STUDY OF PLATELET INDICES IN TYPE 2 DIABETES MELLITUS PATIENTS

By

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Under the Guidance of

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ABSTRACT

BACKGROUND:

Altered platelets have been reported in patients with diabetes mellitus and has been considered as a 'prothrombotic state' with enhanced platelet reactivity. They have been implicated in the pathological processes of diabetes mellitus and associated with increased risk of vascular complications in these patients. Platelet indices correlate with functional status of platelets and is an emerging risk factor of vascular complications in diabetes.

OBJECTIVE:

To determine and compare mean platelet volume (MPV),platelet distribution width(PDW) and platelet-large cell ratio(P-LCR) in Type 2 diabetes mellitus(DM) with age and sex matched healthy controls.

METHODS:

A cross sectional hospital based study of platelet parameters MPV, PDW and P-LCR was carried out on 280 cases diagnosed with Type 2 diabetes mellitus and 280 age and sex matched controls from November 2013 to June 2015 considering the inclusion and the exclusion criteria. Anticoagulated blood (ethylene diamine tetracetic acid) was collected and analyzed in an automated blood cell counter Sysmex-XN 1000 for platelet indices. The blood glucose levels and HbA1c level were also measured. Statistical evaluation was performed by using Student's t test and Pearson correlation test.

RESULTS:

The average age of presentation of type 2 diabetes melllitus was 53±5.7 years. The mean duration of diabetes was 4.7±2.5years.MPV, PDW and P-LCR were significantly higher in diabetics compared to non diabetics (11.3±1.0fl vs 9.0±0.6fl, 14.2±2.5fl vs 10.7±0.7fl, 35.0±8.1% vs 23.0±2.4%).Among the diabetics, MPV,PDW and P-LCR were higher in those with complications as compared to those without complications, which was not statistically significant. Platelet parameters MPV, PDW and P-LCR also showed statistically significant positive correlation with fasting, random, post prandial blood glucose levels, HbA1c level and complications (p<0.05).

CONCLUSION:

The higher values of MPV,PDW and P-LCR indicates that they serve as an early risk indicator of initial vascular complications in diabetes mellitus patients and can be used as a simple and cost effective tool to assess vascular events.

KEYWORDS:

Platelet indices, mean platelet volume, platelet distribution width, platelet-large cell ratio, diabetes mellitus.

LIST OF ABBREVATIONS USED

WHO	World Health Organization
DM	Diabetes Mellitus
NO	Nitric Oxide
MPV	Mean Platelet Volume
PDW	Platelet Distribution Width
P-LCR	Platelet Large Cell Ratio
FBS	Fasting Blood Sugar
RBS	Random Blood Sugar
PPBS	Post Prandial Blood Sugar
HbA1c	Glycosylated hemoglobin
MODY	Maturity Onset Diabetes Of the Young
ADA	American Diabetes Association
GAD	Glutamic acid decarboxylase autoantibodies
IGT	Impaired Glucose Tolerance
HNF	Hepatocyte Nuclear factor
TNF	Tumor Necrosis Factor
IRS	Insulin receptor substrate
PKC	Protein Kinase C
AGEs	Advanced Glycation End Products
GLUT4	Glucose Transporter Type 4
OGTT	Oral Glucose Tolerance Test
IFG	Impaired Fasting Glucose
HDL	High Density Lipoprotein

LDL	Low Density Lipoprotein
BMI	Body Mass index
IDF	International Diabetes Federation
PCOD	Polycystic Ovarian Disease
STEMI	ST-Elevation Myocardial Infarction
NSTEMI	Non-ST Elevation Myocardial Infarction
cAMP	Cyclic Adenosine Monophosphate
PVI	Platelet Volume Indices

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INTRODUCTION

DEFINITION

World Health Organization (WHO) defines diabetes mellitus (DM) as a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage or dysfunction and failure of various organs of the body.[1]

