337.88	32	0.8	0.14	5.03	2.16	15.1	MOD
84	50	F	27.6	10	12	8	182.9	28	0.8	0.09	4.42	2.1	15.5	MOD
85	48	F	22	8	12.4	9.1	214.47	26	0.3	0.16	4.71	2.6	15.3	MOD
86	46	M	21.6	8.5	14.1	8	182.9	28	0.6	0.08	5.2	2.9	14.8	MOD
87	50	F	21.1	8	11.8	7.4	165.68	28	0.8	0.09	4.41	1.85	15.6	MILD
88	50	F	27.3	11	13.5	8.2	188.64	29	0.8	0.16	4.77	1.29	14.2	MILD
89	50	F	26.1	10	13.5	12.6	314.92	27	0.8	0.09	4.88	1.41	14.5	MILD
90	47	F	24.8	12	11.6	10.6	257.52	27	0.8	0.12	4.84	1.98	15.1	MOD
91	48	M	27.6	9	14.9	7.5	168.55	30	0.6	0.11	5.42	3.16	14.9	MOD
92	47	F	22.6	6.5	11.9	10.5	254.65	23	0.6	0.14	4.63	1.98	14.5	MOD
93	46	M	29	11	13.3	13.4	337.88	32	0.8	0.13	4.99	2.16	15.1	MOD
94	50	F	27	10	12	8	182.9	28	0.8	0.09	4.42	2.1	15.6	MOD
95	48	F	22	8	12.4	9.1	214.47	26	0.3	0.12	4.71	2.6	15.3	MOD
96	46	M	21.6	8.5	14.1	8	182.9	28	0.6	0.11	5.2	2.9	14.8	MOD
97	50	F	24	10	13.2	11	269	27	0.8	0.14	4.88	1.41	14.5	MILD
98	46	M	29	8	13.5	13	326.4	32	0.8	0.12	5.03	2.26	16	MOD
99	49	F	21.6	8	12.7	7.9	180.03	30	0.8	0.16	4.68	1.64	15.2	MOD
100	50	M	27	10	13.2	8	182.9	28	0.8	0.08	4.22	2.6	15.6	MOD

KEY TO MASTER CHART

		Reference Interval
BMI	Body Mass Index	18.5-24
HB	Heamoglobin	M=13-18, F=11.5-16.5 g/dL
HBA1C	Glycated Heamoglobin	N=4-6%, good control= 6-7%, Fair=7-8%, Poor >8%
D-DIAB	Duration of Diabetes	
ABG	Average Blood Glucose	N=140mg/dL
UREA	Urea	15-40 mg/Dl
CREAT	Creatinine	M=0.7-1.4mg/dL, F=0.6-1.2 mg/dL
U.SPOT PCR	Urine Spot Protein Creatinine Ratio	N=<0.2
N/L	Neutrophil : Lymphocyte Ratio	N= <2
RDW	Red Cell Distribution Width	N=11.6-14%
RBC count	Red Blood Cell Count	M= 4.5-5.9, F= 3.8-5.2 millions/ cmm
NPDR-GR	Non-Proliferative Diabetic Retinopathy Grade	Mild/ Moderate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Dissertation submitted to
TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
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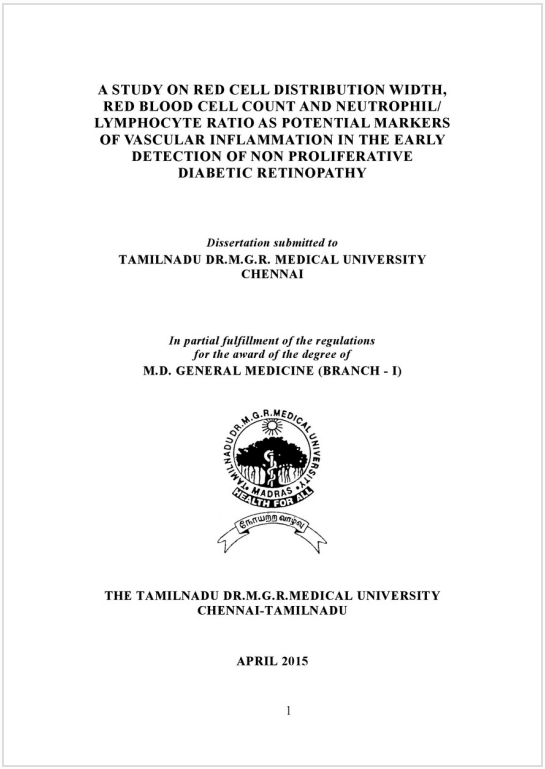

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