"STUDY OF MEAN PLATELET VOLUME IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND ITS CORRELATION WITH MICROVASCULAR COMPLICATIONS."

By:

DR RAKESH GARLAPATI M.B.B.S

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Under The Guidance Of

Dr PRABHAKAR K M.B.B.S., M.D.

Professor & Head Of Department

DEPARTMENT OF GENERAL MEDICINE

SRI DEVARAJ URS MEDICAL COLLEGE, TAMAKA, KOLAR, KARNATAKA.

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Date

Place

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Professor & HOD

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DR Prabhakar K
Professor & HOD
Department of General Medicine

Date :
Place : Kolar

Dr. Harendra Kumar M.L
Principal

Date :
Place : Kolar

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ABSTRACT

BACKGROUND:

Diabetes mellitus (DM) is a major global health problem. According to estimates of the World Health Organization, there were 346 million people suffering from diabetes worldwide in 2013. WHO projects that diabetes will be 7th leading cause of death in 2030. Type 2 DM accounts for 80% of all DM. Platelet volume is a marker of platelet function and activation. It can be quantified as mean platelet volume (MPV). Normal mean platelet volume in healthy subjects ranges between 7.2 and 11.7 fL. DM is characterized by enhanced platelet activation and coagulation proteins and reduced fibrinolytic activity. The increased platelet activity is emphasized to play a role in development of vascular complications in diabetes. An increased platelet count and activity have been reported in diabetes as demonstrated by increase in GP IIb/IIIa, Ib/IX, Ia/IIa, CD 62 and CD 63 and is not influenced by glycemic control. An activated megakaryocyte- platelet system in diabetes has been reported to be responsible for larger than normal platelets circulating in DM patients .MPV is simple and effective and cheap test that may predict micro vascular complications in type 2 DM.

OBJECTIVES:

1. To estimate mean platelet volume in type 2 DM.
2. To record the micro vascular complications.
3. To correlate mean platelet volume with micro vascular complications.

MATERIAL AND METHODS:

- This study was done in R L Jalappa Hospital, Tamaka, Kolar. 132 subjects those fulfilling the inclusion and exclusion criteria were included in the study and were divided into three groups: Group 1: non diabetics, group 2: diabetics without microvascular complication and group 3: diabetics with microvascular complications.

RESULTS:

- A total of 132 cases fulfilling the inclusion and exclusion criteria visiting medicine OPD at R.L. Jalappa Hospital and Research centre, Tamaka, Kolar district, Karnataka during the period of from June 2016 - July 2017 were enrolled in this clinical study. MPV was higher in diabetic patients with microvacular complications (11.44 ± 0.8657 fL), when compared to diabetic patients without microvascular complications (7.793 ± 0.480 fL) and non-diabetics (5.43±0.34) and this difference was statistically significant (p value<0.0001)
- FBS, PPBS and HbA1c were significantly higher in diabetics with microvascular complications, when compared to other two groups. FBS, PPBS and HbA1c were found to have a positive correlation with MPV (p<0.0001 for all three parameters)
- Among the diabetic cases, patients with microvascular complications had a higher MPV than diabetic patients without microvascular complications (p<0.001).

CONCLUSION:

MPV can be used as a simple and cost effective tool to monitor glycemic control and complications in diabetic patients. It would be feasible even in rural centers which will have cell count analyzers. However, larger studies are required to assess the utility of MPV in diabetes.

LIST OF ABBREVIATIONS USED

WHO	-	World Health Organisation
MPV	-	Mean Platelet Volume
T2DM	-	Type 2 diabetes mellitus
HbA1c	-	Glycated hemoglobin
OGTT	-	Oral glucose tolerance test
FPG	-	Fasting plasma glucose
ADA	-	American diabetes association
FFA	-	Free Fatty Acid
PPG	-	Post Prandial Glucose
IFG	-	Impaired fasting glucose

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INTRODUCTION

Diabetes mellitus (DM) is a major global health problem.[1] In 2014 the global prevalence of DM was established to be 9% among adults aged 18 years.[2] The global prevalence of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. According to estimates of the World Health Organization, there were 346 million people suffering from diabetes worldwide in 2013.[3] WHO projects that diabetes will be 7th leading cause of death in 2030[4]. Type 2 DM accounts for 80% of all DM[5]. Diabetes mellitus(DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. DM includes the group of common metabolic disorders that share the phenotype of hyperglycemia[1].

Platelet volume is a marker of platelet function and activation. It can be quantified as mean platelet volume (MPV). Normal mean platelet volume in healthy subjects ranges between 7.2 and 11.7 fL. Platelet volume is a marker of platelet function and activation. It can be quantified as Mean Platelet Volume (MPV) by clinical hematology analyzers. It has been reported that platelets from diabetic patients synthesize more thromboxane than normal platelets. Hyperglycemia has been found to be associated with larger platelets[5], which release more prothrombotic factors such as thromboxane A2[6]. The increased platelet activity is emphasized to play a role in development of vascular complications in diabetes[7]. An increased platelet count and activity have been reported in diabetes as demonstrated by increase in GP IIb/IIIa, Ib/IX, Ia/IIa, CD 62 and CD 63[8,9] and is not influenced by glycemic control[10].An

activated megakaryocyte- platelet system in diabetes has been reported to be responsible for larger than normal platelets circulating in DM patients [10].MPV is simple and effective and cheap test that may predict micro vascular complications in type 2 DM. Platelets are essential for hemostasis. In diabetics, the levels of fibrinogen and plasminogen activator inhibitor-1 are increased, platelet adherence to vascular endothelium and aggregation is increased and insulin is a natural antagonist of platelet hyperactivity. Thus, defects in insulin action in diabetes leads to disordered platelet activity, which in turn leads to macrovascular and microvascular events.

OBJECTIVES OF THE STUDY

- To estimate mean platelet volume in type 2 DM.
- To record the micro vascular complications.
- To correlate mean platelet volume with micro vascular complications

REVIEW OF LITERATURE

HISTORY OF DIABETES MELLITUS

1500 BC: Ebers Papyrus, an ancient Egyptian medical document, describes a condition of "too great emptying of the urine", perhaps, referring to diabetes mellitus.[8]

Around the same time, ancient Indian scholars and physicians had described diabetes as "*a mysterious disease causing thirst, enormous urine output, and wasting away of the body with flies and ants attracted to the urine of people*." They named the condition "madhumeha" or "honey urine"[8,9,10]

230 BC: Apollonius of Memphis, for the first time, used the term "diabetes", which in Greek means "to pass through" (dia- through, betes- to go), as the disease drained more fluid than a person could consume. [8,9]

30 BC- 50 AD: Aulus Cornelius Celsus had given a complete description of diabetes in his work entitled *De Medicina*. [8]

Second Century AD: Aretaeus of Cappadocia, a Greek physician was the first to distinguish between diabetes mellitus and diabetes insipidus[8]

Fifth Century AD: Charaka and Sushruta, Indian physicians, were the first to differentiate between the two types of diabetes. [8]

1776: British physiologist Matthew Dobson showed that the sweet substance in the urine and serum of diabetic patients was sugar and put forward the theory that diabetes is a systemic disease, as against that popular notion at that time that diabetes could be a disease of the kidneys. [8,10]

1788: Thomas Cawley was the first to suggest the link between pancreas and diabetes [8]

1798: John Rollo added the term "mellitus" which means honey in Latin, due to the sweet taste of urine and he used this term to distinguish it from diabetes insipidus (Insipidus means tasteless in Latin) [8,11]

1815: Eugene Chevreul proved that the sugar in urine of diabetics was glucose [8]

1848: Von Fehling first developed quantitative test for glucosuria, thus making it a criterion for diagnosing diabetes. [8]

1869: Paul Langerhans, a German Pathologist described small clusters of pancreatic cells, which were not drained by pancreatic ducts. These were named as Islets of Langerhans, later. [8]

1889: Oscar Minkowski and Joseph Von Mehring, from Germany, observed that removal of pancreas in dogs immediately led to the development of diabetes[8]

1893: Edouard Laguesse, a French investigator showed that an internal secretion from pancreas played a pivotal role in the pathogenesis of diabetes. [8]

1907: Georg Zuelzer produced a pancreatic extract named "acomatol" which was found to decrease glucosuria and increase pH in diabetic dogs. [8]

1921: Fredrick Banting, Charles Best and his physiology professor John.J.R.MacLeod ligated pancreatic ducts of dogs and later used the extract of atrophied pancreatic glands on diabetic dogs and it was found to decrease glucosuria in diabetic dogs dramatically. [8,12]

1922: Banting and Best used their extract for the first time on a human, as an intramuscular injection, which caused an abscess. Later, a second injection using an improved preparation, as suggested by Collip lead to dramatic improvement and fall in glucose levels. [8,12]

1923: Nobel prize was awarded for the discovery of Insulin to Banting and MacLeod, who shared their portions of the prize with Best and Collip, respectively[8,13]

1928: Synthalin, a guanidine derivative, was the first orally administered drug for the treatment of diabetes, but later withdrawn due to hepatic and renal toxicity[8,14]

1939: Ruiz and Silva were the first ones to observe the hypoglycemic properties of sulphonamide antibiotics[8]

1955: Sir Frederick Sanger characterized the amino acid sequence of human insulin, making it the first protein to whose sequence was determined. He was awarded the 1958 Nobel Prize in Chemistry for this work. Subsequently, Hans Christian Hagedorn discovered the prolonged effect of insulin by adding protamine to the insulin molecule[8,15]

1969: Dorothy Hodgkin described the three dimensional structure of porcine insulin using X ray crystallography[8]

1978: Gene coding for human insulin was cloned by Genentech[8]

1978: Robert Crea and David Goeddel et al produced human insulin using recombinant DNA technology. Insulin thus became the first genetically manufactured drug and hormone to be approved by the FDA. [8,16]

1996: FDA approved the first recombinant DNA human insulin analogue, the insulin lispro. At present, more than 300 human insulin molecule analogues have been identified. [8]

DIABETES MELLITUS

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Diabetes can be a primary problem or secondary, as a consequence of another medical condition. Diabetes can be classified into the following categories [17,18]:

- **Type 1 diabetes:** There is beta cell destruction, usually leading to absolute insulin deficiency. It can be immune-mediated or idiopathic. Type 1 DM most commonly develops before the age of 30, however 5 to 10% of individuals who develop DM after the age of 30 years have type 1 DM.
- **Type 2 diabetes:** It is due to a progressive loss of insulin secretion on the background of insulin resistance.
- Gestational diabetes mellitus (GDM): Diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes
- Other types of diabetes include genetic defects of beta cell development or function characterised by specific mutations (Maturity-onset diabetes of the young: MODY 1 to 6), genetic defects in insulin action, drug-induced, diseases of exocrine pancreas, and gestational diabetes mellitus.

SPECTRUM OF DIABETES MELLITUS

Table - 1 : Spectrum of Diabetes Mellitus

Types of Diabetes	**Normal Glucose Tolerance**	**Pre-diabetes/ impaired fasting glucose/ impaired glucose Tolerance**	**Diabetes Mellitus**
FPG	<5.6 mmol/L (100 mg/dL)	5.6-6.9 mmol/L (100-125 mg/dL)	≥ 7 mmol/L (126 mg/dL)
2-h-PG	<7.8 mmol/L (140 mg/dL)	7.8-11 mmol/L (140-199 mg/dL)	≥ 11.1 mmol/L (200 mg/dL)
HbA1c	<5.6 %	5.7-6.4 %	≥ 6.5 %

EPIDEMIOLOGY

Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. This reflects an increase in associated risk factors such as being overweight or obese. Over the past decade, diabetes prevalence has risen faster in low and middle-income countries (especially Asia) than in high-income countries.[2]

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". [19,20] According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. The so called "Asian Indian Phenotype" refers to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, greater abdominal adiposity i.e., higher waist circumference despite lower body mass index, lower adiponectin and higher high sensitive C-reactive protein levels. This phenotype makes Asian Indians more prone to diabetes and premature coronary artery disease. At least a part of this is due to genetic factors. However, the primary driver of the epidemic of diabetes is the rapid epidemiological transition associated with changes in dietary patterns and decreased physical activity as evident from the higher prevalence of diabetes in the urban population. Even though the prevalence of microvascular complications of diabetes like retinopathy and nephropathy are comparatively lower in Indians, the prevalence of premature coronary artery disease is much higher in Indians compared to other ethnic groups. The most disturbing trend is the shift in age of onset of diabetes to a younger age in the recent years. [19]

According to a national study done by Ramachandran et al in 2000, age standardised prevalences of diabetes and impaired glucose tolerance were 12.1% and 14.0% respectively, with no gender difference. Diabetes and impaired glucose tolerance showed increasing trend with age. Subjects under 40 years of age had a higher prevalence of impaired glucose tolerance than diabetes (12.8% vs 4.6%)[21]

According to WHO's global report, diabetes has caused 1.5 million deaths world-wide in 2012. Higher than optimal blood glucose levels were responsible for an additional 2.2 million deaths, thus making a total of 3.7 million deaths related to blood glucose levels in 2012. 43% of these deaths were below 70 years of age. [2]

In India, the CUPS (Chennai Urban Population Study) and CURES (Chennai Urban Rural Epidemiology Study) have provided valuable data on complications related to diabetes. The prevalence of coronary artery disease (CAD) was 21.4% among diabetic patients as compared to 9.1% in non-diabetic controls [22] . The prevalence of peripheral vascular disease (PVD) was 6.3% among diabetics compared to 2.7% in non-diabetics[23]. The CURES eye study showed that prevalence of diabetic retinopathy is 17.6%, lower compared to reports from the West[24]. Prevalence of overt nephropathy was 2.2% in Indians whereas microalbuminuria was present in 26.9% [19,25].

Overall, Asian Indians have a greater predilection for cardiovascular complications whereas prevalence of microvascular complications is lower than Europeans [19].

CRITERIA FOR DIAGNOSIS OF DIABETES MELLITUS[17,18].

A1C ≥ 6.5 %. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay*

OR

FPG≥ 126 mg/dL (7 mmol/L) Fasting is defined as no calorie intake for at least 8 hours*

OR

2-h-PG≥ 200 mg/dL (11.1 mmol/L during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in 300 ml of water*

OR

A random plasma glucose ≥ 200 mg/dL (11.1 mmol/L) in a patient with classical symptoms of hyperglycemia or hyperglycemic crisis,

*In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing

The same tests are used for screening as well as diagnostic purposes

PREDIABETES[17,18]

FPG 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) (IFG)

OR

2-h-PG in the 75g OGTT 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11 mmol/L) (IGT)

OR

A1C 5.7-6.4%

For all three tests, risk is continuous, extending below the lower limit of the range and becoming disproportionately greater at higher ends of the range

WHO and numerous other diabetes organizations define IFG cut-off at 110 mg/dL (6.1 mmol/L) [2]

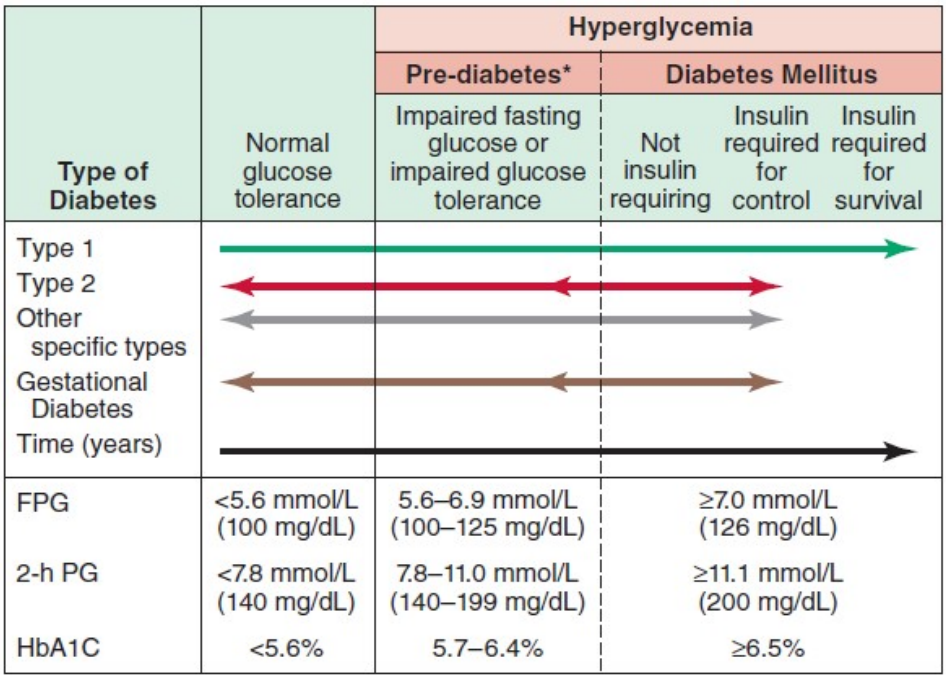


Fig - 1: Spectrum of glucose homeostasis and diabetes mellitus (DM)[1]

ADA recommends screening for diabetes in individuals who come under the following categories: [1,18]

1. Age >45 years (Test every 3 years)
2. Obesity, with BMI ≥25 kg/m^2 (Screen at an earlier age) and have any of the following risk factors:

    - Family history of diabetes
    - Physical inactivity
    - Race/ethnically predisposed
    - Previously identified with IFG/IGT/HbA1c of 5.7-6.4%
    - History of GDM or delivery of baby>4kg
    - Hypertension (BP≥140/90)

- HDL Cholesterol < 35mg/dL and/or triglyceride level>250mg/dL
- Polycystic ovarian syndrome
- Acanthosis nigricans
- History of cardiovascular disease

If results are normal, testing should be repeated once in 3 years. In individuals at high risk, testing may be repeated more frequently. Those with prediabetes need to be tested every year.

REGULATION OF GLUCOSE HOMEOSTASIS[1,26,27]

Normal glucose homeostasis is regulated by three inter-related processes:

1. Glucose production in the liver
2. Glucose uptake and utilization by peripheral tissues, mainly the skeletal muscle
3. Actions of insulin and counter-regulatory hormones on glucose uptake and metabolism

During fasting state, low insulin and high glucagon levels facilitate hepatic gluconeogenesis and glycogenolysis and decrease glycogen synthesis. Thus fasting plasma glucose levels are determined by hepatic glucose output. After a meal, insulin levels rise and glucagon levels fall. Insulin promotes glucose uptake and utilization in tissues. The skeletal muscle is the major insulin-responsive site for post prandial glucose utilization.

Insulin is produced in the rough endoplasmic reticulum of the beta cells of the pancreatic islets. It is initially synthesized as a single-chain 86-amino-acid precursor polypeptide, preproinsulin. Subsequent proteolytic processing removes the amino-terminal signal peptide, giving rise to proinsulin. Proinsulin is structurally related to insulin-like growth factors I and II, which bind weakly to the insulin receptor. Cleavage of an internal 31-residue fragment from proinsulin generates the C peptide and the A (21 amino acids) and B (30 amino acids) chains of insulin, which are connected by disulfide bonds. The mature insulin molecule and C peptide are stored together and co-secreted from secretory granules in the beta cells and released after physiologic stimulation. The most important stimulus for insulin synthesis and release is glucose. An increase in blood glucose leads to its uptake into pancreatic β cells,

facilitated by insulin-independent glucose transporter GLUT-2. Metabolism of glucose generates ATP, which inhibits the activity of ATP sensitive potassium channel, leading to membrane depolarisation and influx of Ca^{2+}, which stimulates the release of insulin.

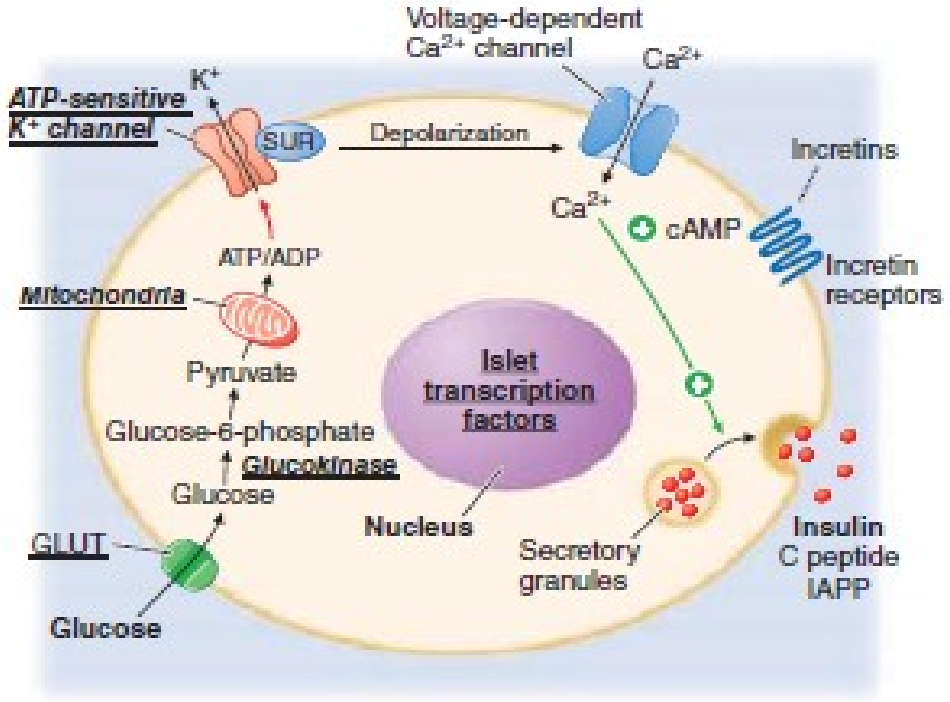


Fig - 2 : Mechanisms of glucose-stimulated insulin secretion and abnormalities in diabetes

INSULIN SYNTHESIS AND SECRETION

Many hormones play a role in glucose homeostasis. Of these, the most important hormones responsible for promoting insulin secretion are the incretins, two of which have been identified: glucose-dependent insulinotropic polypeptide (GIP) secreted by K cells of small bowel, and glucagon-like peptide-1 (GLP-1), secreted by L cells of distal ileum and colon. The rise in incretin levels after food intake is known as incretin effect. These hormones increase insulin secretion, decrease glucagon

secretion and delay gastric emptying, promoting satiety. GIP and GLP-1, are degraded in circulation by dipeptidyl peptidase (DPPs), especially DPP4. In type 2 diabetics, this incretin effect is blunted, and efforts to restore incretin function can lead to weight loss and glycemic control.

Insulin is the most potent anabolic hormone known[26]. In the adipocytes, it increases glucose uptake, enhances lipogenesis and inhibits lipolysis. In the striated muscles, it increases glucose uptake, promotes glycogen synthesis and protein synthesis. In the liver, it increases glycogen synthesis and lipogenesis and inhibits gluconeogenesis. In addition, it also increases cell growth and differentiation and has mitogenic functions including initiation of DNA synthesis in certain cells.