Patient with type 2 diabetes mellitus (DM) have a 2- to 4-fold increased risk of premature cerebral, coronary, and peripheral vascular disease which together constitute the leading cause of death in these patients. The increased risk is independent of and additive to other cardiovascular risk factors, such as hypertension, albuminuria, obesity, cigarette smoking, and dyslipidemia, relative to nondiabetic patients with these comorbidities. Type 2 diabetes is associated with insulin resistance and hyperinsulinemia and is often part of a metabolic syndrome called "Syndrome X," which comprises hypertension, dyslipidemia, decreased fibrinolysis, and increased procoagulation factors .This metabolic syndrome may also be seen in obese insulin-resistant individuals who do not yet have overt diabetes.[2]

People with diabetes, exhibit increased platelet reactivity. Hyperglycemia contributes to greater platelet reactivity through direct effects and by promoting glycation of platelet proteins. Both insulin resistance and insulin deficiency increase platelet reactivity. Insulin inhibits activation of platelets. Therefore, relative or absolute deficiency of insulin would increase platelet reactivity. Diabetes is associated with oxidative stress and inflammation. Resultant endothelial dysfunction promotes activation of platelets by decreasing nitric oxide production that attenuates platelet

reactivity. Oxidative stress accentuates this effect by attenuating activity of nitrous oxide and promoting platelet activation.[3]

Platelet activity and aggregation potential, which are essential components of thrombogenesis and atherosclerosis, can be conveniently estimated by measuring Mean platelet volume (MPV), Platelet distribution width (PDW) and Platelet large cell ratio (P-LCR).

Mean platelet volume (MPV) is an indicator of average size and activity of the platelets and is reported to be high in diabetes mellitus and is considered as a risk factor for heart disease. Similarly platelet distribution width (PDW) is an indicator of variation in platelet size which may be a sign of active platelet release. Platelet large cell ratio (P-LCR) is directly related to PDW and MPV.[4]

The relationship between these platelet indices and diabetes mellitus is poorly understood.[5] Although several measurements of platelet activity have emerged as potential contributors to atherothrombosis, many of them are time consuming, expensive and use a high sample volume. Alternatively, MPV, PDW and P-LCR can be easily determined on routine automated hemograms which are available at low cost. Patients with larger platelets can easily be identified during routine haematological analysis and could possibly benefit from timely treatment.

OBJECTIVE OF THE STUDY

To determine and compare platelet indices (MPV, PDW, P-LCR) in Type 2 diabetes mellitus with age and sex matched healthy controls.

REVIEW OF LITERATURE

DIABETES MELLITUS:

Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia. The chronic hyperglycemia and metabolic dysregulation may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes and blood vessels. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. Most severe forms may present with ketoacidosis or a non–ketotic hyperosmolar state may develop which lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made.[6]

TABLE 1 : DIAGNOSTIC CRITERIA:

The diagnosis of diabetes is established by noting elevation of blood glucose by any one of three criteria.[7]

HbA1c ≥ 6.5%. The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program certified and standardized to the Diabetes Control And Complications Trial assay. OR FPG (Fasting Plasma Glucose) ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h. OR
2-h plasma glucose ≥ 200 mg/dl (11.1mmol/l) during an Oral Glucose Tolerance Test (OGTT). The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dl (11.1 mmol/l).

EPIDEMOLOGY OF DIABETES MELLITUS:

Diabetes Mellitus currently affects more than 171 million people worldwide and will affect an estimated 366 million by 2030, with the most rapid growth in low and middle-income countries. By 2030, it is estimated that the number of people with diabetes more than 64 years of age will be more than 82 million in developing countries and more than 48 million in developed countries. The worldwide prevalence of diabetes in all age groups was estimated to be 2.8% in 2000 and will rise to 4.4% in 2030. By 2030, India will be the country with the maximum number of diabetics in the world. In 2014, 40.9 million people were affected with diabetes in India and the projected estimate for the year 2030 is 80 million. An improvement in economic standards with consequent adoption of more sedentary lifestyle has contributed to this epidemic, especially of Type 2 Diabetes. [8] (Figure 1)