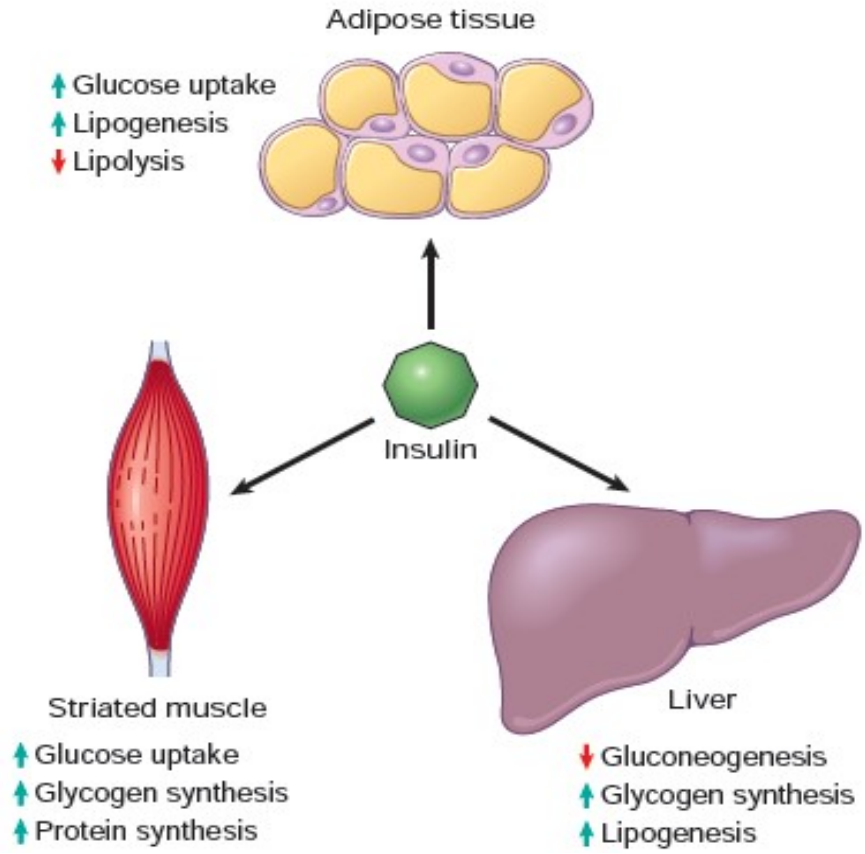


Fig - 3 : Metabolic actions of insulin in striated muscle, adipose tissue and liver

PATHOGENESIS OF TYPE-2 DIABETES MELLITUS[1,26]

It is a complex interplay of genetic and environmental factors, and a pro-inflammatory state. The most important environmental risk factor for T2DM is obesity. Insulin resistance is the failure of target tissues to respond normally to insulin. There is failure to inhibit gluconeogenesis in the liver leading to high fasting blood glucose levels. Failure in glucose uptake and glycogen synthesis in skeletal muscle, after a meal, leads to post-prandial hyperglycemia. There is also failure to inhibit lipoprotein lipase in adipose tissue, leading to excess circulating free fatty acids (FFAs), which in turn, amplify the insulin resistance state. It is reported that there is functional defect in insulin signaling pathway in the insulin resistance state. Reduced tyrosine phosphorylation of the insulin receptor reduces the GLUT-4 levels on cell surface. Exercise increases the translocation of GLUT-4 to the surface of skeletal muscles and thus helps improve insulin sensitivity.

Obesity can impact insulin sensitivity in the following ways:

1. **Free fatty acids:** Central adipose tissue is more lipolytic than peripheral tissues and thus central obesity has deleterious effects. Excess FFAs overwhelm the intracellular fatty acid oxidation pathways, leading to toxic intermediates, which attenuate signalling through insulin receptor pathways.

2. **Adipokines :** They are a variety of proteins secreted by adipose tissue. Some promote hyperglycemia, whereas some like leptin and adiponectin decrease blood glucose by enhancing insulin sensitivity in peripheral tissues. These beneficial adipokines are reduced in obesity, leading to insulin resistance

3. **Inflammation :** Excess FFAs in macrophages and β cells can activate inflammasome, a cytoplasmic complex, leading to secretion of proinflammatory cytokines. These act on sites of insulin action and produce insulin resistance.

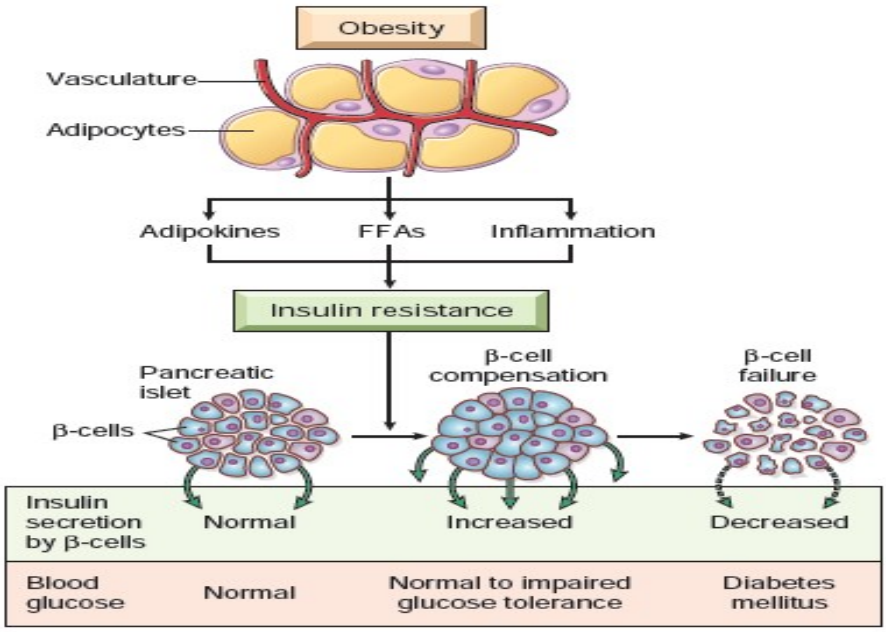


Fig. 4: Development of type 2 Diabetes mellitus. Insulin resistance associated with obesity is induced by adipokines, free fatty acids, and chronic inflammation in adipose tissue. Pancreatic β cells compensate for insulin resistance by hypersecretion of insulin. But at some point β cells fail and diabetes ensues

β cell dysfunction is virtually a requirement for development of overt diabetes. Several mechanisms contribute to the development of β cell dysfunction:

1. Excess FFAs cause lipotoxicity to β cells
2. Impact of chronic hyperglycemia- glucotoxicity
3. An abnormal “incretin effect”
4. Amyloid deposition within islets
5. Impact of genetics

LONG TERM COMPLICATIONS OF DIABETES MELLITUS[1,26]

They can broadly be classified into vascular and nonvascular complications, vascular again divided into microvascular and macrovascular. Microvascular complications are diabetes specific, whereas macrovascular complications are similar to those in non-diabetics, but with a higher frequency.

MICROVASCULAR COMPLICATIONS

1. EYE DISEASE
    - RETINOPATHY (Non-proliferative/ proliferative)
    - MACULAR EDEMA
2. NEPHROPATHY (Albuminuria and declining renal function)
3. NEUROPATHY
    - SENSORY AND MOTOR (mono- and polyneuropathy)
    - AUTONOMIC

MACROVASCULAR COMPLICATIONS

1. CORONARY HEART DISEASE
2. PERIPHERAL ARTERIAL DISEASE
3. CEREBROVASCULAR DISEASE

OTHER COMPLICATIONS

- Gastrointestinal (Gastroparesis, Diarrhoea)
- Genitourinary (Uropathy/ Sexual Dysfunction)

- Dermatological, including Scleredema Diabeticorum
- Infectious
- Cataract
- Glaucoma
- Cheiroarthropathy (Thickened skin and limited joint mobility)
- Periodontal disease
- Hearing loss

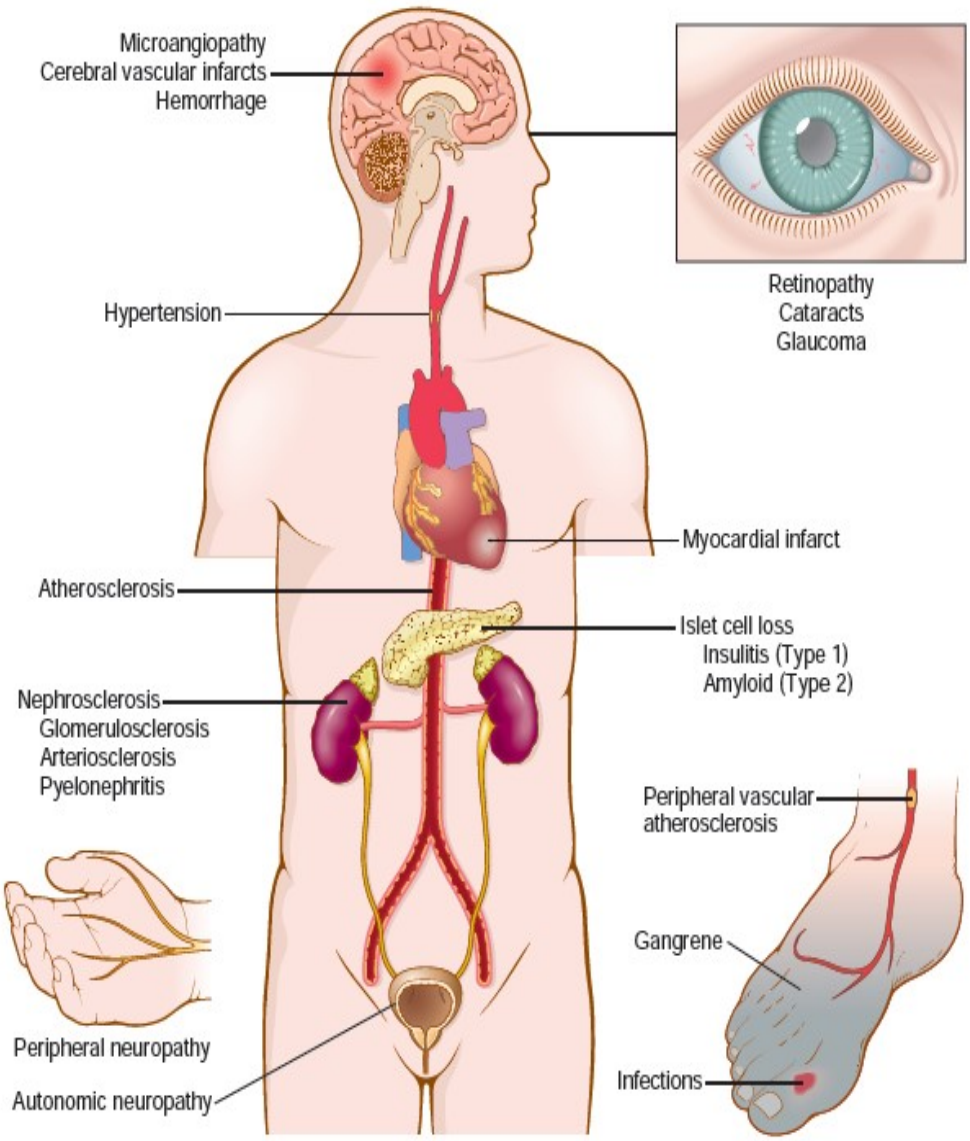


Fig - 5 : Long-term complications of diabetes

PATHOGENESIS OF CHRONIC COMPLICATIONS[26,28,29]

There are four main hypotheses to explain the deleterious effects of persistent hyperglycemia on peripheral tissues. In all of these, increased glucose flux through various intracellular metabolic pathways is thought to generate harmful precursors which contribute to end organ damage.

1. FORMATION OF ADVANCED GLYCATION END PRODUCTS (AGEs)

Advanced glycation end products (AGEs) are formed as a result of non-enzymatic reactions between intracellular glucose derived dicarbonyl precursors (glyoxal, methylglyoxal, and 3-deoxyglucosone) with the amino groups of both intracellular and extracellular proteins. The natural rate of AGE formation is greatly accelerated in the presence of hyperglycemia. AGEs bind to a specific receptor (RAGE) that is expressed on inflammatory cells (macrophages and T cells), endothelium, and vascular smooth muscle. The detrimental effects of the AGE-RAGE signaling axis within the vascular compartment include:

- Release of cytokines and growth factors, including transforming growth factor-β (TGF-β), which leads to deposition of excess basement membrane material, and vascular endothelial growth factor (VEGF), implicated in diabetic retinopathy
- Generation of reactive oxygen species (ROS) in endothelial cells.
- Increased pro-coagulant activity of endothelial cells and macrophages
- Enhanced proliferation of vascular smooth muscle cells and synthesis of extracellular matrix

Apart from receptor mediated effects, AGEs can directly cross link extracellular matrix proteins. Cross-linking in large vessels decreases their elasticity, thus making them vulnerable to shear stress and endothelial injury. These cross-linked proteins are resistant to proteolytic digestion. AGE-modified matrix components also trap non-glycated plasma or interstitial proteins. In large vessels, trapping of LDL enhances cholesterol deposition in the intima, thus accelerating atherosclerosis. In capillaries including renal glomeruli, plasma proteins like albumin bind to glycated basement membrane, and contribute to the basement membrane thickening which is characteristic of diabetic microangiopathy

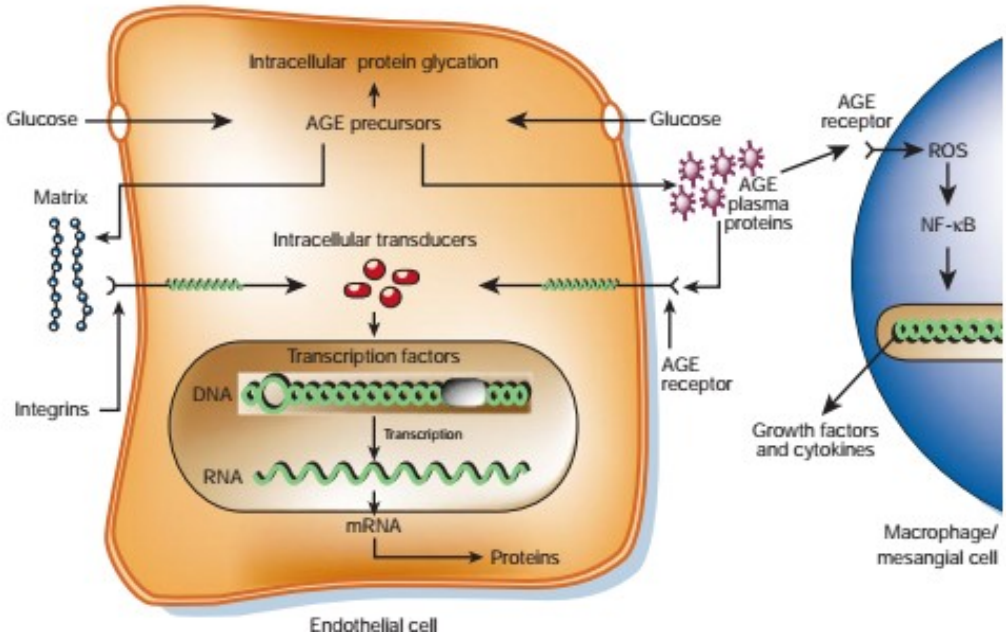


Fig. 6 : Mechanisms by which intracellular production of advanced glycation end-product (AGE) precursors damage vascular cells[28]

Modification of plasma proteins by AGE precursors creates ligands that bind to AGE receptors, inducing changes in gene expression in endothelial cells, mesangial cells and macrophages

2. ACTIVATION OF PROTEIN KINASE C

Calcium-dependent activation of intracellular protein kinase C (PKC) and the second messenger diacyl glycerol (DAG) is an important signal transduction pathway. Intracellular hyperglycemia stimulates the de novo synthesis of DAG from glycolytic intermediates, and hence causes excessive PKC activation. The downstream effects of PKC activation are numerous, including production of vascular endothelial growth factor (VEGF), transforming growth factor- β (TGF-β) and plasminogen activator inhibitor-1 (PAI-1) by the vascular endothelium; affecting expression of endothelial nitric oxide synthetase (eNOS), Endothelin-1 (ET-1); and by activating NF-kB and NADPH oxidases.

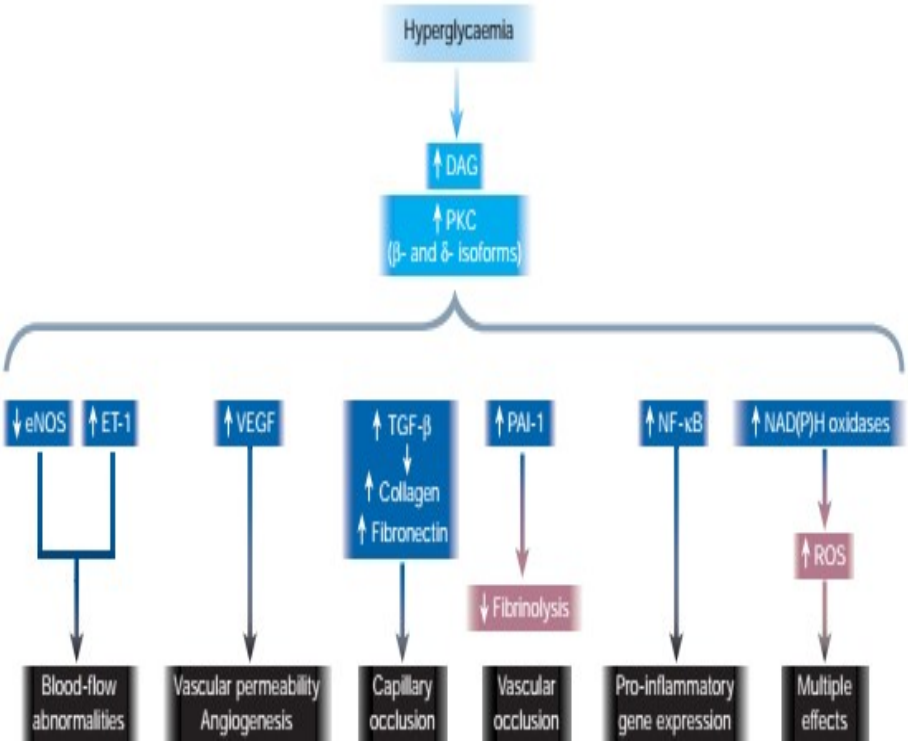


Fig. 7 : Consequences of hyperglycaemia-induced activation of protein kinase C (PKC)

3. OXIDATIVE STRESS AND DISTURBANCES IN POLYOL PATHWAYS

Even in some tissues that do not require insulin for glucose transport (e.g., nerves, lenses, kidneys, blood vessels), persistent hyperglycemia in the extracellular milieu leads to an increase in intracellular glucose. This excess glucose is metabolized by the enzyme aldose reductase to sorbitol, a polyol, and eventually to fructose, in a reaction that uses NADPH (the reduced form of nicotinamide dinucleotide phosphate) as a cofactor. NADPH is also required by the enzyme glutathione reductase in a reaction that regenerates reduced glutathione (GSH). GSH is one of the important antioxidant mechanisms in the cell, and any reduction in GSH increases cellular susceptibility to ROS (Reactive oxygen species). In the face of sustained hyperglycemia, progressive depletion of intracellular NADPH by aldol reductase compromises GSH regeneration, increasing cellular susceptibility to oxidative stress. Sorbitol accumulation in the lens contributes to cataract formation.

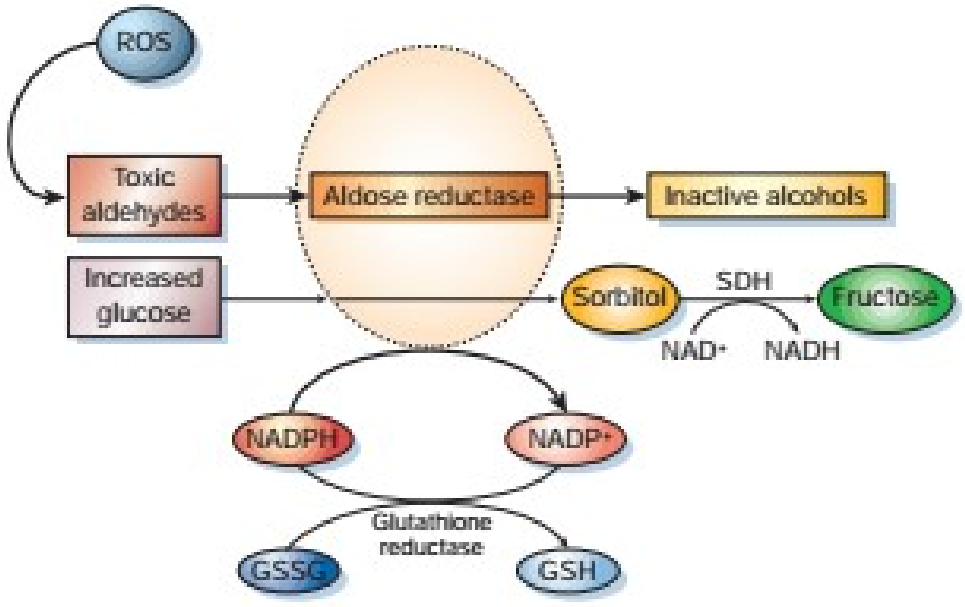


Fig - 8 : Aldose reductase and the polyol pathway

4. HEXOSAMINE PATHWAYS

It is postulated that hyperglycemia induced flux through the hexosamine pathway increases intracellular levels of fructose-6-phosphate, which is a substrate for glycosylation of proteins, leading to generation of excess proteoglycans. These glycosylation changes are accompanied by abnormal expression of TGFβ or PAI-1, which further exacerbate the end-organ damage.

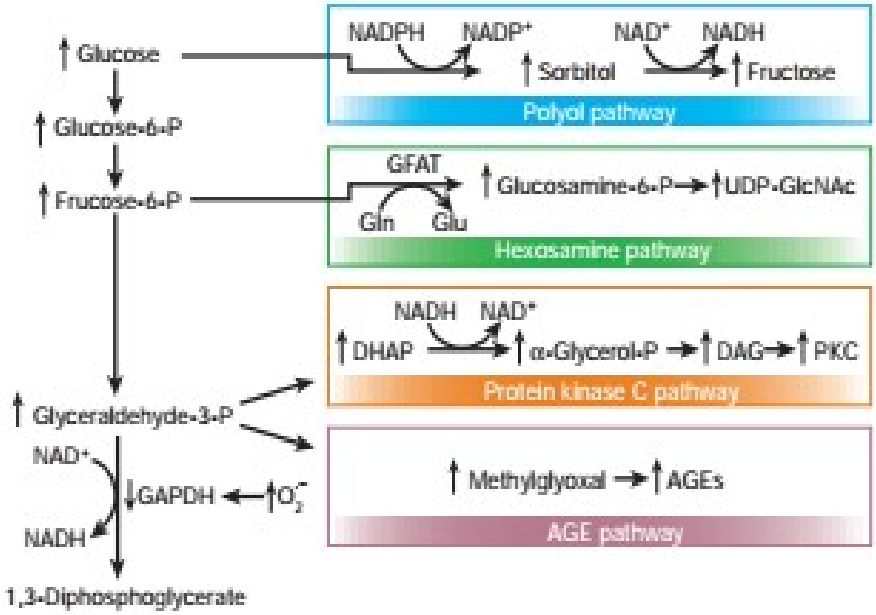


Fig. 9 : Potential mechanism by which hyperglycaemia-induced mitochondrial superoxide overproduction activates four pathways of hyperglycemic damage

When intracellular hyperglycemia develops in target cells of diabetic complications, it causes increased mitochondrial production of ROS. The ROS cause strand breaks in nuclear DNA, which activate PARP. PARP then modifies GAPDH, thereby reducing its activity. Finally, decreased GAPDH activity activates the polyol

pathway, increases intracellular AGE formation, activates PKC and subsequently NF-κB, and activates hexosamine pathway flux.

DIABETIC RETINOPATHY[30-37]

Diabetic retinopathy (DR) is a retinal microvasculopathy resulting from prolonged hyperglycemia. Prevalence of DR increases with the duration of diabetes and nearly all type 1 diabetics and 60% of type 2 diabetics develop DR after 20 years.[33] Major risk factors are degree of glycemic control, duration of diabetes, hypertension and dyslipidemia. Clinically, diabetic retinopathy is separated into non-proliferative and proliferative disease stages. In the early stages, hyperglycemia can lead to intramural pericyte death and thickening of the basement membrane, which contribute to changes in the integrity of blood vessels within the retina, altering the blood-retinal barrier and vascular permeability [31]. As DR progresses, increasingly large and widespread areas of retinal ischemia develop, caused by capillary occlusion and intravascular coagulation, which induces angiogenic growth factors like VEGF. New vessels arise from the post capillary venule in areas of ischemic retina. New vessel growth originates either within 1 DD of the optic disc (neo vascularisation at the disc [NVD]) or more than 1 DD away from the edge of the optic disc (neo vascularisation elsewhere [NVE])

PATHOGENESIS OF DIABETIC RETINOPATHY

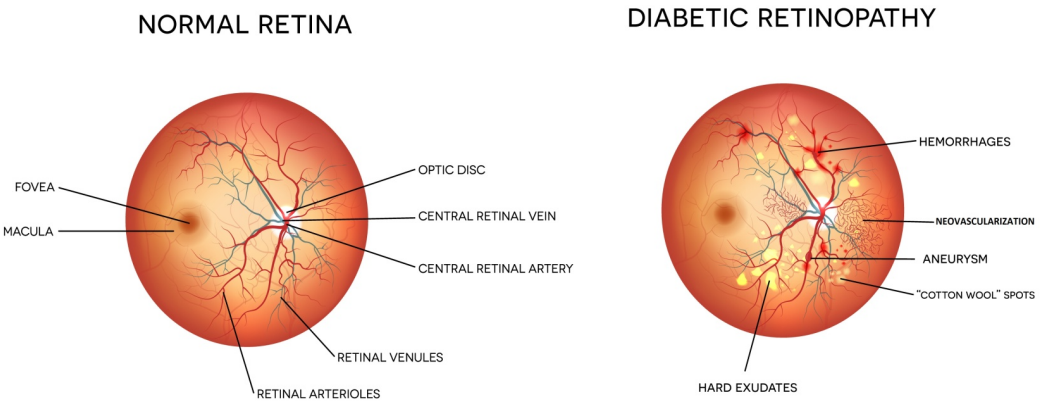


Fig. 10 : Fundus changes in diabetic retinopathy

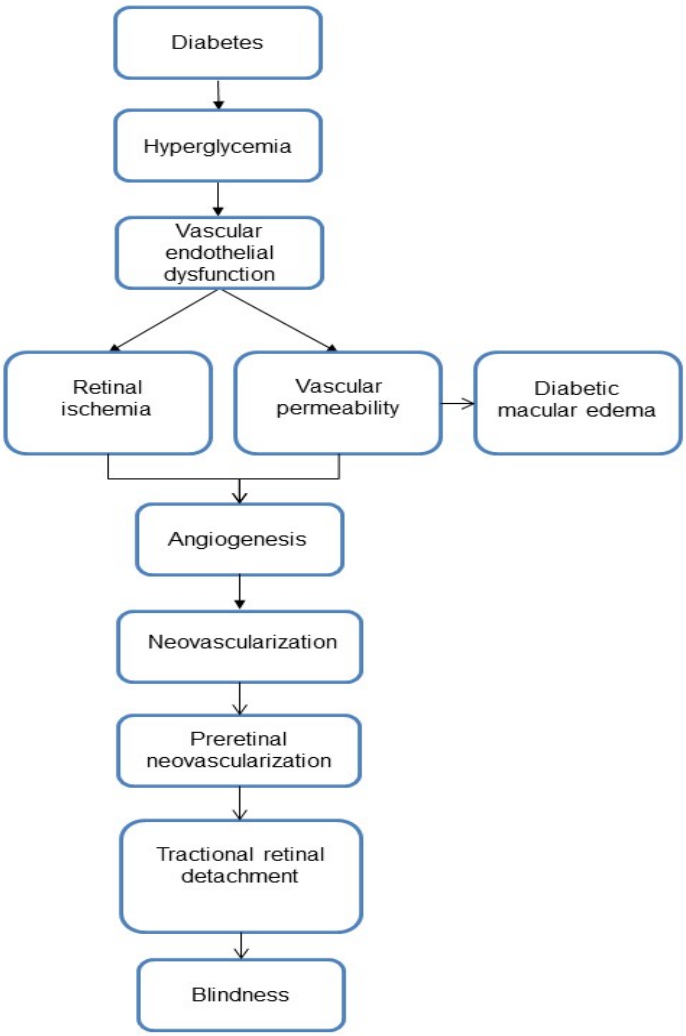


Fig. 11 : Pathogenesis of diabetic retinopathy

Table - 2 : International Clinical Diabetic Retinopathy Disease Severity SCALE[32]

DISEASE SEVERITY LEVEL	FINDINGS OBSERVABLE ON DILATED OPHTHALMOSCOPY
No retinopathy	No abnormalities
Mild NPDR	Microaneurysms only
Moderate NPDR	More than just microaneurysms, but less than severe NPDR
Severe NPDR	Any of the following and no signs of PDR: ➢ More than 20 intraretinal haemorrhages in each of 4 quadrants ➢ Definite venous beading in 2 or more quadrants ➢ Prominent IRMA in one or more quadrants
PDR	One or both of the following: Neovascularisation Vitreous/preretinal hemorrhage

Table - 3 : Diabetic macular edema disease severity scale

Proposed disease severity level	**Findings on dilated ophthalmoscopy**
Diabetic macular edema apparently absent	No apparent retinal thickening or hard exudates in posterior pole
Diabetic macular edema apparently present	Some apparent retinal thickening or hard exudates in posterior pole
If diabetic macular edema is present, it can be categorized as follows:	
Mild diabetic macular edema	Some retinal thickening or hard exudates in posterior pole but distant from the centre of the macula
Moderate diabetic macular edema	Retinal thickening or hard exudates approaching the centre of macula but not involving it
Severe diabetic macular edema	Retinal thickening or hard exudates involving the centre of macula

DIABETIC NEPHROPATHY[1,38]

Diabetic nephropathy is the leading cause of chronic kidney disease (CKD), ESRD (End stage renal disease), and CKD requiring renal replacement therapy. In India, diabetic nephropathy accounts for 46% of CKD in the elderly population. Screening for kidney damage (albuminuria) can be most easily performed by urinary albumin–to–creatinine ratio (UACR) in a random spot urine collection.

Table - 4 Diabetic Nephropathy stages based on urine Albumin Excretion (UAE)

Stages	Urine (Timed Collection) (µg/min)	24 hrs urine (mg/24 hrs)	Random urine Sample Albumin:Creatinine Ratio (ACR)
Normal	<20	<30	<30
Micro-albuminuria	20-199	30-299	30-299
Macro-albuminuria	≥200	≥300	≥300

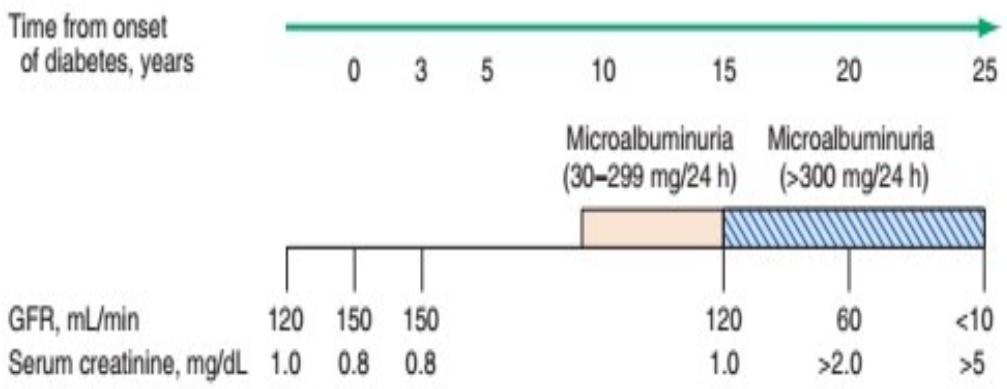


Fig. 12 : Time course of development of diabetic nephropathy

Mogensen classified diabetic nephropathy into 5 stages[39]. Several modifications have been done over the years. The table below summarizes the natural history of diabetic nephropathy:

Table - 5 : Stages of diabetic nephropathy

Stages	**Designation**	**Characteristics**	**GFR**	**albumin excretion**	**BP**	**Chronology**
Stage-1	Stage of hyper-filtration	Glomerular hyperfiltration	Increased	May be increased	Normal	At the time of diagnosis
Stage-2	Silent stage/stage of structural changes	Thickened Basement Membrane, expanded mesangium	Normal	Reversible, subclinical micro-albuminuria	Normal	first 5 yrs
Stage-3	Incipient diabetic nephropathy	Micro-albuminuria	Begins to fall	30-299 mg/day	Increased	6-15 yrs
Stage-4	Overt diabetic nephropathy	Macro-albuminuria	Persistant decline of GFR	>300 mg/day	Hypertension	15-25 yrs
Stage-5	End stage diabetic kidney disease	ESRD	GFR<10 ml/min	Decreasing	Hypertension	>25 years

Urinary albumin excretion rate within microalbuminuria range in at least 2 out of 3 consecutive, non-ketotic, uninfected urine samples is the accepted definition of persistent microalbuminuria[40].

PATHOGENESIS OF DIABETIC KIDNEY DISEASE[40,41,42]

Diabetic nephropathy occurs as a result of an interaction between hemodynamic and metabolic factors[43].

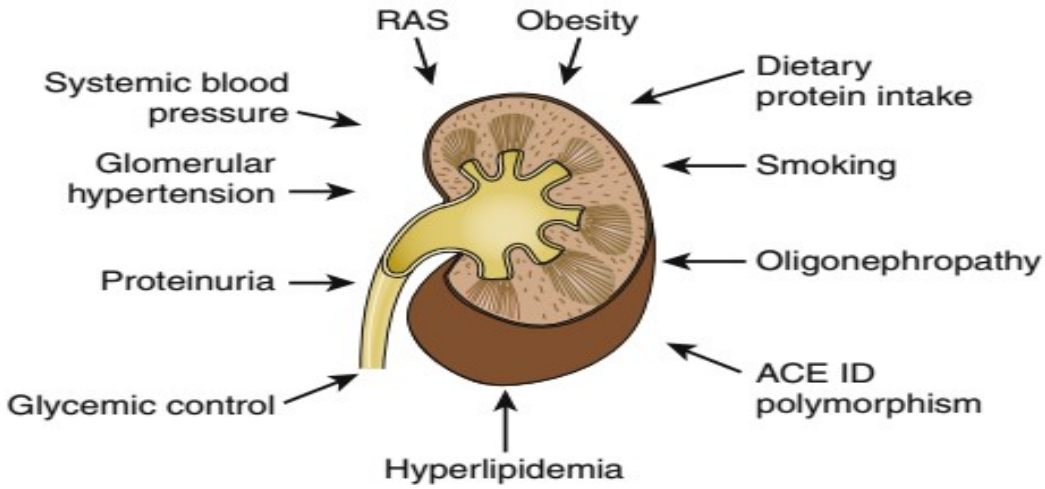


Fig. 13 : Putative promoters of progression of diabetic nephropathy

Hemodynamic factors that contribute to the development of diabetic nephropathy include increased systemic and intra-glomerular pressure, as well as activation of vasoactive hormone pathways including the renin angiotensin system and endothelin[44].

These hemodynamic pathways activate intracellular second messengers such as protein kinase C (PKC), Mitogen activated protein (MAP kinase)[45], nuclear transcription factors such as NF-kB and various growth factors such as the pro-sclerotic cytokine, TGF-β and the permeability enhancing growth factor, VEGF. Glucose dependent pathways are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation[46] and the accumulation of advanced glycation end products (AGEs). In combination, these pathways ultimately

lead to increased renal albumin permeability and extracellular matrix accumulation, resulting in increasing proteinuria, glomerulosclerosis and ultimately tubulointerstitial fibrosis.

Glomerular hyperperfusion and hyperfiltration are the early signs resulted from decreased resistance in both the afferent and efferent arterioles of the glomerulus. Afferent arteriole seems to have a greater decrease in resistance than the efferent. Many factors have been reported to be involved in this faulty auto-regulation, including nitric oxide, prostanoids, vascular endothelial growth factor (VEGF), TGF β, and the rennin angiotensin system, specifically angiotensin

These early hemodynamic changes alleviate albumin leakage from the glomerular capillaries and overproduction of mesangial cell matrix, as well as thickening of the glomerular basement membrane and injury to podocytes[47]. In addition, increased mechanical strain from these hemodynamic changes can induce localized release of certain cytokines and growth factors[48].

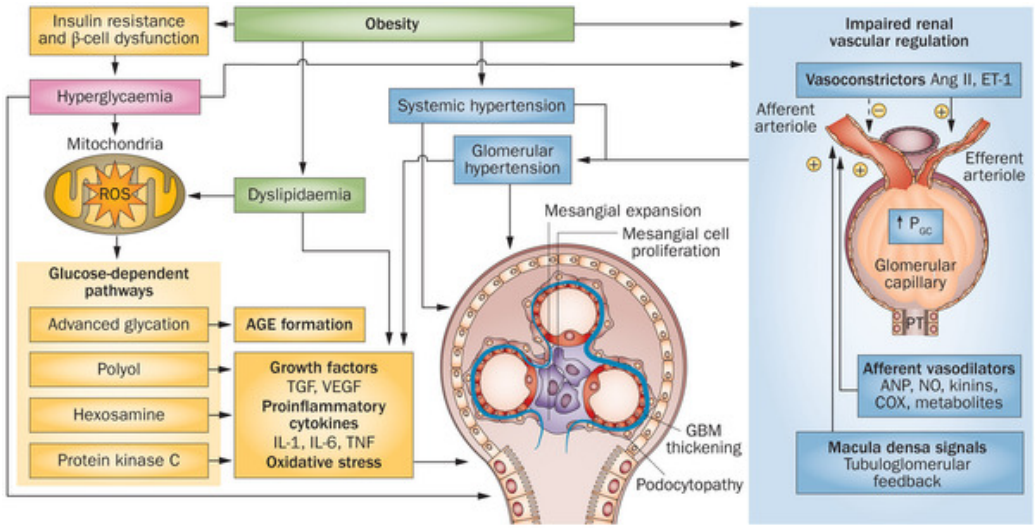


Fig.14 - Pathogenesis of kidney disease in patients with diabetes

Pathological lesions[26] seen in kidney in diabetes include:

1. Glomerular lesions

- Capillary basement membrane thickening
- Diffuse mesangial sclerosis
- Nodular glomerulosclerosis or Kimmelstiel-Wilson disease

2. Renal vascular lesions, principally, arteriolosclerosis

3. Pyelonephritis, including necrotizing papillitis.

DIABETIC NEUROPATHY

It is defined as the presence of symptoms/signs of peripheral nerve involvement in diabetic patients after excluding other causes of peripheral neuropathy

Classification of diabetic neuropathies[49,50]:

1. Symmetrical polyneuropathies
    - Distal sensory or sensorimotor polyneuropathy
    - Large-fibre neuropathy
    - Small-fibre neuropathy
    - Autonomic neuropathy
2. Asymmetrical neuropathies:
    - Cranial neuropathy (single or multiple)
    - Truncal neuropathy (thoracic radiculopathy)
    - Limb mononeuropathy (single or multiple)
    - Lumbosacral radiculoplexopathy (asymmetrical proximal motor neuropathy)
    - Entrapment neuropathy

3. Combinations:
    - Polyradiculoneuropathy
    - Diabetic neuropathic cachexia
    - Symmetrical polyneuropathies

Distal symmetrical polyneuropathy is the most common type of diabetic neuropathy[1]. It most frequently presents with distal sensory loss and pain, but up to 50% of patients do not have symptoms of neuropathy. Symptoms may include a sensation of numbness, tingling, sharpness, or burning that begins in the feet and spreads proximally. Neuropathic pain develops in some of these individuals, occasionally preceded by improvement in their glycemic control. Pain typically involves the lower extremities, is usually present at rest, and worsens at night. Both an acute (lasting <12 months) and a chronic form of painful diabetic neuropathy have been described. Physical examination reveals sensory loss, loss of ankle deep-tendon reflexes, and abnormal position sense.

Diabetic polyradiculopathy is a syndrome characterized by severe disabling pain in the distribution of one or more nerve roots. It may be accompanied by motor weakness. Intercostal or truncal radiculopathy causes pain over the thorax or abdomen. Involvement of the lumbar plexus or femoral nerve may cause severe pain in the thigh or hip and may be associated with muscle weakness in the hip flexors or extensors (diabetic amyotrophy). Fortunately, diabetic polyradiculopathies are usually self-limited and resolve over 6–12 months.

Mononeuropathy (dysfunction of isolated cranial or peripheral nerves) is less common than polyneuropathy in DM and presents with pain and motor weakness in the distribution of a single nerve.

Mononeuropathies can occur at entrapment sites such as carpal tunnel or be non-compressive. A vascular etiology for non-compressive mononeuropathies has been suggested, but the pathogenesis is unknown. Involvement of the third cranial nerve is most common and is heralded by diplopia. Physical examination reveals ptosis and ophthalmoplegia with normal pupillary constriction to light. Sometimes other cranial nerves, such as IV, VI, or VII (Bell's palsy), are affected. Peripheral mononeuropathies or simultaneous involvement of more than one nerve (mononeuropathy multiplex) may also occur.

DIABETIC AUTONOMIC NEUROPATHY

Individuals with long-standing DM may develop signs of autonomic dysfunction involving the cholinergic, noradrenergic, and peptidergic systems (peptides such as pancreatic polypeptide and substance P). DM-related autonomic neuropathy can involve multiple systems, including the cardiovascular, gastrointestinal, genitourinary, sudomotor, and metabolic systems. Orthostatic hypotension, resting tachycardia and heart rate unresponsiveness to respiration are the hallmarks of diabetic autonomic neuropathy [50].

Symptoms and signs of autonomic neuropathy [51]:

1. **Cardiovascular**
    - Postural hypotension
    - Resting tachycardia
    - Painless myocardial infarction
    - Sudden death (with or without association with general anaesthesia)
    - Prolonged QT interval

2. **Gastrointestinal**

- Oesophageal motor incoordination
- Gastric dysrhythmia, hypomotility (gastroparesis diabeticorum)
- Pylorospasm.
- Uncoordinated intestinal motility (diabetic diarrhoea, spasm)
- Intestinal hypomotility (constipation)
- Gallbladder hypo contraction (diabetic cholecystopathy)
- Anorectal dysfunction (faecal incontinence)

3. **Genitourinary**

- Diabetic cystopathy (impaired bladder sensation, atonic bladder, post micturition dribbling, detrusor hyporeflexia or hyperreflexia)
- Male impotence
- Ejaculatory disorders
- Reduced vaginal lubrication, dyspareunia

4. **Respiratory**

- Impaired breathing control
- Sleep apnoea

5. **Thermoregulatory**

- Sudomotor
- Vasomotor

6. **Pupillary**

- Miosis
- Disturbances of dilatation
- Argyll Robertson pupil

PATHOPHYSIOLOGY OF DIABETIC NEUROPATHY [52]

The pathogenesis of diabetic neuropathy involves a complex interaction between metabolic and microvascular injury to nerve, as shown below:

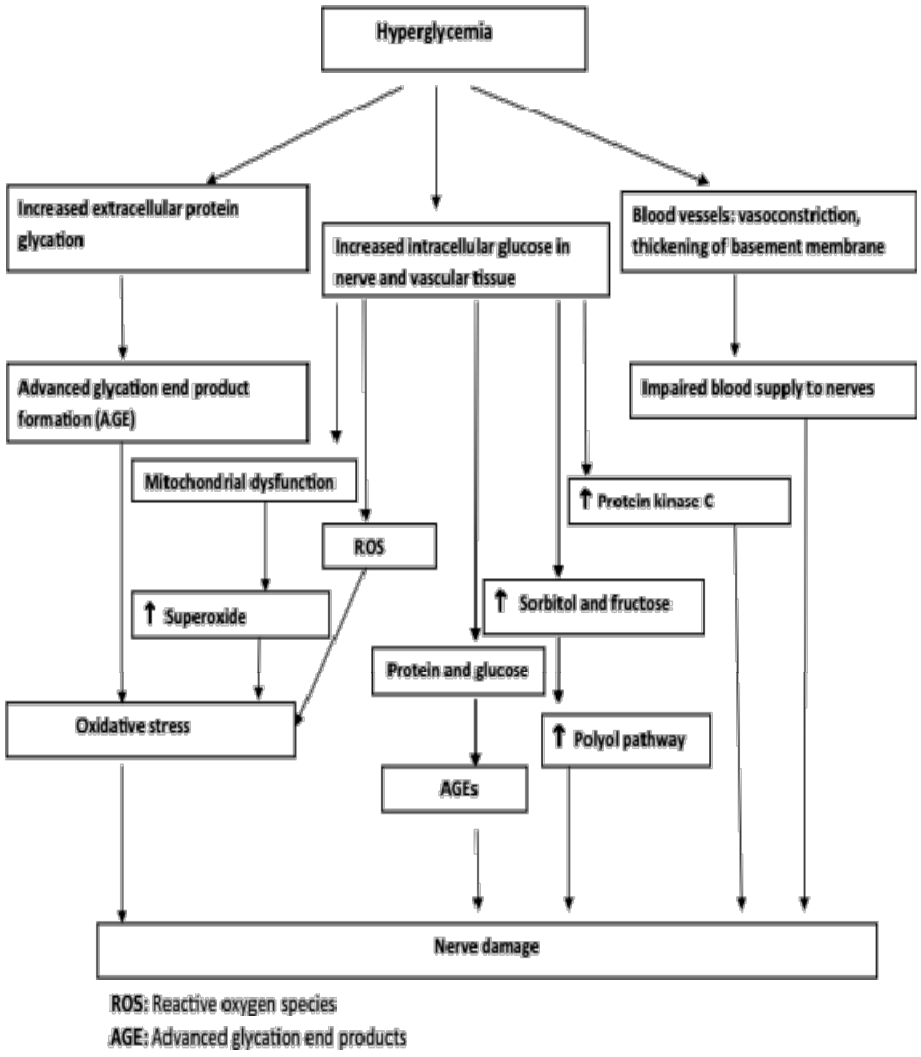


Fig. 15 : Pathogenesis of diabetic neuropathy

GLYCATED HEMOGLOBIN: HbA1c

In normal human erythrocytes, HbA comprises > 90% of the total hemoglobin. Besides HbA, human erythrocytes contain other hemoglobin components. Some of these, such as HbA2 and foetal hemoglobin (HbF), are the products of alternate globin chain genes and others such as HbA1c are post translational modifications of HbA. Glycated hemoglobin (HbA1c) is derived from the non-enzymatic addition of glucose to amino groups of hemoglobin. HbA1c is a specific glycated hemoglobin that results from the attachment of glucose to the N-terminal valine of the hemoglobin β-chain [66]. This process is a purely intracellular process and is influenced by the concentration of glucose within RBC. Glycation may occur at other sites, producing HbA1 other than HbA1c. Glycated Hemoglobin (GHb) is a better term than glycosylated or glycohemoglobin because, HbA1c production depends on a nonenzymatic amadori chain reaction, but glycosylation means enzymatic reaction between glucose and free amino acids of hemoglobin. Total GHb means HbA1c, HbA1a, HbA1b, and HbS, HbC, HbF, and so on. Of all these variants, HbA1c is most sensitive and specific, least affected by different factors and most dependable.