There is marked difference in the prevalence of diabetes between urban and rural areas of India. The prevalence of diabetes in urban India was 12.1% in 2002 as compared to rural India which had prevalence of 6.4%.[9] The Crude prevalence rate in the urban areas of India is thought to be 9 per cent. The mean age of onset is 42.5years. The prevalence of Impaired Glucose Tolerance (IGT) is thought to be around 8.7 per cent in urban areas and 7.9 per cent in rural areas, although this estimate may be too high. It is thought that around 35 per cent of IGT sufferers go on to develop type 2 diabetes so India is genuinely facing a healthcare crisis.[10]

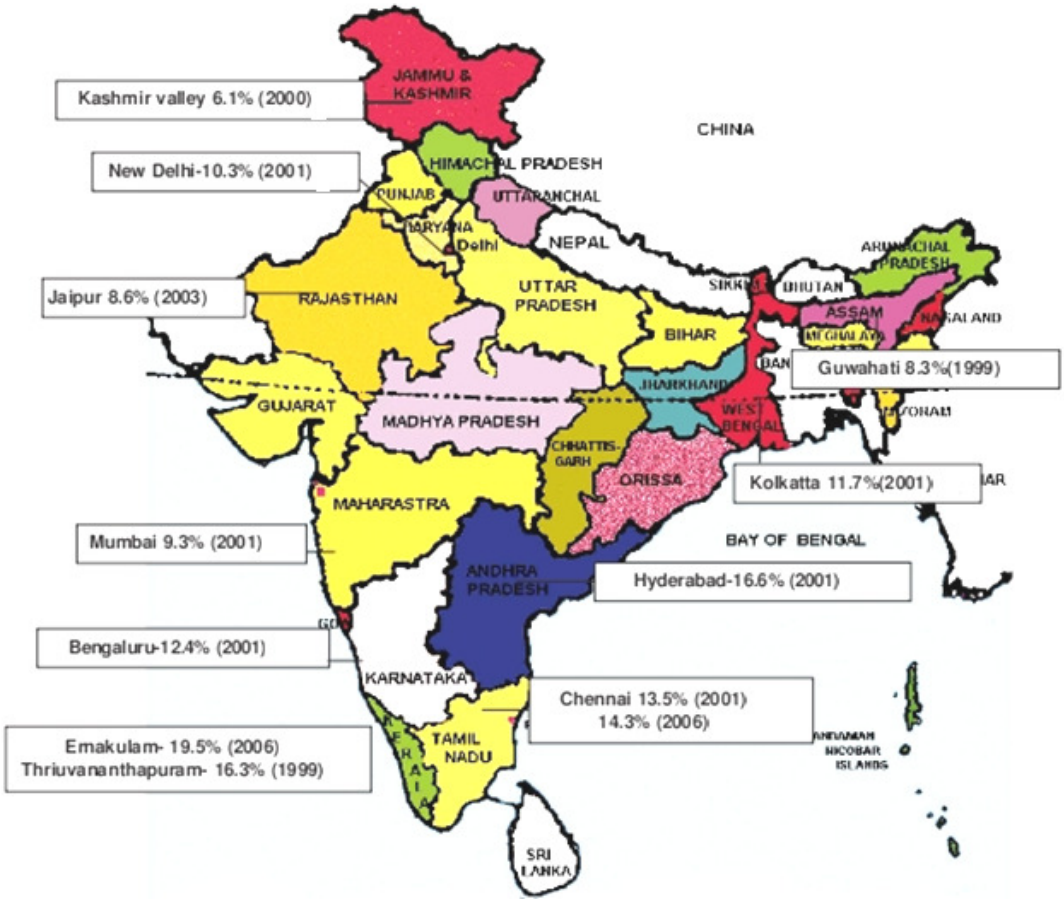


Figure 1 : Distribution of Diabetes Mellitus in India[8]