HbA1c reflects blood glucose concentrations over the preceding 8–12 weeks and is commonly used as an indication of average blood glucose concentration [67].

MEASUREMENT OF HbA1c [68,69,70]:

Numerous assays were subsequently developed to measure glycated hemoglobins. The principle of all methods is to separate the glycated and non-glycated forms of hemoglobin. This can be accomplished based on differences in charge (usually by HPLC) or structure (usually immunoassays or boronate affinity chromatography). Assay techniques for HbA1c include affinity chromatography,

capillary electrophoresis, high performance liquid chromatography (HPLC), cation-exchange chromatography and immmunoassays. The "gold standard"of these methods is assay by HPLC. The most widely adopted system is that of the National Glycohemoglobin Standardization Program (NGSP), which standardizes glycated hemoglobin test results so that values reported by clinical laboratories are comparable to those reported in the two largest clinical trials on the effects of intensive diabetes treatment, namely the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) [68]. NGSP-certified methods are used worldwide.

The International Federation for Clinical Chemistry (IFCC) developed a reference method for measuring HbA1c in 2009. An N-terminal hexapeptide is cleaved from the β-chain of hemoglobin by the enzyme endoproteinase Glu-C. Glycated and non-glycated hexapeptides are separated from one another by high performance liquid chromatography and separately quantified by either mass spectrometry or capillary electrophoresis. The IFCC reference system produces values that are 1.5–2.0% absolute HbA1c units lower than those measured by the NGSP[70], presumably due to the greater specificity of the IFCC method. However, the IFCC method is time consuming, technically complex and carries higher cost, thus is not designed to be used for routine analysis of patient samples.

FACTORS AFFECTING HbA1c [71,72]:

Table -6 : Factors that modify HbA1c

Increase HbA1c level	Decrease HbA1c level
HbF and HbG	HbS and HbC
Uremia	Hemolytic Anemia
Lead poisoning	Pregnancy
Hypertriglyceridemia	Acute or Chronic blood loss
Alcoholism	
Opiate Addiction	
Iron deficiency state	
Post-splenectomy	
Hyperbilirubinemia	
Chronic Aspirin therapy	

- Mechanism by which a hemoglobin variant causes a falsely low HbA1c is by causing hemolysis and a shortened red cell survival. In fact, any condition that shortens red cell survival or necessitates blood transfusions will falsely lower HbA1c levels, as for example hemolytic anemias, blood loss, or treatment with multiple transfusions.

- However, not all anemias will lower HbA1c levels. Both iron deficiency anemia and pernicious anemias, for example, are associated with a longer RBC survival, which is associated with an increase in HbA1c levels. In addition, in the case of iron deficiency, malondialdehyde, which is increased in iron deficiency anemia, enhances the glycation of hemoglobin and the combination of these two mechanisms regularly results in a false increase in HbA1c levels in patients with iron deficiency anemia.

- Chronic renal failure (CRF) is also associated with a shortened red cell survival and falsely low HbA1c levels, but in CRF, other mechanisms are also in play that may instead raise the HbA1c. These include decreased levels of erythropoietin, increased glycation, higher levels of carbamylated hemoglobin, and variable exposure to higher levels of glucose during dialysis.

- Vitamins C and E have been reported to lower A1C measurements, possibly by inhibiting glycation[72]

- Lower A1C levels are found in diabetic and non diabetic pregnant women, probably due both to lower fasting blood glucose and a shortened erythrocyte lifespan[72]

- There are also genetic variances in the speed of glycation, one of the explanations given for the observation that in the African-American population, the HbA1c may be higher than expected from the levels of glycemia[72]

- Hyperbilirubinemia: Severely icteric specimens may give falsely elevated HbA1c values with methods relying on charge separation if whole

blood haemolysates are used, since bilirubin migrates with the fast hemoglobin and absorbs at the detecting wavelength. [73]

- Hyperlipidemia: Hyperlipidemia can also cause false elevation of HbA1c , since lipids elute in the first HbA1c fraction and absorb at 415 nm. This issue is method specific. It could be magnified if analysis were performed on postprandial samples. Since Hyperlipidemia is a relatively common finding in diabetic patients, this limitation is important. [73]

HbA1c AS A DIAGNOSTIC TOOL FOR DIABETES

Glycosylated hemoglobin measures the number of glucose molecules attached to hemoglobin. Although glycation depends on the life span of RBC which is normally 120 days considering the age group among different RBC populations on an average, it is accepted that HbA1c reflects glycaemic status of prior 90 days. ADA recommends the use of HbA1c as a diagnostic marker for diabetes and categories for increased risk of diabetes. Persons with HbA1c of 6.5% and above (≥ 6.5% or 48 mmol/mol) are to be diagnosed as diabetes and HbA1c between 5.7-6.4 are considered to have pre-diabetes. The A1C test should be performed using a method that is certified by the NGSP (www.ngsp.org) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay. Although point-of-care A1C assays may be NGSP certified, proficiency testing is not mandated for performing the test, so use of point-of-care assays for diagnostic purposes is not recommended.[18]

ADVANTAGES OF HbA1c TESTING:

- Greater convenience (fasting not required)
- Greater pre-analytical stability
- Less day-to-day perturbations during stress and illness
- Can differentiate between stress hyperglycaemia and pre-existing undetected diabetes in emergency situations
- HbA1c estimation is also useful to plan therapy in newly detected T2DM as per American Association of Clinical Endocrinologists (AACE) recommendation 2015[74]. If a patient presents with an A1C level >9.0% and ymptomatic hyperglycemia, it is recommended that basal insulin should be started along with oral agents.
- Easy tool to assess glycemic control in diabetic patients at the time of follow-up
- Predictor of complications

DIS-ADVANTAGES OF HbA1c TESTING:

- Levels can be misleading in a variety of conditions like anemias and hemoglobinopathies, as already described
- Cost factor
- May not be available in primary health care set-up
- Co-existing severe illness can shorten RBC life span and give misleading results
- A1C does not provide a measure of glycemic variability or hypoglycemia

HbA1c and Mean Blood Glucose:

The international A1C Derived Average Glucose (ADAG) trial was based the correlation with A1C on frequent SMBG (Self-monitoring of blood glucose) and CGM (Continuous glucose monitoring) in 507 adults with type 1,type 2, and no diabetes[75], and an empirical study of the average blood glucose levels at pre-meal, post-meal, and bedtime associated with specified A1C levels using data from the ADAG trial[76]. The American Diabetes Association (ADA) and the American Association for Clinical Chemistry have determined that the correlation in the ADAG trial is strong enough to justify reporting both the A1C result and the eAG result when a clinician orders the A1C test

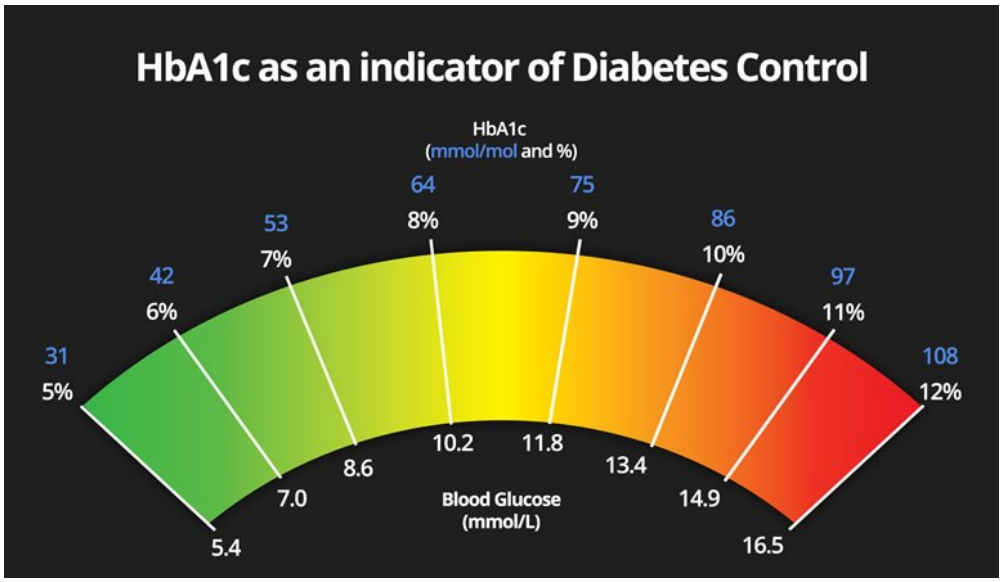


Fig. – 16: HbA1c as an indicator of Diabetes control

HbA1c GOALS (ADA 2017)[77]

- A reasonable A1C goal for many nonpregnant adults is 7% (53mmol/mol).

- More stringent A1C goals (such as 6.5% [48 mmol/mol]) are suggested for selected patients if this can be achieved without significant hypoglycemia or other adverse effects of treatment. Appropriate patients include those with short duration of diabetes, type 2 DM treated with lifestyle or metformin only, long life expectancy, or no significant cardiovascular disease.

- Less stringent A1C goals (such as 8% [64 mmol/mol]) may be appropriate for patients with a history of severe hypoglycemia, limited life expectancy, advanced microvascular or macrovascular complications, extensive co-morbid conditions, or long-standing diabetes in whom the general goal is difficult to attain despite diabetes self-management education, appropriate glucose monitoring, and effective doses of multiple glucose-lowering agents including insulin.

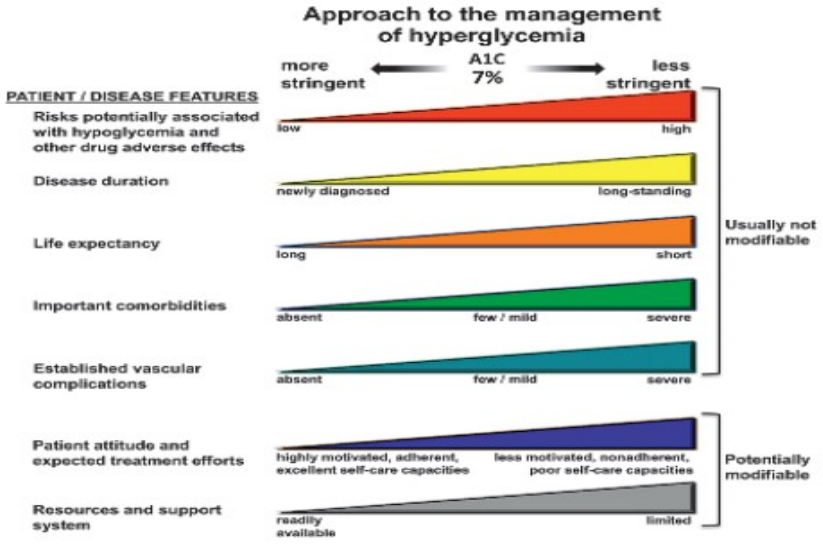


Fig. 17 : Depicted are patient and disease factors used to determine optimal A1C targets. Characteristics and predicaments toward the left justify more stringent efforts to lower A1C; those toward the right suggest less stringent efforts. [78]

HbA1c AND COMPLICATIONS IN T2DM:

The Kumamoto Study[79] and UK Prospective Diabetes Study (UKPDS)[80,81] confirmed that intensive glycemic control was associated with significantly decreased rates of microvascular and neuropathic complications in patients with type 2 diabetes. Also, three landmark trials (Action to Control Cardiovascular Risk in Diabetes [ACCORD], Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation [ADVANCE], and Veterans Affairs Diabetes Trial [VADT]) showed that lower A1C levels were associated with reduced onset or progression of microvascular complications[82-84]. In type 2 diabetes, there is evidence that more intensive treatment of glycemia in newly diagnosed patients may reduce long-term CVD rates.

HbA1c TESTING: ADA 2016 RECOMMENDATIONS [77]

- Perform the A1C test at least two times a year in patients who are meeting treatment goals (and who have stable glycemic control)
- Perform the A1C test quarterly in patients whose therapy has changed or who are not meeting glycemic goals.

PLATELETS[85,86]

Platelets as small, anucleate fragments with occasional reddish granules, measuring approximately 2 μm in diameter with a volume of approximately 8fL and exhibiting considerable variation in size and shape. [85] They play an important role in hemostasis and thrombosis. While not immune cells, per se, they often participate in the response to tissue injury in cooperation with inflammatory cell types. They have a ring of microtubules around their periphery and an extensively invaginated membrane

with an intricate canalicular system in contact with the ECF. Their membranes contain receptors for collagen, ADP, vessel wall von Willebrand factor, and fibrinogen. Their cytoplasm contains actin, myosin, glycogen, lysosomes, and two types of granules:

1) Dense granules, which contain the nonprotein substances that are secreted in response to platelet activation, including serotonin, ADP, and other adenine nucleotides

2) α-granules, which contain secreted proteins. These proteins include clotting factors and platelet-derived growth factor (PDGF). PDGF is also produced by macrophages and endothelial cells. It is a dimer made up of A and B subunit polypeptides. PDGF stimulates wound healing and is a potent mitogen for vascular smooth muscle. Blood vessel walls as well as platelets contain von Willebrand factor, which, in addition to its role in adhesion, regulates circulating level of factor VIII.

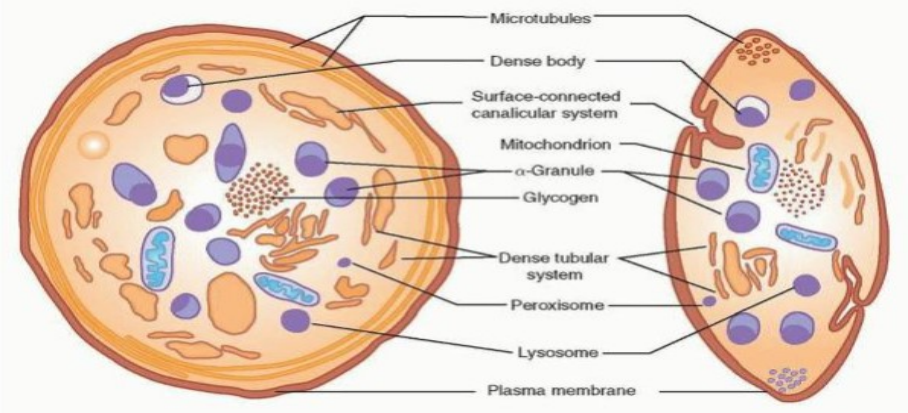


Fig - 18 : Diagram of a human platelet displaying components visible by electron microscopy and cytochemistry[85]

When a blood vessel wall is injured, platelets adhere to the exposed collagen and von Willebrand factor in the wall via receptors on the platelet membrane. Von Willebrand factor is a very large circulating molecule that is produced by endothelial cells. Binding produces platelet activations, which release the contents of their granules.

The released ADP acts on the ADP receptors in the platelet membranes to produce further accumulation of more platelets (platelet aggregation). Humans have at least three different types of platelet ADP receptors: P2Y1, P2Y2, and P2X1. Aggregation is also fostered by platelet-activating factor (PAF), a cytokine secreted by neutrophils and monocytes as well as platelets. This compound also has inflammatory activity. It acts via a G protein-coupled receptor to increase the production of arachidonic acid derivatives, including thromboxane A2.

The normal platelet count varies between 150,000 and 400,000/µl, and normal platelet size (mean platelet volume) varies between 7.5 and 10.5 fL[85]. Platelets are released into the blood from long proplatelet extensions of megakaryocytes. Platelets labeled with ^{51}Cr (chromate) have been used to estimate platelet lifespan in humans at 8 to 12 days.

MEAN PLATELET VOLUME [87]

Platelet volume is a marker of platelet function and activation. Although several measurements of platelet activity have emerged as potential contributors to atherothrombosis, many of these measurements are time-consuming, expensive, use a high sample volume, or require specialty training [88,89]. Alternatively, mean platelet volume (MPV) is a marker of platelet size that is easily determined on routine automated hemograms and routinely available at a relatively low cost. Subjects with a

higher MPV have larger platelets that are metabolically and enzymatically more active and have greater prothrombotic potential than smaller platelets. MPV correlates with platelet function and activation, whether measured as aggregation, thromboxane synthesis, beta-thromboglobulin release, procoagulant function, or adhesion molecule expression.

MPV is increased in certain vascular risk factor states, including hypercholesterolemia and diabetes mellitus. Several studies have been done to establish the normal range of mean platelet volume. Currently, 7.5 to 10.5 fL is an accepted normal range.[85]

MEASUREMENT OF PLATELET VOLUME:

The optimal method for measuring platelet volume utilizes changes in either electrical impedance (as used in coulter haematology analysers) or light diffraction (as used by Technicon) when a platelet passes through a narrow aperture. Alternative methods include semi-quantitative measurement of diameter on platelet smears, or using flow cytometry, but these are less satisfactory. A platelet histogram is derived from the data, and MPV is calculated as the mode. Differences of nearly 40% have been found when Coulter and Technicon results are compared.

With regard to anti-coagulant of whole blood prior to automated cell counting, studies have been done, comparing EDTA and citrate. Complete blood count specimens are usually anticoagulated in EDTA which causes platelet to swell in a time dependent manner. EDTA is thought to increase intracellular cyclic AMP and change plasma membrane permeability. Some studies have shown the inefficacy of EDTA for testing MPV as it causes a progressive increase in MPV [90]. However, in a study done in Iran by Dastjerdi et al, MPV can be measured accurately by both

methods of anticoagulation; EDTA and citrate if analysis is performed within 1 hour of sampling.[91]

MPV AND DIABETES [6]

Normal blood flow and thrombo-resistance is dependent on vasomotion, blood corpuscular elements, plasma components, and their interaction with the endothelial surface. Rupture of an atherosclerotic plaque exposes sub-endothelial material that promotes platelet activation and the local initiation of the coagulation cascade that can lead to thrombus formation at the site of endothelial disruption. Acute vascular events, such as myocardial infarction and stroke, are due to such atherothrombotic events rather than gradual progression of luminal stenosis caused by atheromatous plaque.

Patients with DM not only have a greater atheromatous plaque burden but also a thrombotic diathesis that is in part due to changes in the coagulation system with increased levels of plasma fibrinogen, increased intravascular thrombin generation, and reduced fibrinolytic potential. Equally importantly, however, platelets from patients with diabetes mellitus have dysregulated signaling pathways that lead to an increased tendency to activate and aggregate in response to a given stimulus (platelet hyper-reactivity). Platelet activation contributes to the pathology by not only triggering thrombus formation but also causing microcapillary embolization and release of constrictive, oxidative, and mitogenic substances that accelerate progression of local vascular lesions.

Platelet hyper-reactivity and increased baseline activation in patients with diabetes is multifactorial and associated with biochemical factors such as hyperglycemia and hyperlipidemia, insulin resistance, and an inflammatory and oxidant state.

BIOCHEMICAL FACTORS AFFECTING PLATELET FUNCTION IN DIABETES

Hyperglycemia is the diagnostic hallmark finding in diabetes mellitus and is associated with macrovascular disease even in the prediabetic stage. Hyperglycemia, particularly postprandial, plays a significant role in the DM-associated development of cardiovascular disease as well as the DM prothrombotic state [92].

In healthy subjects, without DM, the induction of acute hyperglycemia can lead to increased platelet reactivity and platelet activation as evidenced by increased markers such as soluble P-selectin and CD40-ligand [93-95]. Exposure of platelets to hyperosmolar solutions also causes increased reactivity, suggesting that hyperglycemia may have a direct osmotic effect[96]. Both chronic and acute hyperglycemia cause *in vivo* activation of protein kinase C (PKC), a transduction pathway mediator for many proaggregatory platelet agonists[97].

Platelets from patients with DM, unlike those from healthy individuals, also manifest short-term activation of the calcium-sensitive PKC-β isoenzyme by acute hyperglycemia even *in vitro*, in the absence of additional stimuli, indicating an inherent diabetes-related dysregulation of this pathway.

Recurrent episodes of hyperglycemia lead to the production of AGEs [98]. Some of these AGEs cause externalization of platelet membrane phosphatidylserine that leads to surface clotting factor activation and so directly enhance the thrombogenic state [99]. Similarly, the platelets of patients with diabetes have increased glycation levels of surface membrane proteins which cause decreased membrane fluidity and increased platelet sensitivity to agonists. [100,101].

The final common pathway of platelet activation signaling is platelet aggregation mediated by the glycoprotein IIb/IIIa receptor (GPIIb/IIIa) platelet-fibrin interaction. The expression of platelet surface GP IIb/IIIa, as well as of GPIb, which mediates binding to von Willebrand factor, correlates with levels of hemoglobin A_{1C}. Hyperglycemia leads to release of larger platelets with more GPIb and GPIIb/IIIa receptors and higher thromboxane forming capacity [102]. Other platelet surface receptors, such as $P2Y_{12}$, the target of widely used thienopyridine antiplatelet agents, are also present in increased numbers on DM platelets likely as a result of the altered membrane fluidity dynamics [103]. Activation of the platelet $P2Y_{12}$ receptor normally leads to reduced levels of cyclic adenosine monophosphate (cAMP) and subsequently suppressed phosphorylation of vasodilator-stimulated phosphoprotein by specific protein kinases (PKA) that enhances platelet activation and aggregation [104]. Platelets from patients with DM have lower levels of cAMP compared with non-diabetics, with consequently upregulated $P2Y_{12}$ signaling. Platelets of older diabetics in particular have a higher baseline intracellular calcium level with more enhanced calcium mobilization from intracellular stores in response to thrombin agonism compared to non-diabetic patients [103,105]. The higher baseline calcium and lower cAMP make the platelets more reactive such that they activate and aggregate at lower levels of agonist stimulation.

EFFECTS OF INSULIN ON PLATELETS IN DIABETES

Insulin can directly regulate platelet function via a functional insulin receptor (IR) found on human platelets [106]. Binding of insulin to the IR leads to activation of the insulin receptor substrate-1 (IRS-1) through tyrosine phosphorylation which initiates association with the $G_i\alpha$-subunit. The result is a reduced G_i activity that

impairs tonic cAMP suppression, and thus leads to increased cAMP intraplatelet levels, blunting of $P2Y_{12}$ signaling and reduced platelet activity[107,108] Experiments in healthy non-obese individuals confirm that insulin inhibits platelet interaction with collagen and attenuates the platelet aggregation effect of agonists [109].

Obesity, a common feature in T_2DM patients, can exacerbate or induce insulin resistance yet is associated with platelet hyper-reactivity [110] Furthermore, obese patients have evidence of increased platelet activation with increased plasma CD40L, increased levels of platelet derived microparticles (released in blood by platelet activation), and higher thromboxane production [111,112].

The mechanism underlying these discrepant effects of insulin on the platelets of healthy individuals versus patients with insulin resistance appears to be impairment of the insulin receptor signaling pathway that occurs not only in tissues but also in platelets. The reduced platelet insulin sensitivity leads to lower cAMP levels, increased intraplatelet calcium concentration, and platelet hyper-reactivity. Hyperinsulinemia is, therefore, not protective but potentially detrimental to platelet reactivity in patients with insulin resistance.

Several studies have been done to establish the relation between diabetes and platelet indices, over the last few years :

P.C.Sharpe et al[113], in 1993, measured mean platelet volume (MPV) in patients with diabetes mellitus and compared the results with MPV in non-diabetic controls. Mean MPV was significantly increased in the diabetic subjects (8.9 ± 0.07 fL,) compared with non-diabetic subjects (8.0 ± 0.05) ($p < 0.001$).

Hekimsoy Z et al[114], in 2004, measured MPV in 145 consecutive Type 2 diabetic patients and 100 non-diabetic control subjects without known coronary artery disease. MPV was significantly higher and the mean platelet counts were significantly lower in diabetics compared to age- and sex-matched non-diabetic healthy controls [10.62 ± 1.71 fl vs. 9.15 ± 0.86 fL (P=.00), 260.38 ± 68.65 x 10 (9)/L vs. 292.33 ± 79.19 x 10 (9)/L (P=.001)], respectively.

Papanas N. et al[5], in 2004, did a study on 416 patients divided into two groups- Group A comprised 265 type 2 diabetic patients (131 men) with a mean age of 67.4 ± 9.5 years and a mean diabetes duration of 14.5 ± 5.7 years. Group B comprised 151 non-diabetic patients (74 men) with a mean age of 68.6 ± 9.1 years. MPV was significantly higher (P = 0.01) in group A (14.2 ± 2.2 fL) than in group B (7.1 ± 1.2 fL). In group A, MPV was significantly higher (P = 0.043) in patients with retinopathy (15.8 ± 1.3 fL) than in patients without retinopathy (10.9 ± 1.1 fL) and also significantly higher (P = 0.044) in patients with microalbuminuria (15.6 ± 1.2 fL) than in patients without microalbuminuria (10.1 ±1.2 fL). No association, however, was found in group A between MPV and age, gender, duration of diabetes, insulin dependency, BMI, HbA1c , coronary artery disease or Dyslipidemia.

Papanas N et al[115], in 2005, did a study that included 163 patients divided into two groups A (patients with neuropathy) and B (patients without neuropathy). MPV was significantly (P = 0.03) higher in patients of group A (15.2 ± 1.6 fL) than in those of group B (11.3 ± 1.4 fL).

Zuberi B F et al[116], in 2006 did a study on 612 patients in Karachi, Pakistan, and patients were allocated to three groups of 204 patients each, referred to as DM

group, IFG group and non-DM group. These included 337 (55.1%) males and 275 (44.9%) females. MPV in the DM group was 9.34 fL, in the IFG Group 8.98 fL, and in the non-DM group 8.63 fL. Comparison of MPV values for the three groups showed statistically significant inter-group and intra-group differences, with a p-value of 0.00.

Refik Demirtunc[117] et al, in 2009, studied 70 patients with type 2 DM and 40 age and sex-matched healthy individuals were enrolled. Diabetic patients were grouped into those with glycated hemoglobin (HbA1c) levels $\leq$ 7% (Group A, n=35 patients) and those with HbA1c > 7% (Group B, n=35 patients). MPV was significantly higher in patients with DM than in controls (8.7±0.8 fL vs. 8.2±0.7 fL, *P*=.002). In diabetic patients, there was a significant positive correlation between MPV and HbA1c levels (*r*=.39, *P*=.001) but not diabetic vascular complications. Among the two diabetic groups, Group B patients had significantly higher MPV than Group A (9.0 ± 0.7 fL vs. 8.4 ± 0.8 fL, P=.01)

Tavil Y et al[118], in 2010, did a study on 258 patients, who were divided into two groups. Group A consisted of 158 type 2 diabetic patients with coexistent coronary artery disease and Group B, the control group, consisted of 100 subjects without type 2 diabetes with normal coronary angiographies. The MPV was significantly different in the patient group compared to the controls (9.79 ± 1.5 fL vs 8.3 ± 0.9 fL, P<0.001).

Jindal S et al[119], in 2011, did a study which included 75 subjects with DM (50 with one or more microvascular complications) and 50 non-selected patients from the hospital as controls. MPV, PDW and platelet-large cell ratio were all significantly higher in diabetic patients compared to the control subjects (P<0·05 for all).

Thomas Alex Kodiatte et. al[120], in 2012, did a study on 166 male diabetics and 89 female diabetics in the study (255 in total), there were 145 non-diabetic males and 106 non-diabetic females in the study (251 in total). In the diabetic subjects, MPV was significantly higher (8.29±0.735 fL) as compared to the non-diabetic group (7.47±0.726 fL; $P < 0.001$). Among the diabetic subjects, a positive statistical Pearson correlation was seen between MPV and HbA1c levels ($r = 0.29$; $P < 0.001$), FBS levels ($r = 0.269$; $P < 0.001$) and PPBS levels ($r = 0.194$; $P = 0.002$). However, no statistical correlation was seen between MPV and the duration of DM, BMI and the vascular complications in the diabetic group. In the diabetic group, the mean MPV in subjects with complications (8.35±0.73 fL) were higher than that of subjects without complications (8.2±0.74 fL) but independent student t-test did not show any statistical significance ($P = 0.145$). Also, diabetic group was divided based on the HbA1c levels into group A (HbA1c $< 6.5\%$) and group B (HbA1c $\geq 6.5\%$). The mean MPV in group A (7.95±0.72 fL) was significantly lower than that of group B (8.35±0.724 fL; $P = 0.003$)

Shah B. et al[121], in 2012, did a retrospective analysis of 13,021 participants in the National Health and Nutrition Examination Survey from 1999 to 2004. MPV was significantly higher in subjects with diabetes (8.20 vs. 8.06 fL, $P < 0.01$) but not in subjects with metabolic syndrome (8.09 vs. 8.07 fL, $P = 0.24$). There was a significant correlation between MPV and glucose ($P < 0.0001$) and between MPV and hemoglobin A1c ($P < 0.0001$) in subjects with diabetes. These correlations were no longer significant in those without diabetes. The adjusted odds of diabetes rose with increasing MPV levels and were most pronounced in subjects with MPV levels exceeding the 90th percentile (≥9.31 fL). The association between MPV and diabetes was most apparent in those with the poorest glucose control.

Shimodaira et al[122], in 2012, studied 1876 Japanese subjects who were categorized into four groups according to FPG: Q1 (70 mg/dL ≤ FPG < 90 mg/dL, n = 467), Q2 (90 mg/dL ≤ FPG < 95 mg/dl, n = 457), Q3 (95 mg/dL ≤ FPG < 100 mg/dL, n = 442), and Q4 (100 mg/dL ≤ FPG < 126 mg/dL, n = 512). Q1, Q2, and Q3 were defined as normal FPG groups and Q4 was defined as pre-diabetic group. The MPV increased with the increasing FPG levels, in the following order: Q1 (9.89 ± 0.68 fL), Q2 (9.97 ± 0.69 fL), Q3 (10.02 ± 0.72 fL), and Q4 (10.12 ± 0.69 fL). After adjusting for the confounding parameters, MPV of the prediabetic group was higher than that in other groups ($P < 0.001$ for Q4 vs. Q1 and Q2, and $P < 0.05$ for Q4 vs. Q3). MPV in the high-normal glucose group (Q3) was significantly higher than in the low-normal glucose group (Q1). MPV was independently and positively associated with FPG, not only in prediabetic subjects but also in normal FPG subjects ($p = 0.020$ and $p = 0.006$, respectively).

Li S et al[123], in 2012, explored the variance of mean platelet volume (MPV) in subjects with normal glucose tolerance (NGT), impaired glucose regulation (IGR) and type 2 diabetes mellitus (T2DM) and risk factors of MPV changes and analyzed the relationship between MPV and diabetic peripheral artery disease (PAD). A total of 173 subjects were enrolled into this observational cross-sectional study. They were divided into 3 groups: NGT (n = 41), IGR (n = 41) and T2DM (n = 91). The MPV level was highest in the T2DM group (12.3 ± 1.5) fL. And it was significantly higher in the IGR group than in the NGT group (9.7 ± 0.9 vs 8.0 ± 0.9) fL ($P < 0.01$). It was significantly higher in diabetics with HbA1c ≥ 7% (13.2 ± 1.9) fL than in patients those with HbA1c <7% (11.8 ± 1.7) fL ($P < 0.01$). Multiple Logistic regression analysis indicated that MPV was an important risk factor of PAD

Farah Jabeen et al[124], in 2013, evaluated 170 Diabetic patients (Type-2) (93 male & 77 females) and 92 healthy control (42 male & 50 females) in an institute in Pakistan. Mean age of control is 45.5±0.77 years and 51.08±0.7 years for patients. Significant increase in MPV ($P < 0.0001$) and PDW ($P < 0.0033$), was found in diabetic patients as compared to control group.

Wan H et al[125], in 2013, conducted a study on 128 subjects, who were divided into 3 groups based on carotid artery intima-media thickness (IMT): normal IMT group (A, IMT □1.1 mm, n = 26), IMT thickness group (B, IMT >1.1 mm, n = 45), IMT thickening with plaque group (C, $n = 57$). MPV was significantly higher in group A as compared to both group B and group C (7.94 ± 0.97 fL, 7.24 ± 0.71 fL, 7.03 ± 0.79 fL, respectively, P 〈0.001). Thus, level of MPV was correlated with IMT in diabetic patients and MPV could be a useful marker for detecting atherosclerosis risk in diabetic patients.

Ulutas et al[126], in 2014, did a study on 33 male and 32 female diabetics, and 21 non-diabetic males and 19 non-diabetic females. The diabetic group was divided based on the HbA1c levels into Group A (HbA1c ≤7%) and Group B (HbA1c >7%). MPV was significantly higher (8.3±1.3 fL) in Group B as compared to both non-diabetics (7.1±1.0 fL; $p<0.001$) and Group A (7.5±1.1 fL; $p=0.039$). MPV had a high positive Pearson Correlation with HbA1c ($r=0.393$; $p<0.001$) and FSG ($r=0.41$; $p<0.001$), as with diabetes duration in a significance of $p=0.02$ ($r=0.222$)

Ezgi Coşkun Yenigün[127], in 2014, evaluated MPV in patients with type II diabetes mellitus (DM) and its associations with diabetic microvascular and macrovascular complications. A total of 48 patients with type II DM and 30 age and gender matched healthy subjects constituted the study population. 12 of the diabetics

(25 %) had macrovascular complications, 26 patients (54.2 %) had HT, 15 patients (31.3 %) had retinopathy, 16 patients (33.3 %) had nephropathy and 39 patients (81.2 %) had neuropathy. Mean HbA1c was 8.73 ± 2.03 %. MPV was significantly higher in patients with type II DM than the healthy controls (9.25 ± 1.49 and 8.47 ± 0.49, respectively) ($p<0.01$). The diabetic patients were divided into subgroups depending on the presence of microvascular complications. Patients with at least one of the microvascular complications had slightly higher MPV compared to the ones without any of the complications (9.38 ± 1.47 fL and 7.85 ± 0.88 fL, respectively) ($p= 0.048$). In type II diabetic patients there was no association between MPV and age, duration of diabetes, lipid profile, HbA1c , and FBS.

Lutfullah Cakir et al[128], in 2014, studied 96 subjects in a hospital in Turkey (46 patients with type 2 diabetes and 50 healthy controls). MPV was significantly elevated in study group (9.05 [4.3-11.9]) compared to control subjects (8.14 [6.1 – 10.5]) ($p<0.001$).

Vadatti T et al[129], in 2015, conducted a study on 171 type 2 diabetics and 37 controls in Guntur, India. In their study, MPV was significantly higher in diabetics when compared to healthy controls (7.91 ± 0.87 > 6.91 ± 0.71). There was also a statistically significant positive correlation between HbA1c and MPV (r value - 0.5, p value - 0.4).

Kurt H et al[130], in 2015, examined baseline and final HbA1c , MPV and FPGA values of 343 patients. The study group (SG) consisted of 169 patients with diabetes whose HbA1c levels decreased by 1% in 3 months and the control group involving 174 patients whose HbA1c levels did not change. There existed a positive correlation between MPV and HbA1c levels ($r=154$; $p<0.005$). Additionally, positive

correlation was found between MPV and HbA1c changes (r=216; p=0.005), and MPV and FPG changes (r=245; p=0.001) in SG.

Xiangyu chen et al[133],The relationship between type 2 diabetes and platelet indicators. No relationship between the presence of diabetes with PDW and PLT. The MPV was independently associated with the presence of diabetes.

Buch A et al [134] showed that MPV was found to be high in diabetics when compared to non-diabetics. MPV and PDW are predictive biomarkers of diabetic vascular complications

MATERIALS AND METHODS

SOURCE OF DATA:

Data was collected from patients fulfilling the inclusion and exclusion criteria visiting medicine OPD of R L Jalappa Hospital, Tamaka, Kolar.

METHOD OF COLLECTION OF DATA:

- Detailed history including regarding diabetes, hypertension and drug intake was taken. Family history was enquired. MPV and platelet count in the above diabetic and non-diabetic subjects was done using an automatic blood counter (Alere H 560). Venous blood samples from brachial vein was collected and within 1 hour of collection, samples were analyzed to minimize variations due to sample aging. Renal parameters such as blood urea, serum creatinine, and urine micro albumin and urea albumin/serum creatinine were done[17]. Fundoscopy was done to look for retinopathy changes. At least two micro aneurysms and/or retinal hemorrhages and/or other signs of retinal damage were recognized as diabetic retinopathy. Biothesiometry was done to look for neuropathy.

- Now the study population was divided into three groups:

1. Group A: Normal healthy non-diabetic
2. Group B: Diabetics without microvascular complications.
3. Group C: Diabetics with microvascular complications.

The study was done over a period of 17 months, from june 2016 to July 2017

TYPE OF STUDY:

Cross sectional study

INCLUSION CRITERIA OF CASES:

- Patients diagnosed with type 2 DM with chronic micro vascular complication.
- Adults more than 18 years of age

EXCLUSION CRITERIA OF CASES:

- Male patient and female patients with hemoglobin below 12mg/dl and below 11mg/dl respectively.
- Diabetics on antiplatelet drugs such as aspirin and clopidogrel
- Pregnant women
- Malignancy
- Collagen Vascular disease
- Infections

INCLUSION CRITERIA OF CONTROLS:

- Non-diabetics above the age of 18 years

EXCLUSION CRITERIA OF CONTROLS:

- Male patient and female patients with hemoglobin below 12mg/dl below 11mg/dl respectively.
- Pregnant women
- Malignancy

- Collagen Vascular disease
- Infection

Study design:

- It is a cross sectional study
- The study was carried out in 132 patients, and they were divided into three groups

1. Group A: Normal healthy non-diabetic
2. Group B: Diabetics without microvascular complications.
3. Group C: Diabetics with microvascular complications.

- Complete case history was taken from all the subjects, and a thorough clinical examination was done
- The following investigations were done for all the subjects:

1. Complete blood count
2. Fasting blood glucose
3. Post prandial blood glucose
4. Electrocardiogram
6. HbA1c (glycosylated hemoglobin)
7. Blood urea
8. Serum creatinine
9. Urine routine examination
10. Urine Protein: Creatinine ratio

11. Fundus examination

12. Biothesiometry

13. Other relevant investigations whenever needed

- MPV was done as a part of complete blood count using Alere H 560 - Automated Hematology Analyzer.
- Venous blood samples were collected in di-potassium EDTA and tested within one hour of collection to minimize variations due to sample aging.
- Samples for plasma glucose estimation and HbA1c were collected in sodium fluoride and di-potassium EDTA, respectively.
- Urine P: Cr ratio was taken into account to evaluate for diabetic nephropathy. Patients having significant proteinuria and nephrotic range proteinuria were considered to have nephropathy.
- Biothesiometry was done to look for features of neuropathy.
- A detailed peripheral vascular examination was done to look for evidence of peripheral vascular disease
- Microvascular complications consist of neuropathy, retinopathy and nephropathy. Patient was considered to have microvascular complication if any one or more of the three were present.

Data analysis:

- Data will be entered into Microsoft excel data sheet and will be analyzed using SPSS 22 version software.
- Results are expressed as Mean ± SD (Min-Max).
- Microsoft Excel 2016 was used to plot graphs and for the masterchart.
- The following were the statistical tests used:
  - Categorical data will be represented in the form of Frequencies and proportions.
  - Continuous data will be represented as mean and standard deviation.
  - Independent samples t test for comparative analysis
  - One way analysis of variance with Duncan's multiple range test for pair wise comparisons
  - Pearson's correlation coefficient
  - Chi square test
- A "p value" of < 0.05 is considered as statistically significant

OBSERVATION AND RESULTS

Statistical analysis was carried out to meet the objectives of the study.

The statistical results are compared at 0.05 level of significance. (p value ≤ 0.05 implies significance)

Group 1= Non diabetic,

Group 2= Diabetic without complication,

Group 3= Diabetic with complication

Table 7: Age Distribution

Age	Frequency	Percent
26-40 years	18	13.6
41-55 years	45	34
56-70 years	60	45.45
>70 years	9	6.8
Total	132	100

Graph:1 Distribution of subject across age

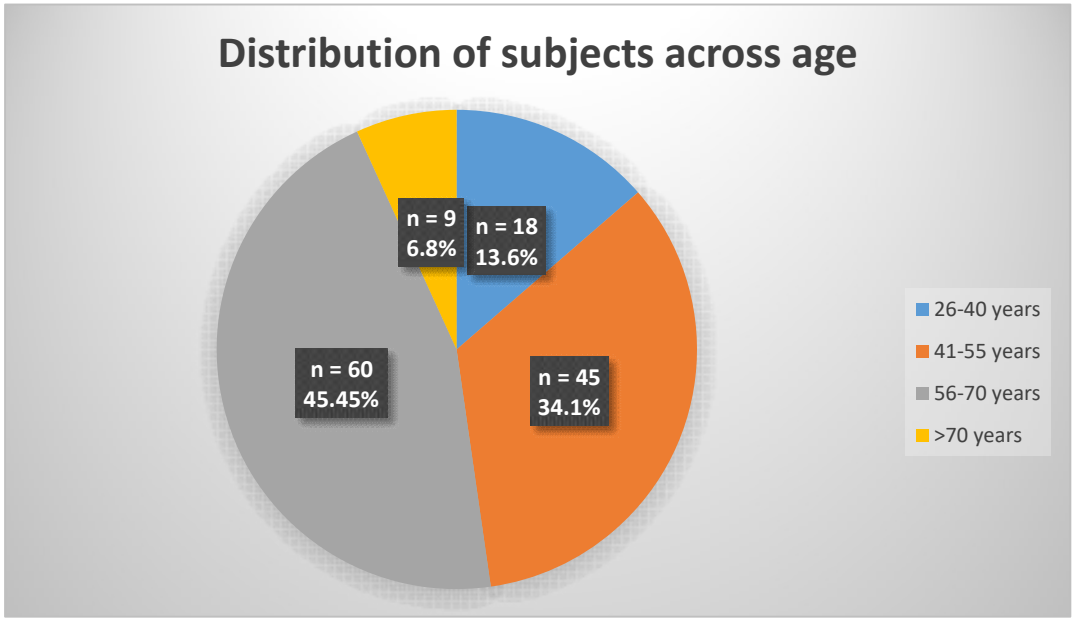


Table 8:- Distribution of subject according to age group among the groups

AGE Group	GROUP			Total
	1	2	3	
26-40yrs	18	0	0	18
	40.9%	.0%	.0%	13.6%
41-55yrs	26	4	15	45
	59.1%	9.1%	34.1%	34.1%
56-70yrs	0	40	20	60
	.0%	90.9%	45.5%	45.5%
>70yrs	0	0	9	9
	.0%	.0%	20.5%	6.8%
Total	44	44	44	132
	100.0%	100.0%	100.0%	100.0%

Mean age of the Group1 was 42.41 $\pm$ 4.5 yrs. Mean age of the Group2 was 59.57 $\pm$ 2.7 yrs. Mean age of the Group 3 was 63.75 $\pm$ 10.3 yrs. P Value = <0.001, There was a statistically significant difference between the Age groups among the groups.