ETIOLOGICAL CLASSIFICATION OF DIABETES[11]:

I. Type 1 diabetes (beta-cell destruction, usually leading to absolute insulin deficiency)

A. Immune mediated

B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

III. Other specific types

A. Genetic defects of beta-cell function

1. Chromosome 12, HNF-1a (MODY3)
2. Chromosome 7, glucokinase (MODY2)
3. Chromosome 20, HNF-4a (MODY1)
4. Others

B. Genetic defects in insulin action

1. Type A insulin resistance

2. Leprechaunism

3. Rabson-Mendenhall syndrome

C. Diseases of the exocrine pancreas

1. Pancreatitis

2. Trauma/pancreatectomy

3. Neoplasia

4. Fibrocalculous pancreatopathy

D. Endocrinopathies

1. Acromegaly

2. Cushing's syndrome

3. Glucagonoma

4. Pheochromocytoma

E. Drug or chemical induced

1. Vacor

2. Pentamidine

3. Nicotinic acid

4. Glucocorticoids

F. Infections

1. Congenital rubella

2. Cytomegalovirus

3. Others

G. Uncommon forms of immune-mediated diabetes

1. "Stiff-man" syndrome

2. Anti-insulin receptor antibodies

H. Other genetic syndromes sometimes associated with diabetes

1. Down syndrome

2. Klinefelter syndrome

3. Turner syndrome

4. Wolfram syndrome

7. Laurence-Moon-Biedl syndrome

IV. Gestational diabetes mellitus

AETIOLOGICAL TYPES

The aetiological types designate defects, disorders or processes which often result in diabetes mellitus.

TYPE 1 DM

Type 1 DM indicates beta–cell destruction of insulin that may ultimately lead to diabetes mellitus in which "insulin is required for survival" to prevent the development of ketoacidosis, coma and death. An individual with a Type 1 DM may be metabolically normal before the disease is clinically manifested. Beta cell destruction can be detected by the presence of anti–Glutamic Acid Decarboxylase Autoantibodies (GAD), islet cell or insulin antibodies which identify the autoimmune processes leading to beta–cell destruction.

Idiopathic diabetes:

Some forms of type 1 diabetic patients have permanent insulinopenia and are prone to ketoacidosis, but lack evidence of autoimmunity. Although only a minority of patients fall into this category,most of them are of African or Asian ancestry. Individuals with this form of diabetes suffer from episodes of ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, is not HLA associated and lacks immunological evidence for beta-cell autoimmunity. An absolute requirement for insulin replacement therapy in affected patients may come and go.[11]

TYPE 2 DIABETES :

This form of diabetes, accounts for 90–95% of those with diabetes. It was previously termed as non–insulin-dependent diabetes or adult onset diabetes. It encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime,

these individuals do not need insulin treatment to survive. Although the specific etiologies are not known, autoimmune destruction of beta-cells does not occur.

Most patients with this form of diabetes are obese, and obesity itself leads to variable degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension, dyslipidemia, and is often associated with a strong genetic predisposition.[12]

GESTATIONAL DIABETES MELLITUS (GDM)

Gestational diabetes is carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy but has been previously unrecognized. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy.

The International Association of Diabetes and Pregnancy Study Groups, the American Diabetes Association (ADA), and multiple obstetrical and diabetes organizations, recommended that high-risk women found to have diabetes at their initial prenatal visit, using standard criteria mentioned previously, receive a diagnosis of overt, not gestational, diabetes.[13]

OTHER SPECIFIC TYPES OF DIABETES :

Genetic defects of the β-cell:

Several forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity onset diabetes of the young (MODY). It is inherited in an autosomal dominant pattern and is characterized by impaired insulin secretion with minimal or

no defects in insulin action. Till date, abnormalities at six genetic loci on different chromosomes have been identified. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1a. A second form is associated with mutations in the glucokinase gene on chromosome 7p resulting in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, which, in turn, stimulates insulin secretion by the β -cell. Thus, glucokinase serves as the "glucose sensor" for the β -cell. Due to the defects in the glucokinase gene, increased plasma levels of glucose are necessary to maintain normal levels of insulin secretion.Other less common forms result from mutations in other transcription factors, including HNF-4a, HNF-1b and insulin promoter factor.[14]

Genetic defects in insulin action:

Genetically determined abnormalities of insulin action result into some unusual cases of diabetes mellitus. Mutations of the insulin receptor leads to metabolic abnormalities which may range from hyperinsulinemia and modest hyperglycemia to symptomatic diabetes. Women may have virilization and enlarged cystic ovaries. Previously, this syndrome was termed Type A insulin resistance. Leprechaunism and Rabson– Mendenhall syndrome are two paediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance .[15]

Diseases of the exocrine pancreas:

Acquired processes which injures pancreas include pancreatitis, trauma, infection, pancreatic carcinoma, and pancreatectomy. With the exception of carcinomas, there should be extensive damage to the pancreas for diabetes to occur. This implies a mechanism apart from simple reduction in beta–cell mass. Extensive

cystic fibrosis and haemochromatosis can also damage beta cells and impair insulin secretion. Fibrocalculous pancreatopathy presents with abdominal pain radiating to the back and pancreatic calcification on X–ray and ductal dilatation.

Endocrinopathies:

Several hormones (e.g. growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Diseases associated with excess secretion of these hormones (e.g. Phaeochromocytoma, Cushings Syndrome, Glucagonoma and Acromegaly) can cause diabetes. Removal of excess hormones resolves the hyperglycemic state.

Drug– or chemical–induced diabetes:

Many drugs can impair insulin secretion and thereby, precipitates diabetes in persons with insulin resistance. In such cases, the classification is ambiguous, as the primacy of beta– cell dysfunction or insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and pentamidine which fortunately causes rare drug reactions can permanently destroy pancreatic beta cells.

Infections:

Certain viruses like Coxsackie B, cytomegalovirus and adenovirus have been associated with beta–cell destruction thereby leading to diabetes.[16]

Uncommon but specific forms of immune– mediated diabetes mellitus:

Diabetes may be associated with several immunological diseases with a pathogenesis or aetiology different from that of Type 1 diabetes. The “stiff man syndrome” is an autoimmune disorder of the central nervous system, characterized by stiffness of the axial muscles with painful spasms. Affected people usually have high titres of the GAD autoantibodies and approximately one-half will develop diabetes.

Patients receiving interferon alpha have been reported to develop diabetes associated with islet cell autoantibodies.

Anti–insulin receptor antibodies occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases can cause diabetes by binding to the insulin receptor, thereby reducing the binding of insulin to target tissues. However, these antibodies also can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia .Patients with extreme insulin resistance and anti–insulin receptor antibodies often have acanthosis nigricans. This syndrome was termed Type B insulin resistance in the past.

Other genetic syndromes sometimes associated with diabetes:

Several genetic syndromes, including chromosomal abnormalities of Down's syndrome, Klinefelter's syndrome and Turner's syndrome are associated with increased incidence of diabetes mellitus. Wolfram's syndrome is an autosomal recessive disorder characterized by insulin–deficient diabetes and the absence of beta cells at autopsy .[17]

PATHOGENESIS OF TYPE 1 DIABETES:

Several autoimmune features of type 1 diabetes mellitus have been explained:

1. Presence of immunocompetent and accessory cells in infiltrated pancreatic islets;
2. Association of susceptibility to disease with the class II (immune response) genes of the major histocompatibility complex (MHC; human leucocyte antigens HLA);
3. Presence of islet cell specific autoantibodies;
4. Alterations of T cell mediated immunoregulation, in particular in CD4+ T cell compartment;

5. The involvement of monokines and TH1 cells producing interleukins in the disease process;
6. Response to immunotherapy and;
7. Frequent occurrence of other organ specific auto-immune diseases in affected individuals or in their family members.