Graph 2:- Graph showing distribution of subject according to age group

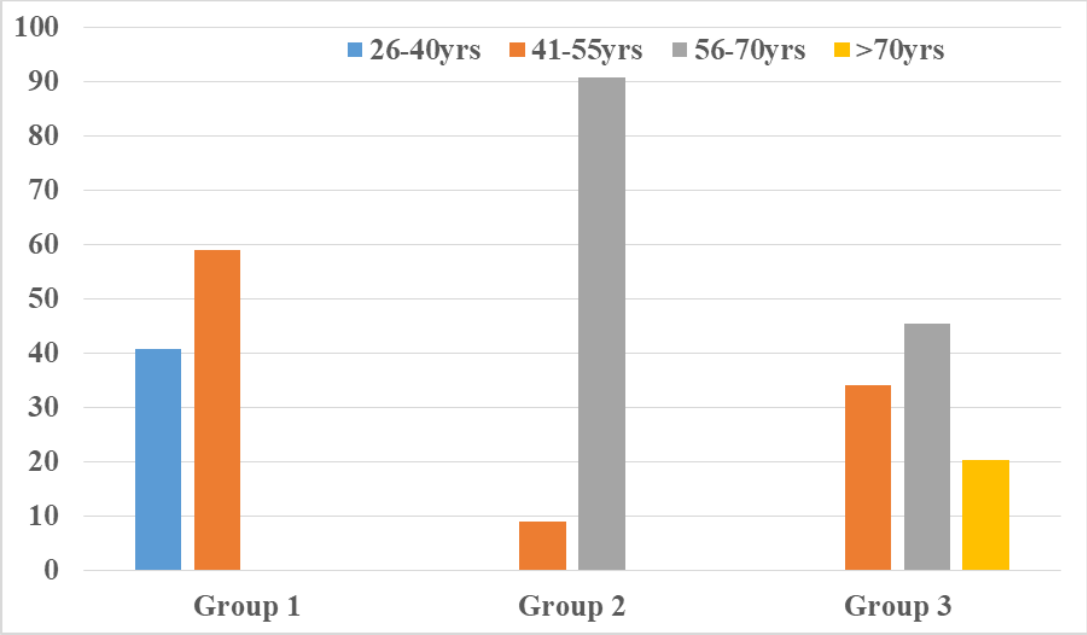


Table 9:- Distribution of subject according to sex among the groups

SEX	Group			Total
	1	2	3	
Female	17	18	17	52
	38.6%	40.9%	38.6%	39.4%
Male	27	26	27	80
	61.4%	59.1%	61.4%	60.6%
Total	44	44	44	132
	100.0%	100.0%	100.0%	100.0%

P value = 0.351, There was no statistically significant difference between sex among the groups.

Graph 3:- Graph showing distribution of subject according to sex among the groups

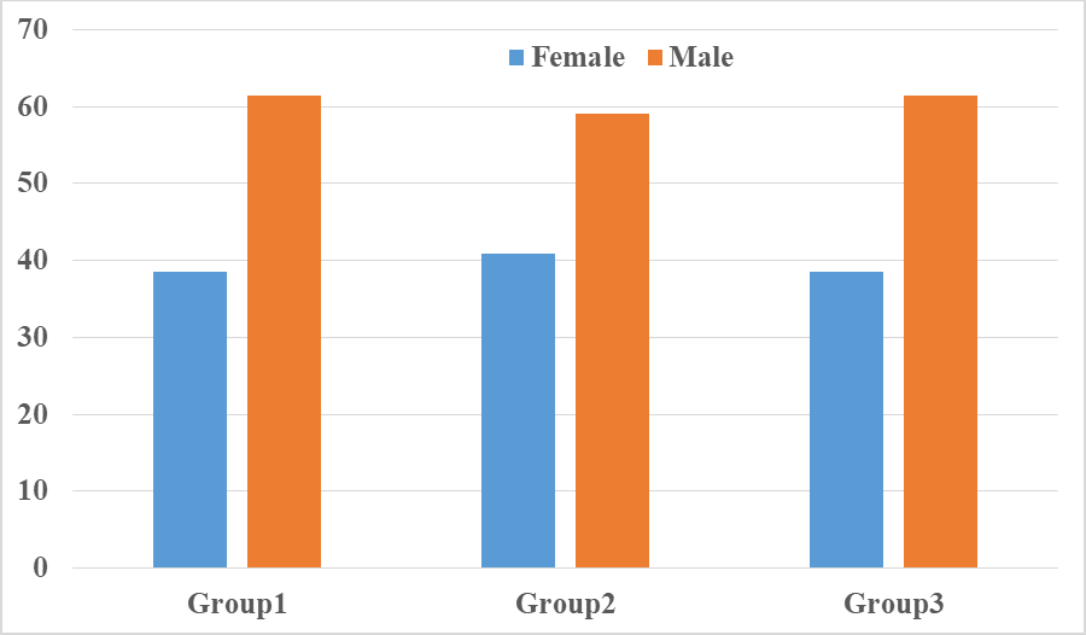


Table 10:- Comparison of mean MPV between the age groups

Age Group	Mean MPV	Std. Deviation	P Value
26-40yrs	5.41	.33	<0.001
41-55yrs	7.81	3.06	
56-70yrs	8.90	1.65	
71yrs	11.34	1.19	

There was a statistically significant difference of Mean MPV between the age groups.

Graph 4:- Graph showing Comparison of mean MPV among the groups

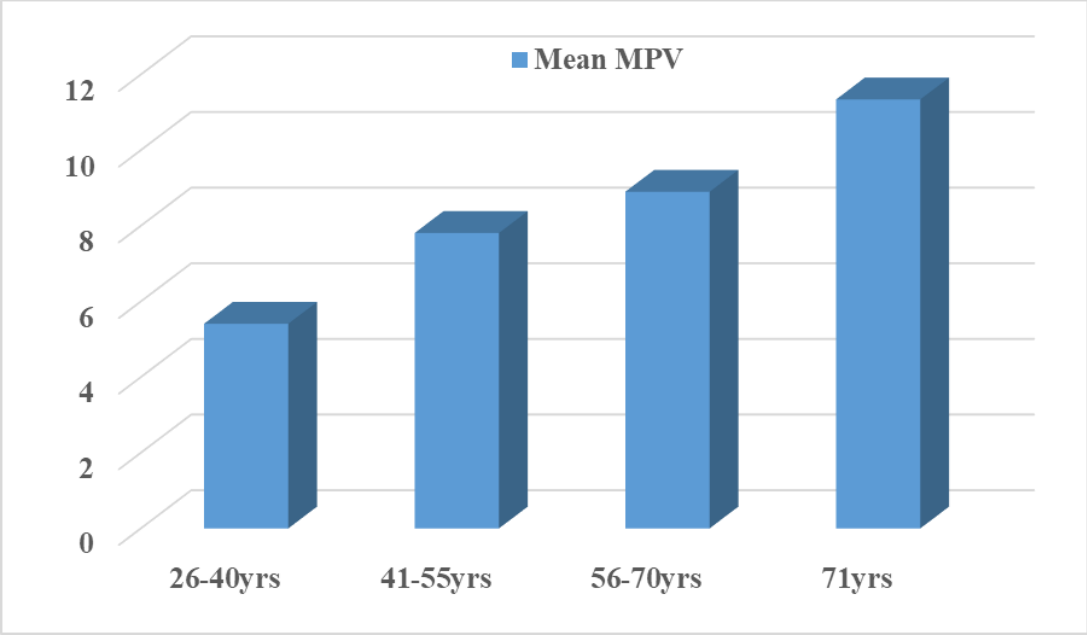


Table 11:- Comparison of mean MPV among sex

	Sex	Mean MPV	Std. Deviation	P Value
MPV	Female	8.161538	2.3850148	.821
	Male	8.262500	2.6689198	

There was no statistically significant difference of Mean MPV among sex

Graph 5:- Graph showing Comparison of mean MPV among sex

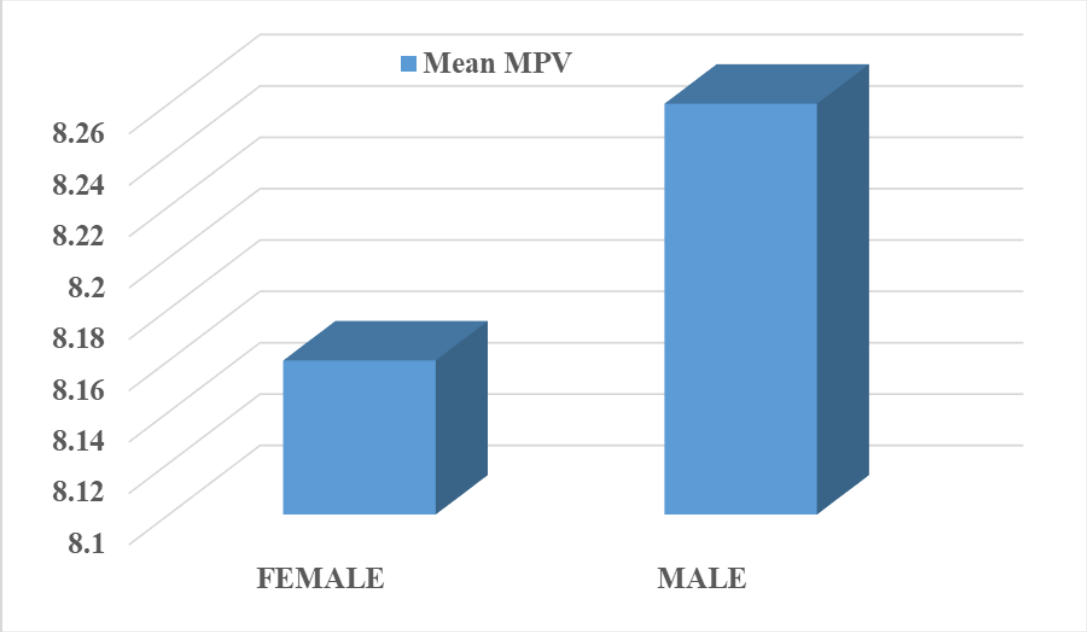


Table 12:- Comparison of mean MPV and duration of DM

Duration of DM	Mean MPV	Std. Deviation	P Value
1-5yrs	8.48	1.59	<0.001
5-10yrs	10.43	1.58	
>10yrs	12.02	.764	

There was a statistically significant difference of Mean MPV with duration of DM

Graph 6:- Graph showing Comparison of mean MPV with duration of DM

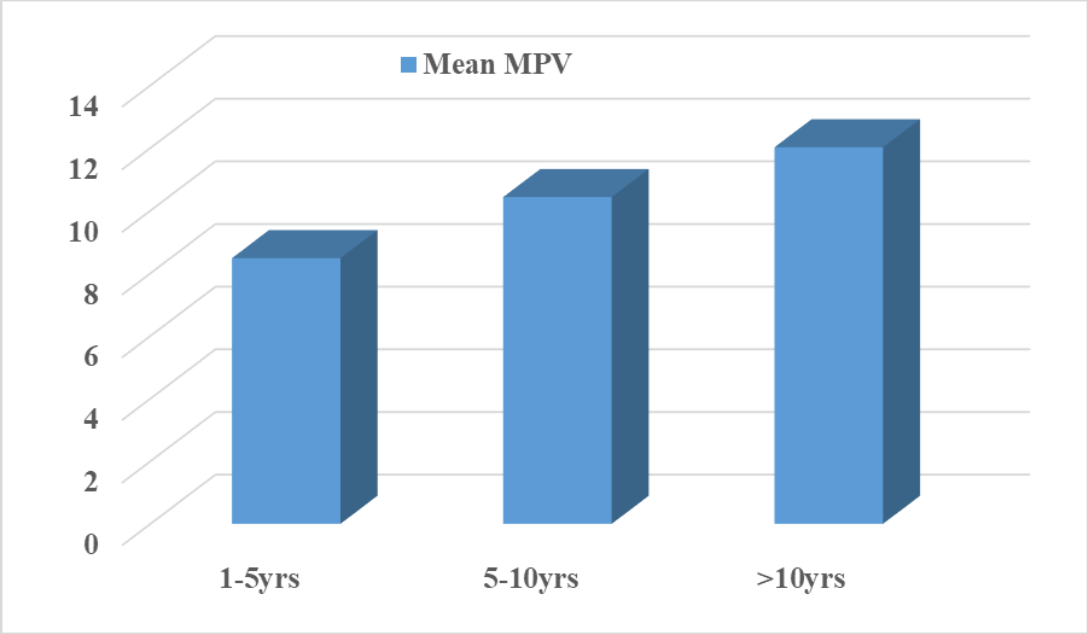


Table 13:- Distribution of subject according to HbA1c

HbA1c	Group			Total
	1	2	3	
≤7	44	5	0	49
	100.0%	11.4%	.0%	37.1%
>7	0	39	44	83
	.0%	88.6%	100.0%	62.9%
Total	44	44	44	132
	100.0%	100.0%	100.0%	100.0%

There was no statistically significant difference between HbA1c and the groups.

Graph 7:- Graph showing Distribution of subject according to HbA1c

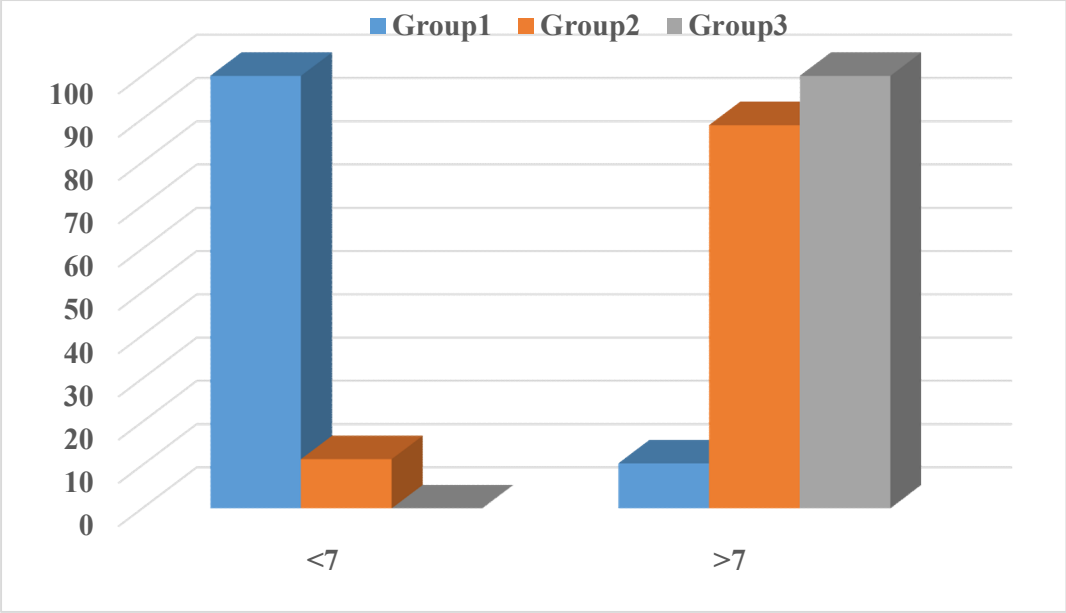
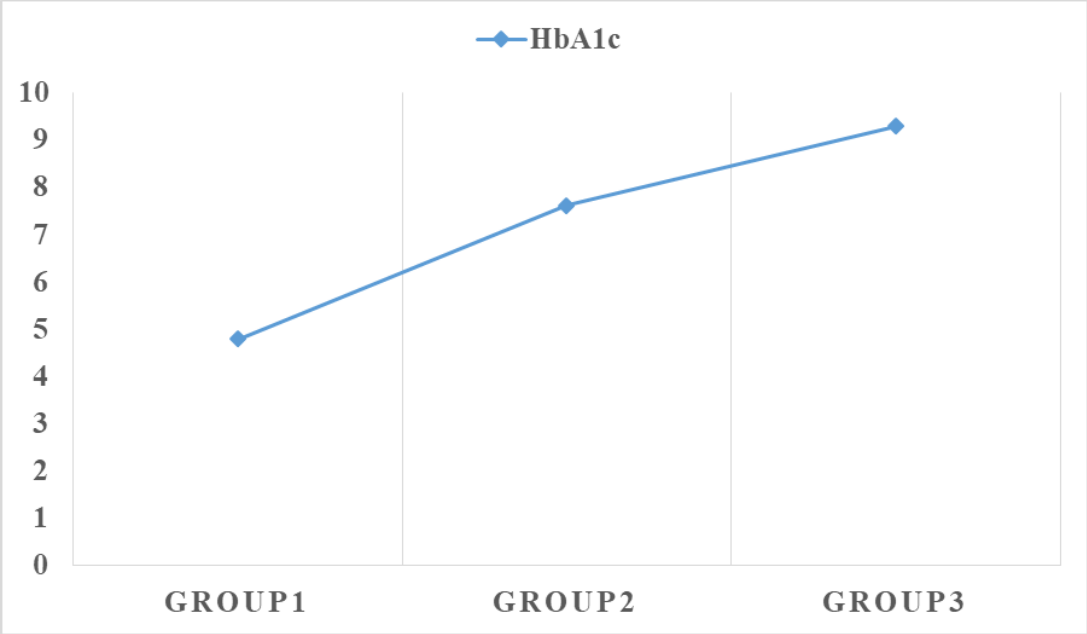


Table 14:- Comparison of MPV, HbA1c, FBS, PPBS, Platelets, Urea among the groups

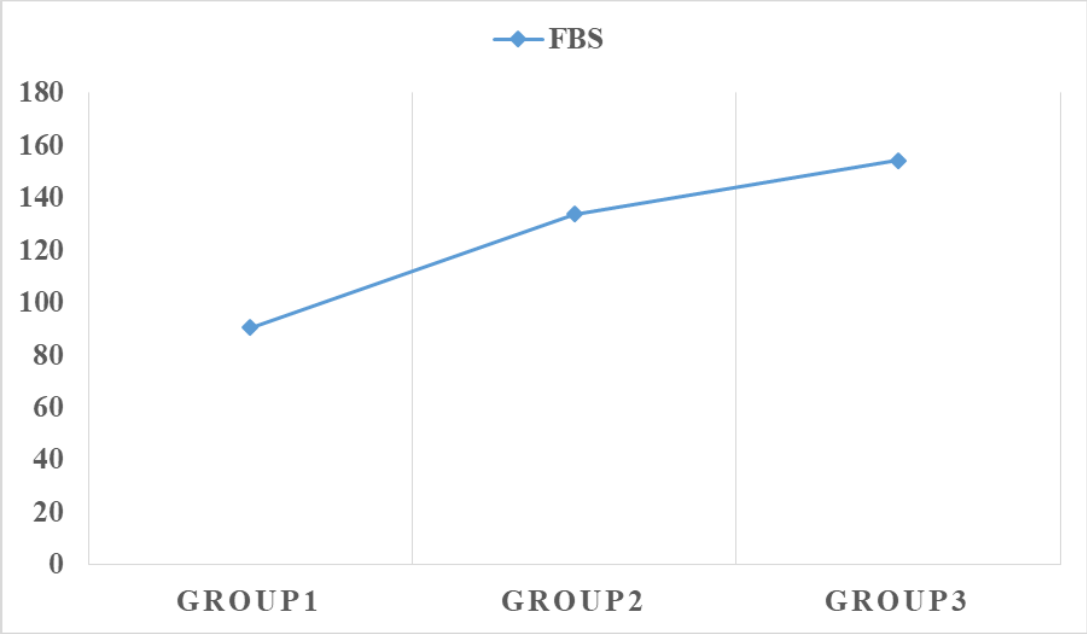
Group		MPV	FBS (mg_dl)	PPBS (mg_dl)	Platelets count (cells/mm3)	Urea (mg/dl)	HBA1c
1	Mean	5.434	90.41	127.95	259386.36	29.36	4.820455
	Std. Deviation	.3409	5.350	4.870	69251.216	5.405	.4032309
2	Mean	7.793	133.82	229.00	272522.73	32.05	7.625000
	Std. Deviation	.480	3.668	10.742	80380.343	5.158	.3348898
3	Mean	11.44	154.16	275.11	251045.45	36.73	9.352273
	Std. Deviation	.8657	10.835	21.994	87730.813	5.078	.8560289
Tota l	Mean	8.22	126.13	210.69	260984.85	32.71	7.265909
	Std. Deviation	2.55	27.652	63.326	79373.211	6.010	1.9607924
	P value	<0.001	<0.001	<0.001	0.444	<0.001	<0.001

The difference in MPV, HbA1c, Urea, FBS, and PPBS among the groups were found statistically significant (<0.001). The difference in platelets among the groups was not statistically significant

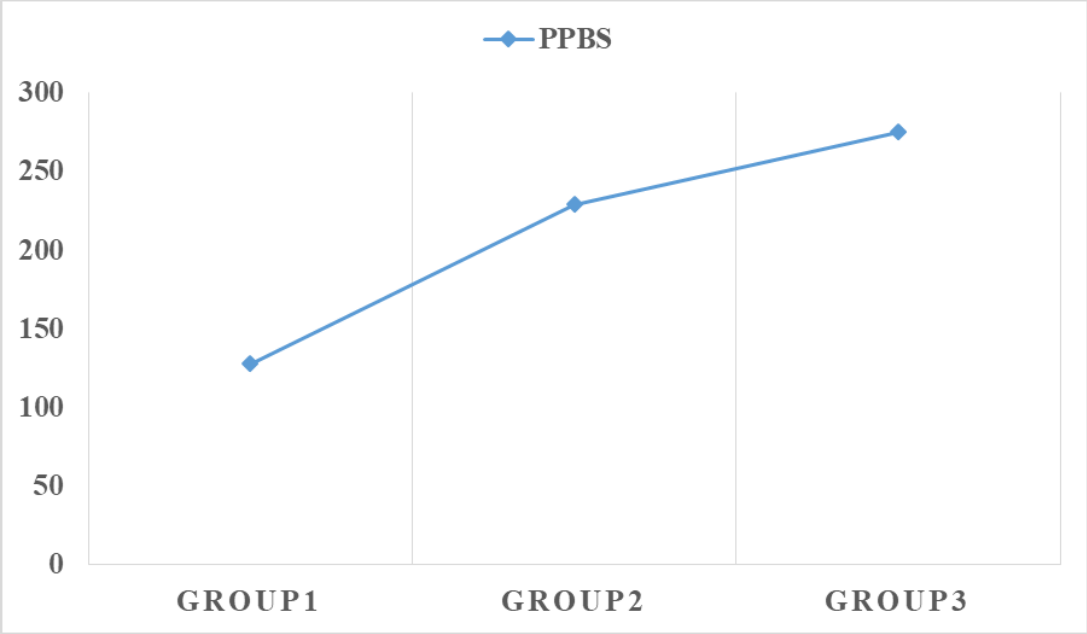
Graph 8:- Graph showing comparison of HbA1c among the groups