At the onset of overt hyperglycemia, a mixture of pseudoatrophic islets with cells producing glycogen (α cells), somatostatin (d cells) and pancreatic poly-peptide cells, and islets containing both β-cells and infiltrating lymphocytes and monocytes may be seen.

Lymphocytic infiltration is found only in the islet containing residual β-cells and is likely that the chronicity with which type 1 DM develops reflects this heterogeneity of islet lesions. β-cells are rapidly destroyed when pancreas is transplanted among identical twin donors in the absence of immunosupression, and massive insulitis develops rapidly with infiltrating T lympocytes indicating an anamnestic autoimmune reaction.[18]

Gill and Haskins study concluded that absolute prerequisite for the development of type 1 DM appear to be activation of islet antigen - specific CD4+ T cell which are sufficient to induce insulitis while CD8+ T cells contribute to the severity of the damage.[19] Poulsen *et al*[20] suggested in their study that Interleukin 1 and tumor necrosis factor (TNFα), two cytokines mainly produced by macrophages, induce structural changes of β-cells and suppression of their insulin releasing capacity. Therefore, autoimmune destruction of pancreatic β cells leads to a deficiency of insulin secretion that leads to the metabolic derangements associated with type 1 diabetes.[21] (Figure 2)

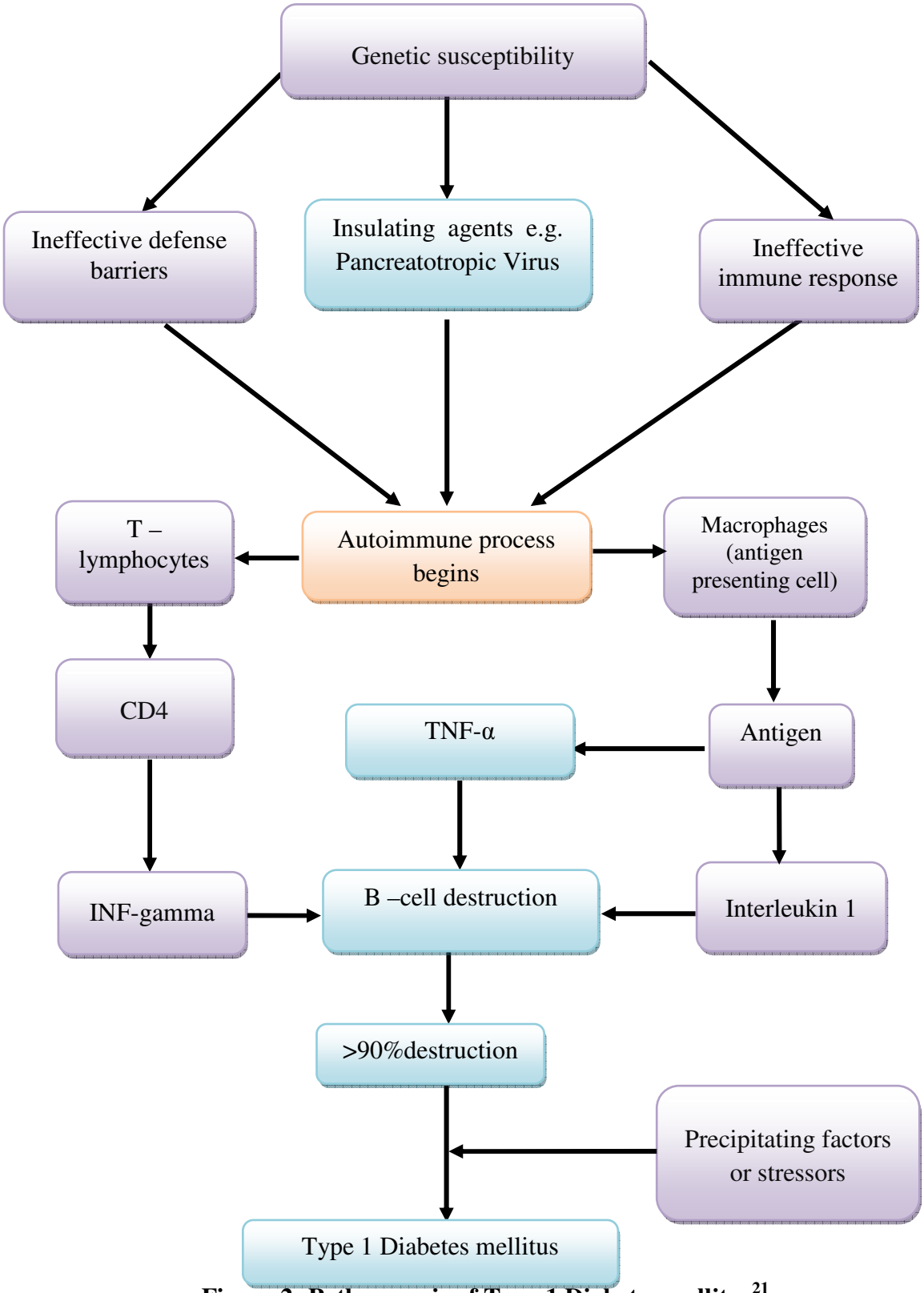


Figure 2: Pathogenesis of Type 1 Diabetes mellitus[21]

PATHOGENESIS OF TYPE 2 DIABETES MELLITUS:

Impaired insulin secretion and insulin resistance contribute more or less jointly to the development of type 2 DM.

Impaired insulin secretion

Impaired insulin secretion is a decrease in glucose responsiveness, observed before the clinical onset of disease. More specifically, impaired glucose tolerance (IGT) is induced by a decrease in glucose-responsive early-phase insulin secretion, and a decrease in additional insulin secretion after meals causes postprandial hyperglycemia. Although an over-response is seen in obese persons, they show a decrease in early-phase secretory response which is extremely important as a basic pathophysiological change during the onset of disease in all ethnic groups. Impaired insulin secretion is generally progressive, and its progression involves glucose toxicity and lipo-toxicity. When untreated, these are known to cause a decrease in pancreatic cell mass. While patients in early stages after disease onset chiefly show an increase in postprandial blood glucose as a result of increased insulin resistance and decreased early-phase secretion, the progression of the deterioration of pancreatic cell function subsequently causes permanent elevation of blood glucose.[22] (Figure 3)

Insulin resistance

Insulin resistance which develops and expands prior to disease onset, is a condition in which insulin in the body does not exert sufficient action proportional to its blood concentration. The major target organs are liver and muscles. The GLUT2 gene, expressed in liver and pancreatic beta cells, and GLUT4, expressed in skeletal muscle and adipocytes, are strong candidate genes for the genetic susceptibility to type 2 DM.[23] Genetic factors like insulin receptor and insulin receptor substrate (IRS)-1 gene polymorphisms not only directly affect insulin signals but also

polymorphisms of thrifty genes such as the adrenergic receptor gene and the uncoupling protein gene, associated with visceral obesity and promote insulin resistance. Recent attention has focused on the involvement of adipokines in insulin resistance. While TNF, leptin, resistin, and free fatty acids act to increase resistance, adiponectin improves resistance.[24]

Clinical tests to assess the extent of insulin resistance include homeostasis model assessment for insulin resistance (homa-ir), insulin sensitivity test (loading test), steady-state plasma glucose, minimal model analysis, and insulin clamp technique.[25]