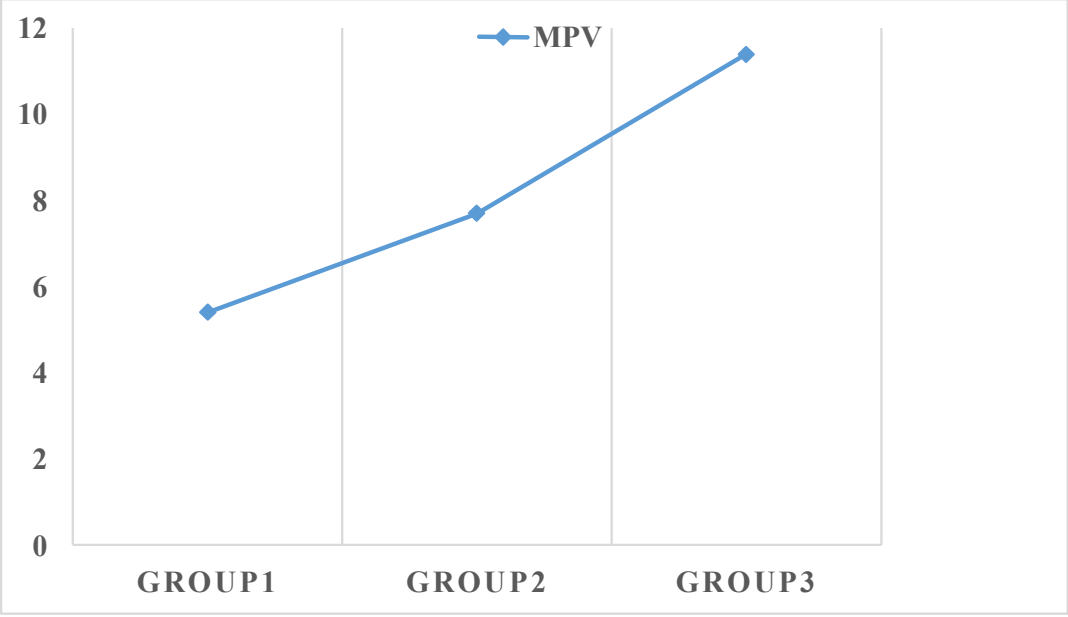
Graph 9:- Graph showing comparison of FBS among the groups



Graph 10:- Graph showing comparison of PPBS among the groups



Graph 11: - Graph showing comparison of MPV among the groups



Graph 12: - Graph showing comparison of urea among the groups

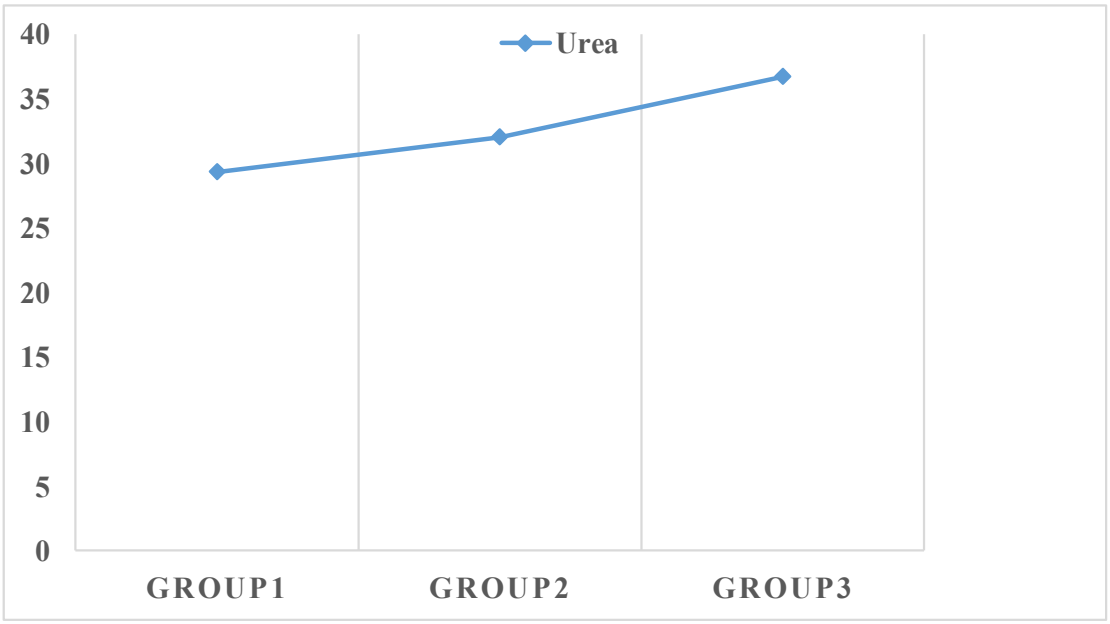


Table 15:- Correlation of MPV with FBS, PPBS, urea, Platelets and HbA1c.

		MPV
FBS(mg_dl)	Pearson Correlation	.894**
	Sig. (2-tailed)	.000
PPBS(mg_dl)	Pearson Correlation	.903**
	Sig. (2-tailed)	.000
Platelets count (cells/mm3)	Pearson Correlation	-.043
	Sig. (2-tailed)	.621
Urea (mg/dl)	Pearson Correlation	.494**
	Sig. (2-tailed)	.000
HBA1c	Pearson Correlation	.917**
	Sig. (2-tailed)	.000

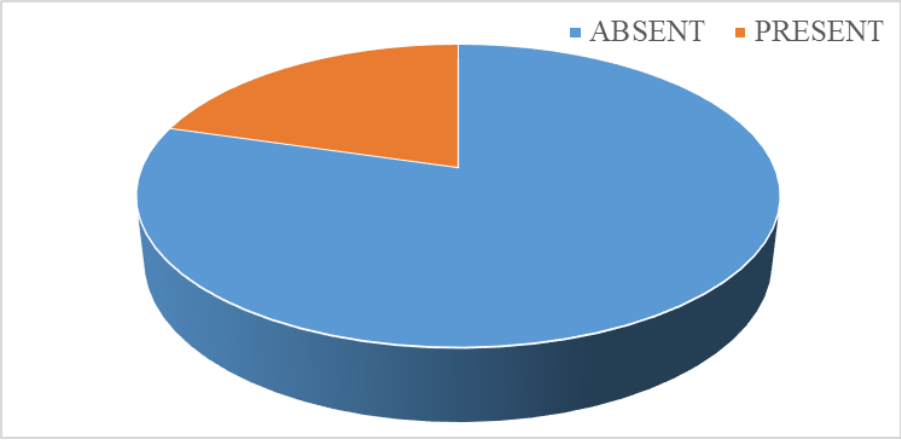
MPV had a positive correlation with Urea, FBS, PPBS and HbA1c. These was found to be statistical significant. MPV had a negative correlation with platelets counts that was not .statistical significant

Table 16: - Mean MPV levels and Nephropathy

	Nephropathy	Number	Mean	Std. Deviation	P value
MPV	1	70	9.232857	1.9764352	<0.001
	2	18	11.111111	.9348727	

The Mean difference in MPV between those with Nephropathy and those without Nephropathy was statistically significant

Graph 13 :- Graph showing distribution of diabetic subjects according to nephropathy



Graph 14:- Graph showing MPV between those with nephropathy and those without nephropathy

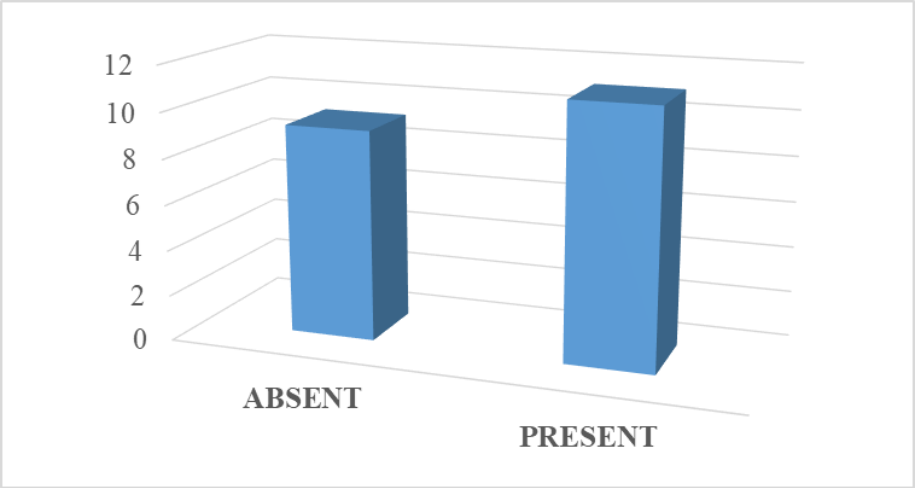
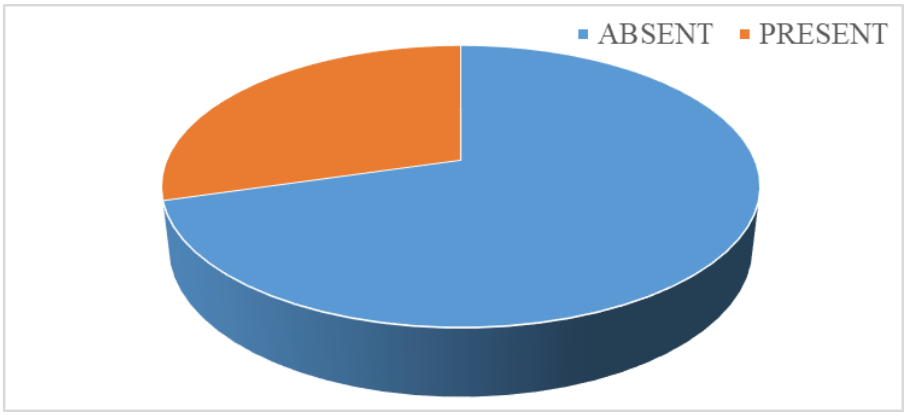


Table 17: - Mean MPV levels and Retinopathy

	Retinopathy	Number	Mean	Std. Deviation	P value
MPV	1	62	8.832258	1.7531026	<0.0001
	2	26	11.488462	.8529136	

The Mean difference in MPV between those with Retinopathy and those without Retinopathy was statistically significant

Graph 15:- Graph showing distribution of diabetic subjects according to Retinopathy



Graph 16:- Graph showing MPV between those with Retinopathy and those without Retinopathy

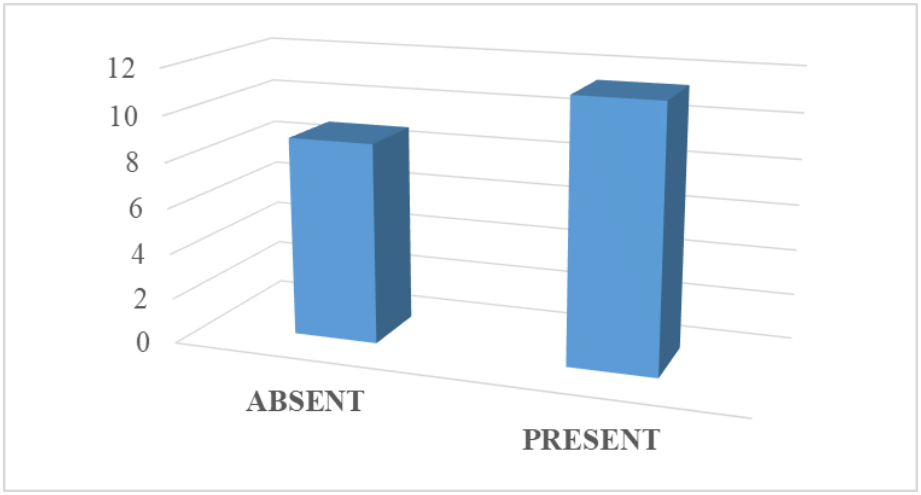
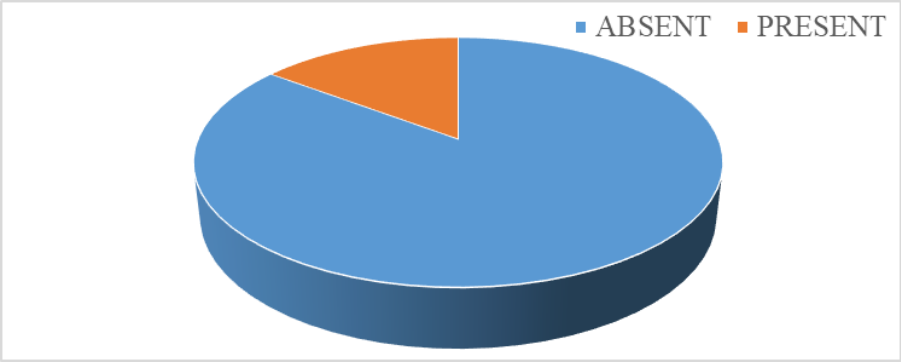


Table 18: - Mean MPV levels and Neuropathy

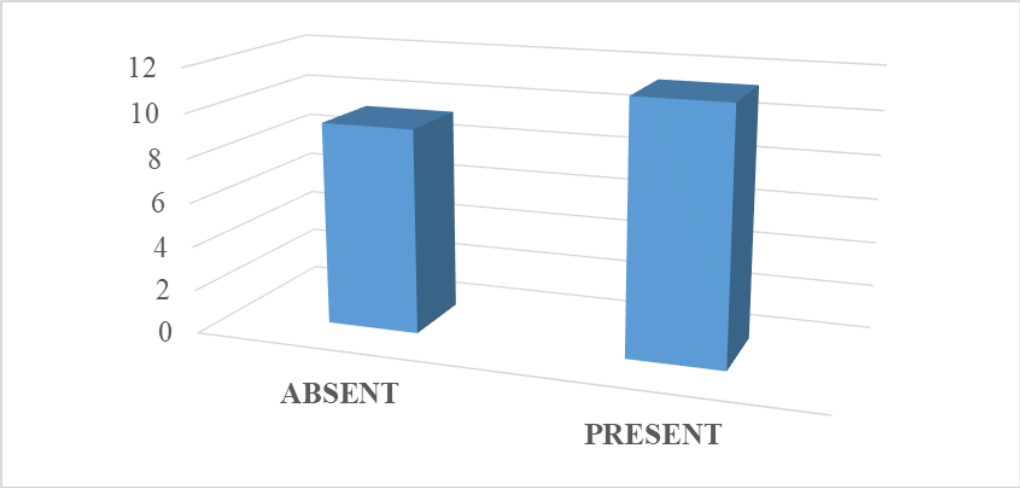
	Neuropathy	Number	Mean	Std. Deviation	P value
MPV	1	75	9.312000	1.9583473	.000
	2	13	11.376923	.5673827	

The Mean difference in MP Vbetween those with Neuropathy and those without Neuropathy was statistically significant

Graph 17:- Graph showing distribution of diabetic subjects according to Neuropathy



Graph 18:- Graph showing MPV between those with Neuropathy and those without Neuropathy



DISCUSSION

India leads the world with the largest number of diabetic patients. Also, the morbidity and complications associated with diabetes are well known. This warrants a detailed knowledge of pathogenesis of the disease. The aim of this study was to evaluate the platelet activity in diabetic patients. MPV is an indicator of the average size and activity of platelets. Larger platelets are younger, more reactive and have greater potential to aggregate. High MPV is emerging as a new risk factor for the vascular complications of DM of which atherothrombosis plays a major role.[120] The main aim of this study was to evaluate MPV in diabetics and to determine if MPV was significantly higher in diabetics, and also establish its association with complications and glycemic control.

ASSOCIATION BETWEEN AGE AND MPV:

All subjects were above 18 years of age. In this study the age group range was 26 to >70 years, with maximum patients being in 56-70 years (45.45%). The mean age of group 1 was 42.41±4.5 years, group 2 was 59.57±2.7 years and mean age of group 3 was 63.75± 10.3 years. There was statistically significant difference between the age group among the groups.

According to this study there was no statistically significant difference between sex among the groups. According to this study, there is statistically significant association between age groups and MPV ($p<0.001$).

The results of previous studies like Ulutas et al [126], who established that there is no significant difference in MPV in relation to age (p value= 0.90). According to Ezgi Coşkun Yenigün et al[127], there was no significant difference in MPV in the

various age groups (p value = 0.62). In a study done by Papanas et al [5] also, there was no association between MPV and age.

STUDY	Ulutas et al	Ezgi Coşkun Yenigün et al
p-VALUE	p=0.90	p= 0.62

ASSOCIATION BETWEEN GENDER AND MPV:

In this study, there were 80 males and 52 females. Out of 80 males, Group 1 had 27, group 2 had 26 and group 3 had 27. Out of 52 females, group 1, 2 and 3 had 17, 18 and 19 respectively in each group.

Males had a mean MPV of 8.1615±2.38fL, whereas females had a mean MPV of 8.26±2.66 fL. There was no significant difference in MPV between males and females (p=0.821). This was similar to the results of studies done by Ezgi Coşkun Yenigün et al [127] (p=0.62) and Papanas et al [5]

COMPARISON OF PLATELET COUNT IN DIABETICS AND NON-DIABETICS

In our study, comparative analysis between these three groups with respect to platelet count did not show any significant difference (p=0.444). The mean platelet count in group 1 was 259386.36±69251.216 per mm^3, group 2 was 272522.73±80380.343 per mm^3 and in group 3 it was 251045.45±87730.813 per mm^3. This result was similar to that of study done by Kodiatte et al [120], where there difference in platelet count between cases and controls was not found to be significant (p=0.256). According to Yenigün et al [127], platelet count was somewhat lower in the diabetic group; however, this difference was not significant (249.729 ± 73.479 /µL

and 279.466 ± 73.294 /µL, in cases and controls, respectively) (p= 0.10). Similar findings were seen in the results of study done by Lutfullah Cakir et al [128].

Study	Platelet Count (X 10^9 /L)		p Value
	Cases	Controls	
KODIATTE ET AL	277.46±81.13	269.79±70	0.256
EZGI COŞKUN YENIGÜN ET AL	249.729 ± 73.479	279.466 ± 73.294	0.10
LUTFULLAH CAKIR ET AL	294 ± 77	271 ± 72	0.13

COMPARISON OF MPV IN DIABETICS AND NON-DIABETICS

The main goal of this study was to determine the mean platelet volume in non-diabetics and diabetics. In this study, the mean MPV in group 1 ie in patients without diabetes was 5.434±).3409 fL and mean MPV in group 2 ie in patient with diabetes and without microvascular complications was 7.793±0.480 fL. The MPV in group 3 ie diabetics with microvascular complications was 11.44±0.8657. This difference was statistically significant (p value<0.0001), thus reinforcing the concept that platelet activity, reflected by mean platelet volume is higher in diabetic patients, compared to non-diabetic individuals. These findings were in agreement with several studies done over the last few years.

P.C.Sharpe et al[113] demonstrated that MPV was higher in diabetics (8.9±0.07 fL) compared to non-diabetic subjects (8.0±0.05) with a p-value of <0.001. This study was one of the earliest in this regard, and was done in 1993. Hekimsoy Z et al[114], in 2004, showed that MPV was significantly higher in diabetics (10.62±1.71 fL), compared to non-diabetics (9.15±0.86 fL) with a p-value of 0.00. Zuberi et al[116]

divided their study groups into three- diabetic (DM group), prediabetic (IFG group) and non-diabetic group. They demonstrated that MPV was highest in DM group (9.34 fL), followed by IFG group (8.98 fL) and then followed by non-diabetic group (8.63 fL). These differences were statistically significant (p =0.00).

Two recent studies done in India also came with similar findings: Kodiatte et al[120] did a study in Kolar, Karnataka, in 2012 and demonstrated that MPV was higher in diabetics (8.29±0.735 fL), when compared to non-diabetics (7.47±0.73 fL) [p value < 0.001]. Vadatti et al[129] did a study in Guntur, Andhra Pradesh, in 2015, and they demonstrated that MPV was significantly higher in diabetics, when compared to non-diabetics (7.91 ± 0.87 fL vs 6.91 ± 0.71 fL respectively [p value < 0.00001])

	STUDY	**P.C.Sharpe et al**	**Hekimsoy Z et al**	**Papanas et al**	**Kodiatte et al**
MPV (in fL)	**Cases**	8.9±0.07	10.62±1.71	14.2±2.2	8.29±0.735
	Controls	8.0±0.05	9.15±0.86	7.1±1.2	7.47±0.73
	P value	<0.001	0.00	0.01	< 0.001

MPV AND GLYCEMIC CONTROL

In our study, mean MPV was highest in group 3 (11.44±0.8657) followed by group 2 (7.793±0.480) and MPV was lowest in group 1 (5.434±0.3409) and this inter-group difference was statistically significant (p value<0.0001).

In the study done by Kodiatte et al[120], the diabetic subjects were divided into 2 groups: Group A with HbA1c <6.5 % and Group B with HbA1c ≥6.5%, they found that the mean MPV in Group-A (7.95±0.72 fL) was significantly lower, compared to Group-B (8.35±0.724), p-value being 0.003.

In the study done by Demirtunc et al[117], diabetics with HbA1c >7% had a mean MPV of 9.0±0.7 fL which was significantly higher than the group with diabetic patients having HbA1c ≤7%, who had a mean MPV of 8.4±0.8 fL, p-value being 0.01.

Studies done by Li S et al[123], Ulutas et al[126] and Kurt et al[130] showed similar results. However, studies done by Papanas et al[5] and Yenigün et al[127] did not show a significant association between MPV and HbA1c.

Study	**MPV among diabetic patients, divided based on glycemic control**		
	HbA1c ≤7	**HbA1c >7**	**p-VALUE**
Demirtunc et al	8.4±0.8	9.0±0.7	0.01
Ulutas et al	7.1±1.0	8.3±1.3	0.039

Also, in this study, there was significant correlation between MPV and HbA1c and p value <0.0001

MPV AND FBS

In this study, there was a positive correlation between MPV and FBS. In the study done by Kodiatte et al[120], correlation between MPV and FBS was statistically significant (p value < 0.001 and r = 0.269).

In the study done by Shimodaira et al[122], MPV in patients with pre-diabetes was found to be higher than that in normal subjects, and was positively associated with FPG levels in pre-diabetic and normal subjects

MPV AND MICRO-VASCULAR COMPLICATIONS

In the current study, the mean platelet volume was more in group 3 ie diabetes with microvascular complications (11.44±0.3409) when compared to other groups and the difference was statistically significant.

Similar results were obtained in the study done by Yenigün et al[127] where diabetics with microvascular complications had a higher MPV (9.38 ± 1.47 fL) compared to the diabetics without microvascular complications (8.47 ± 0.49 fL), this difference was statistically significant (p value=0.048)

Similar results were obtained in the study of Papanas et al[5], where mean MPV of diabetics with microvascular complications was significantly higher compared to that of diabetics without microvascular complications.

However, in the study done by Kodiatte et al[120], mean MPV in diabetics with microvascular complications was more compared to that of diabetics without microvascular complications, however this difference was not statistically significant (p=0.145).

In our study, mean difference in MPV between those with nephropathy (11.11±0.934) and those without nephropathy (9.23±1.97) was statistically significant (p<0.001).

Mean difference in MPV between those with retinopathy (11.48±0.85) and those without retinopathy (8.83±1.75) was statistically significant (p<0.001).Mean difference in MPV between those with neuropathy (11.37±0.56) and those without neuropathy (9.31±1.95) was statistically significant (p<0.001)

STUDY	**MPV (in fL)**		
	Cases with microvascular complications	**Cases without microvascular complications**	**p value**
Yenigün et al	9.38 ± 1.47	8.47 ± 0.49	0.048
Kodiatte et al	8.35±0.73	8.2±0.74	0.145

Inference: Diabetic patients having a higher mean platelet volume should be carefully examined to look for any microvascular complications. Greater the platelet hyperactivity, greater will be the predisposition for developing microvascular complications.

MPV AND DURATION OF DIABETES

In this study, the association of MPV with duration of diabetes was statistically significant, (p<0.001). However, there was a significant association between the duration of diabetes and micro and macrovascular complications. Longer the duration, greater is the likelihood to develop complications.

In studies by Papanas et al[5] and Kodiatte et al[120]. No significant association was found, between MPV and duration of diabetes in these studies.

CONCLUSION

- Type 2 diabetic patients have a higher MPV, compared to non-diabetic controls.
- Higher MPV indicates increased platelet activity and greater aggregation of platelets, which in turn predisposes the diabetic patients to vascular complications
- Microvascular complications of diabetes are associated with a higher MPV, as compared to diabetic patients without vascular complications. The increased platelet activity and aggregation contribute to an increased risk of atherosclerosis and associated complications.
- Diabetic patients having good glycemic control have a lower MPV when compared to diabetics with poor glycemic control.
- There is positive correlation between MPV and FBS, PPBS and HbA1c
- There is association between MPV and duration of diabetes, according to this study.
- MPV can be used as a simple and cost-effective tool to monitor glycemic control and complications in diabetic patients. It would be feasible even in rural centers which have cell count analyzers. However, its usefulness and sensitivity, when used on a larger scale, requires further large-scale studies and analysis.

LIMITATIONS OF THIS STUDY

- This study population may not be representative of the general population, as all our subjects were patients visiting the hospital
- There were confounding variables like hypertension and dyslipidemia which may interfere with some variables in the study
- This was a small scale study, hence, further studies are required to assess the utility of MPV in diabetes

SUMMARY

- This study was done in R L Jalappa Hospital, Tamaka, Kolar. The main aim of the study was to understand the association between MPV and microvascular complications of type 2 diabetes mellitus. 132 subjects were divided into three groups: Group 1: non diabetics, group 2: diabetics without microvascular complication and group 3: diabetics with microvascular complications.
- MPV was higher in diabetic patients with microvacular complications (11.44 ± 0.8657 fL), when compared to diabetic patients without microvascular complications (7.793 ± 0.480 fL) and non diabetics (5.43±0.34) and this difference was statistically significant (p value<0.0001)
- FBS, PPBS and HbA1c were significantly higher in diabetics with microvascular complications, when compared to other two groups. FBS, PPBS and HbA1c were found to have a positive correlation with MPV (p<0.0001 for all three parameters)
- Among the diabetic cases, patients with microvascular complications had a higher MPV than diabetic patients without microvascular complications (p<0.001).
- When individual microvascular complications were assessed, the following were the results obtained:

1. Neuropathy: MPV was significantly higher in diabetic patients with neuropathy compared to diabetic cases without neuropathy, (p value<0.001)
2. Retinopathy: MPV was higher in diabetic patients with retinopathy compared to diabetics without retinopathy , this difference was statistically significant (p value<0.001)

3. Nephropathy: MPV was higher in diabetic cases with nephropathy compared to diabetic cases without nephropathy, however, this difference was of statistical significance (p value<0.001)

- MPV had significant association with duration of diabetes (p value<0.001)
- There was a significant association between the duration of diabetes and microvascular complications. Longer the duration, more the likelihood to develop complications.

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INFORMED CONSENT FORM

STUDY NUMBER:

SUBJECT'S NAME: **HOSPITAL NUMBER:**

AGE:

If you agree to participate in the study we will collect information (as per proforma) from you or a person responsible for you or both. We will collect the treatment and relevant details from your hospital record. This information collected will be used for only dissertation and publication. This study has been reviewed by the institutional ethical committee. The care you will get will not change if you don't wish to participate. You are required to sign/ provide thumb impression only if you voluntarily agree to participate in this study.

I understand that I remain free to withdraw from the study at any time and this will not change my future care. I have read or have been read to me and understood the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information that will be collected and disclosed during the study. I have had the opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction. I, the undersigned agree to participate in this study and authorize the collection and disclosure of my personal information for dissertation.

Subject name

(Parents / Guardians name)

DATE: SIGNATURE /THUMB IMPRESSION

ANNEXURE

PROFORMA

Name:

Age:

Sex:

OP/IP Number:

Current symptoms:

Past history:

Duration of diabetes:

Treatment taken:

Other Co-morbidities, if any:

Other medications:

Personal history:

- Smoker : Y/N
- Alcoholic: Y/N
- Tobacco chewer: Y/N

Family history of diabetes:

- Date of admission:
- Date of discharge:

CLINICAL EXAMINATION

VITALS:

- PULSE
- BP
- TEMPERATURE
- RR

GENERAL EXAMINATION

- Pallor: Y/N
- Icterus: Y/N
- Lymphadenopathy: Y/N
- Pedal edema: Y/N
- Cyanosis: Y/N
- Clubbing: Y/N
- Any other significant finding:

SYSTEMIC EXAMINATION:

CVS:

RS:

PER ABDOMEN:

CNS:

SIGNS OF PERIPHERAL NEUROPATHY: Y/N

FUNDOSCOPY:

E/O DIABETIC RETINOPATHY: Y/N

INVESTIGATIONS:

1. Hb-
2. Platelet count-
3. Mean Platelet volume-
4. FBS-
5. PPBS-
6. HbA1c -
7. B. Urea -
8. S. Creatinine-
9. Urine R/E –
10. U P:Cr ratio
11. ECG
12. ANY OTHER RELEVANT INVESTIGATION:

KEY TO MASTER-CHART

A1C	Glycosylated Hemoglobin
B.UREA	Blood Urea
FBS	Fasting Blood Sugar
HbA1c	Glycosylated Hemoglobin
MPV	Mean Platelet Volume
NPDR	Non-Proliferative Diabetic Retinopathy
NRP	Nephrotic Range Proteinuria
PDR	Proliferative Diabetic Retinopathy
PPBS	Post-Prandial Blood Sugar
S.Cr	Serum Creatinine

Diabetes with Microvascular Complications

S No	Hosp No	Age	Sex	Duration of DM	HBA1c	FBS (mg/dl)	PPBS (mg/dl)	Platelets count (cells/mm3)	MPV	Urea (mg/dl)	Creatinine (mg/dl)	Nephropathy	Retinopathy	Neuropathy
1	344848	55	m	5	8	150	222	150,000	11	39	1.2	Absent	Mild NPDR	Absent
2	270123	60	F	6	8.5	155	225	185,000	10.2	52	1.5	Yes	Moderate NPDR	Absent
3	401166	41	m	4	8	160	230	222,000	10	47	2	Yes	Absent	Absent
4	401181	60	F	7	8.4	162	299	177,000	10.7	50	2.1	Yes	Moderate NPDR	Absent
5	33486	60	M	8	9	170	276	168,000	11	30	1.2	Absent	Absent	Yes
6	401179	43	m	7	10	130	287	200,000	11.5	40	1.1	Yes	Severe NPDR	Absent
7	401095	50	m	8	9.4	150	298	195,000	12	42	1	Absent	Absent	Yes
8	112262	40	f	4	9.6	155	255	167,000	12.4	40	1.2	Absent	Mild NPDR	Absent
9	401150	70	m	5	11	160	269	345,000	12.7	38	0.4	Absent	Mild NPDR	Absent
10	395943	42	m	6	9	170	320	288,000	12	34	1.2	Yes	Absent	Absent
11	309956	60	f	7	8	147	300	234,000	10	35	1	Yes	Moderate NPDR	Absent
12	400853	53	m	10	8.5	150	289	176,000	10.5	34	1.1	Absent	Absent	Yes
13	400856	47	f	14	8.9	160	245	198,000	10.6	36	1.5	Yes	Moderate NPDR	Absent
14	348747	61	M	11	9.2	160	275	234,000	10.9	37	1.3	Absent	Absent	Yes
15	315663	45	m	16	9.3	140	280	245,000	12	34	1	Absent	Mild NPDR	Absent
16	369843	46	m	17	9.5	142	250	345,000	12.4	33	1.4	Absent	Mild NPDR	Absent
17	327342	70	f	13	9.4	170	267	457,000	12.3	35	1.2	Absent	Absent	Yes
18	327463	65	m	12	9	165	265	198,000	11.7	40	1.2	Absent	Mild NPDR	Absent
19	159166	52	f	10	8.7	170	253	435,000	11	30	1.3	Yes	Severe NPDR	Absent
20	167659	63	f	9	9	173	266	345,000	10	33	1	Yes	Absent	Absent
21	290533	62	m	8	8.8	163	276	321,000	11.6	44	1.4	Yes	Severe NPDR	Absent
22	285035	40	f	7	8.5	168	280	196,000	11.8	33	1	Absent	Absent	yes
23	375728	60	m	5	8	164	310	186,000	11.9	34	1.2	Absent	Mild NPDR	Absent
24	34000	80	m	10	11	170	322	150,000	11.2	40	1.2	Absent	Absent	Yes
25	173281	60	m	11	10.5	162	320	187,000	13	37	1	Absent	Moderate NPDR	Absent
26	188275	57	m	12	10.6	132	234	175,000	12.7	33	1.1	Absent	Moderate NPDR	Absent
27	187538	60	f	13	11	138	250	215,000	12.5	38	0.9	Absent	Mild NPDR	Absent
28	335931	65	M	12	10.9	130	265	235,000	12.1	37	1.1	Yes	Absent	Absent
29	400893	65	m	10	11	152	256	298,000	10.9	40	1	Absent	Absent	Yes
30	340155	62	M	9	9	166	263	165,000	11	39	1.1	Yes	Severe NPDR	Absent
31	375732	50	f	7	10	134	237	178,000	11.5	37	1.2	Absent	Mild NPDR	Absent
32	280818	40	F	8	8.2	154	239	188,000	11.3	35	1.1	Yes	Severe NPDR	Absent
33	327392	70	f	5	9.2	167	223	290,000	12.2	33	0.9	Yes	Absent	Absent
34	399156	38	m	4	9.3	129	227	274,000	11.7	30	1	Absent	Mild NPDR	Absent
35	132184	35	m	8	9.5	130	228	264,000	11.5	33	1.3	Absent	Absent	Yes
36	400670	40	f	7	9.8	131	232	298,000	11.3	36	1.2	Absent	Mild NPDR	Yes
37	399927	50	f	9	9.9	140	245	398,000	10.4	36	1.2	Yes	Absent	Absent
38	400792	58	f	2	10	145	320	311,000	10	32	1.1	Absent	Mild NPDR	Absent
39	401048	65	m	5	9.4	132	300	423,000	10.7	40	1	Absent	Absent	Yes
40	362530	72	m	6	9	148	294	381,000	10.4	37	1.3	Yes	Moderate NPDR	Absent
41	3156630	44	m	9	9.4	143	288	189,000	13.4	37	0.9	Yes	Absent	Absent
42	401695	50	m	8	9.4	140	250	165,000	12	25	0.9	Absent	Moderate NPDR	Yes
43	344348	28	F	7	9.5	159	270	175,000	11.8	33	1.2	Absent	Absent	Yes
44	344348	50	m	7	9.2	132	280	420,000	11.6	38	1	Yes	Moderate NPDR	Absent

Diabetes without Microvascular complications

S No	Name and Hosp No	Age	Sex	Duration of DM	HBA1c	FBS (mg/dl)	PPBS (mg/dl)	Platelets count (cells/mm3)	MPV	Urea (mg/dl)	Creatinine (mg/dl)	Nephropathy	Retinopathy	Neuropathy
1	402079	38	m	1	7	130	222	150,000	6.5	30	1	Absent	Absent	Absent
2	402074	55	f	2	7.5	146	225	168,000	6.7	32	1.1	Absent	Absent	Absent
3	409157	52	m	3	8	150	230	180,000	7	34	1	Absent	Absent	Absent
4	342665	50	m	2	7.8	138	250	200,000	7.5	28	0.8	Absent	Absent	Absent
5	409314	56	f	3	8	140	250	167,000	6.9	29	1.2	Absent	Absent	Absent
6	401978	48	m	4	7.9	130	270	198,000	7.5	30	1.1	Absent	Absent	Absent
7	409123	86	f	2	6.9	128	208	205,000	8	35	0.8	Absent	Absent	Absent
8	409285	66	f	1	7	135	209	235,000	8.5	37	1	Absent	Absent	Absent
9	398868	60	m	3	7.1	132	215	378,000	8	36	0.4	Absent	Absent	Absent
10	400383	42	m	4	7.5	133	220	410,000	8.1	34	0.5	Absent	Absent	Absent
11	409419	48	m	2	7.7	140	235	350,000	7.9	27	0.8	Absent	Absent	Absent
12	409217	43	f	1	8	142	240	272,000	7.8	26	0.7	Absent	Absent	Absent
13	409882	49	f	3	8	130	245	356,000	8	28	0.6	Absent	Absent	Absent
14	409368	47	f	4	7.9	135	275	168,000	8.1	23	0.9	Absent	Absent	Absent
15	409111	50	f	2	7.8	140	280	185,000	7.3	23	1.1	Absent	Absent	Absent
16	340297	45	m	1	7.5	142	250	298,000	7.5	20	1	Absent	Absent	Absent
17	408936	58	F	3	7.9	132	257	325,000	7.7	21	0.7	Absent	Absent	Absent
18	409246	56	m	2	8	133	240	205,000	7.9	28	0.4	Absent	Absent	Absent
19	378895	75	m	2	7.3	135	234	215,000	8	30	0.5	Absent	Absent	Absent
20	10732	73	m	3	7.5	137	225	256,000	8.1	33	1	Absent	Absent	Absent
21	410306	85	M	3	7.9	139	240	269,000	8.2	36	1.1	Absent	Absent	Absent
22	410534	48	m	5	7.6	144	230	324,000	8.3	33	1.2	Absent	Absent	Absent
23	410462	57	m	6	7.5	132	236	278,000	8.5	34	1.3	Absent	Absent	Absent
24	390888	64	f	5	7.4	135	246	247,000	8	35	1.2	Absent	Absent	Absent
25	323944	65	m	6	7.2	136	243	219,000	8.5	37	1.1	Absent	Absent	Absent
26	397007	56	m	6	7.8	132	234	239,000	8	33	1	Absent	Absent	Absent
27	410192	44	f	4	7.6	138	250	367,000	8	38	1	Absent	Absent	Absent
28	408017	60	f	5	7.8	140	265	420,000	7.7	37	1.1	Absent	Absent	Absent
29	216407	50	m	4	7.9	141	256	356,000	7.9	38	0.5	Absent	Absent	Absent
30	407751	70	M	5	8	145	263	219,000	8	39	0.8	Absent	Absent	Absent
31	407863	65	m	6	7	142	237	325,000	8.1	37	1.3	Absent	Absent	Absent
32	363685	70	m	3	7.8	139	239	410,000	8.2	35	1.2	Absent	Absent	Absent
33	326386	50	m	3	7.6	130	223	367,000	8.3	32	0.9	Absent	Absent	Absent
34	405383	38	F	5	7.4	129	227	189,000	8.5	30	1.1	Absent	Absent	Absent
35	407993	60	m	4	7.5	130	228	387,000	7.3	33	1	Absent	Absent	Absent
36	408020	70	f	3	7.4	131	232	245,000	7.4	20	0.6	Absent	Absent	Absent
37	407999	65	m	8	7.8	132	245	289,000	7.5	36	0.5	Absent	Absent	Absent
38	408040	48	m	4	7.9	133	232	185,000	7	32	0.8	Absent	Absent	Absent
39	407994	40	f	8	8	137	231	189,000	7.3	40	0.7	Absent	Absent	Absent
40	402036	45	m	4	7.6	139	220	256,000	7.6	37	1	Absent	Absent	Absent
41	407995	70	m	5	8	138	210	345,000	7.7	33	0.9	Absent	Absent	Absent
42	407969	50	f	6	8	140	215	415,000	7.8	32	0.8	Absent	Absent	Absent
43	407857	40	f	7	7.5	141	217	232,000	8	31	1.2	Absent	Absent	Absent
44	392720	45	f	1	7	132	202	298,000	8.1	38	1	Absent	Absent	Absent

Non Diabetics

S No	Hosp No	Age	Sex	Duration of DM	HBA1c	FBS (mg/dl)	PPBS (mg/dl)	Platelets count (cells/mm3)	MPV	Urea (mg/dl)	Creatinine (mg/dl)	Nephropathy	Retinopathy	Neuropathy
1	415798	68	m	0	5.1	90	126	150,000	5	20	1	Absent	Absent	Absent
2	415858	35	m	0	5.2	96	132	225,000	5.2	23	1.1	Absent	Absent	Absent
3	415860	65	m	0	4.8	90	128	223,000	5.4	25	0.9	Absent	Absent	Absent
4	415743	65	F	0	5	92	130	186,000	5.3	28	0.8	Absent	Absent	Absent
5	312833	55	f	0	5.3	97	134	197,000	5.6	29	1	Absent	Absent	Absent
6	415597	62	M	0	5.4	99	138	235,000	5.5	30	1.1	Absent	Absent	Absent
7	415633	58	F	0	4.6	88	126	278,000	5	31	0.8	Absent	Absent	Absent
8	415775	40	M	0	4.2	84	122	345,000	5.1	33	0.9	Absent	Absent	Absent
9	382144	65	M	0	5	92	130	264,000	5.3	32	0.4	Absent	Absent	Absent
10	41584	40	F	0	4.4	85	124	245,000	5.2	35	0.5	Absent	Absent	Absent
11	416141	60	m	0	4.8	90	128	476,000	5.9	36	0.6	Absent	Absent	Absent
12	372700	60	f	0	5.1	95	131	372,000	6	31	0.7	Absent	Absent	Absent
13	416517	43	m	0	5.2	96	132	243,000	5.9	19	0.6	Absent	Absent	Absent
14	416162	45	f	0	4.7	89	127	297,000	5.5	23	0.5	Absent	Absent	Absent
15	393343	63	f	0	5	92	130	256,000	5.7	23	0.7	Absent	Absent	Absent
16	415905	51	f	0	4.2	84	122	326,000	5.4	20	0.5	Absent	Absent	Absent
17	188722	60	m	0	4.4	85	123	228,000	5.6	21	0.7	Absent	Absent	Absent
18	164915	60	m	0	4.8	90	128	230,000	5.3	28	0.4	Absent	Absent	Absent
19	325834	56	m	0	5.1	95	131	250,000	5.7	34	0.5	Absent	Absent	Absent
20	416468	60	f	0	5.2	96	132	278,000	6	33	1	Absent	Absent	Absent
21	416491	40	m	0	4.6	88	126	187,000	5.8	36	1.1	Absent	Absent	Absent
22	414473	65	f	0	4.4	85	124	196,000	5.9	37	1.2	Absent	Absent	Absent
23	389870	55	m	0	4	78	118	165,000	5.8	32	1.3	Absent	Absent	Absent
24	389870	41	f	0	5.2	96	132	188,000	5.7	18	0.5	Absent	Absent	Absent
25	372714	72	f	0	5	92	130	266,000	5.9	34	0.7	Absent	Absent	Absent
26	414118	70	f	0	5.3	97	133	254,000	5.5	32	0.8	Absent	Absent	Absent
27	412618	71	m	0	4.1	81	120	249,000	5.2	33	0.9	Absent	Absent	Absent
28	414109	55	m	0	4.2	83	122	297,000	5.7	37	1	Absent	Absent	Absent
29	414105	44	m	0	5.1	95	131	259,000	5.3	24	1	Absent	Absent	Absent
30	410790	65	m	0	5	92	130	320,000	4.8	26	1	Absent	Absent	Absent
31	414161	58	m	0	5.3	97	136	345,000	4.9	27	1.1	Absent	Absent	Absent
32	413740	57	m	0	4.5	86	124	216,000	5	28	1.2	Absent	Absent	Absent
33	312736	62	m	0	4.2	84	122	345,000	5.7	29	1.3	Absent	Absent	Absent
34	413987	65	m	0	4.9	90	128	182,000	4.9	30	0.7	Absent	Absent	Absent
35	334757	35	m	0	4	78	118	199,000	5	33	0.7	Absent	Absent	Absent
36	327920	60	m	0	4.6	88	126	389,000	5.3	20	0.6	Absent	Absent	Absent
37	413908	60	f	0	5	92	130	398,000	5.5	36	0.5	Absent	Absent	Absent
38	414483	80	f	0	5.2	96	132	278,000	5.4	32	0.8	Absent	Absent	Absent
39	414453	40	f	0	5.4	98	137	269,000	5.3	31	0.4	Absent	Absent	Absent
40	414911	46	f	0	5.1	95	131	167,000	5.7	37	1	Absent	Absent	Absent
41	414945	52	m	0	5	92	130	188,000	5.3	33	1	Absent	Absent	Absent
42	414944	60	m	0	5	92	130	243,000	5.9	32	1	Absent	Absent	Absent
43	398680	52	m	0	4.6	88	126	275,000	5	31	1.1	Absent	Absent	Absent
44	414916	40	m	0	4.9	90	120	234,000	5	30	1	Absent	Absent	Absent