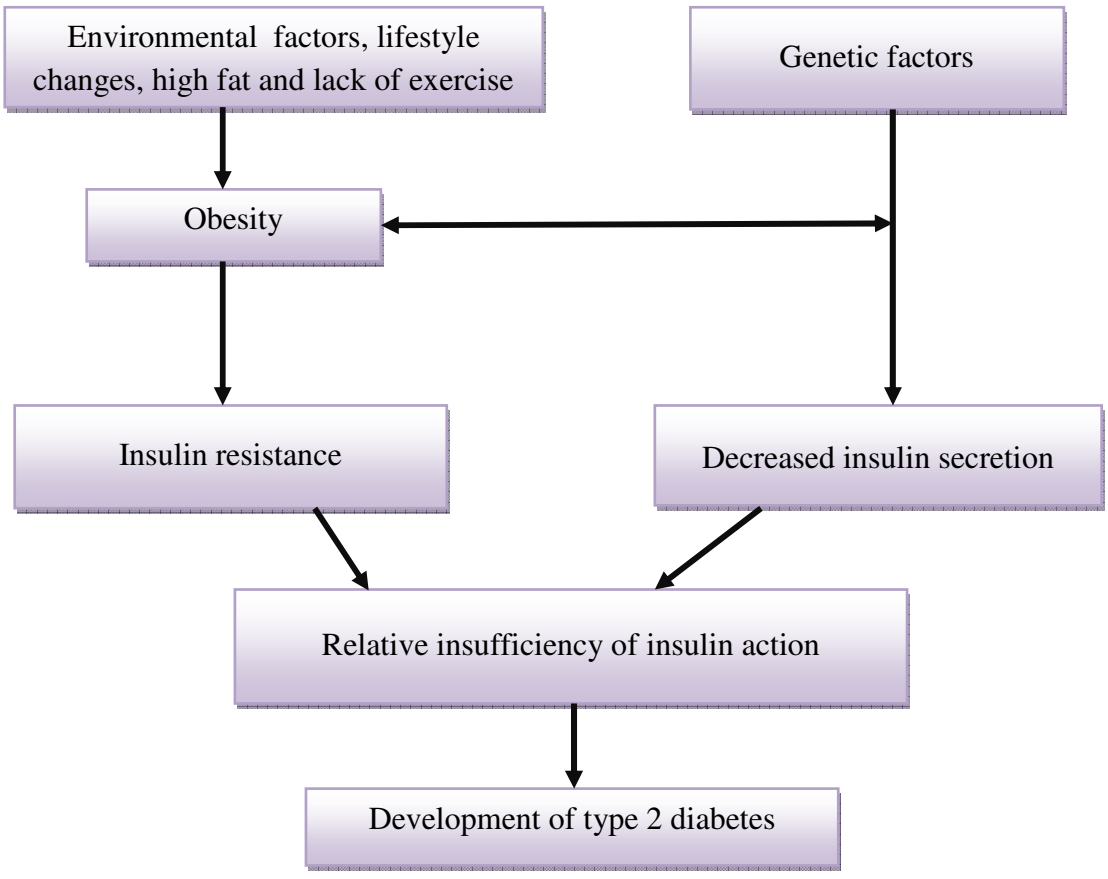


Figure 3 : Pathogenesis of Type 2 Diabetes mellitus[22]

TARGETING SITES OF INSULIN:

Diabetic patients have an increased risk of developing micro and macrovascular complications, and platelets may be involved in this. Altered platelet morphology and function have been reported in patients with diabetes and are likely to be associated with the pathological processes.[26]

There are four cellular systems which are targeting sites of insulin that includes:

- Platelets
- RBCs
- Endothelium and
- Monocytes

Insulin is shown to enhance red blood cell membrane deformability, allowing their passage through capillary beds. Insulin exerts endothelial effects by enhancing NO and prostacyclin production, which acts on smooth muscle to cause vasodilation. In platelets, insulin inhibits platelet adhesion and aggregation. Insulin also inhibits the production of plasminogen activator inhibitor-1. Insulin's action on platelets is to sensitize platelets to the inhibitory actions of prostacyclin and NO on aggregation and to reduce the proaggregatory properties of a number of agonists. There are receptors on monocytes for insulin and insulin may exert anti-inflammatory effects.[27] (Figure 4)

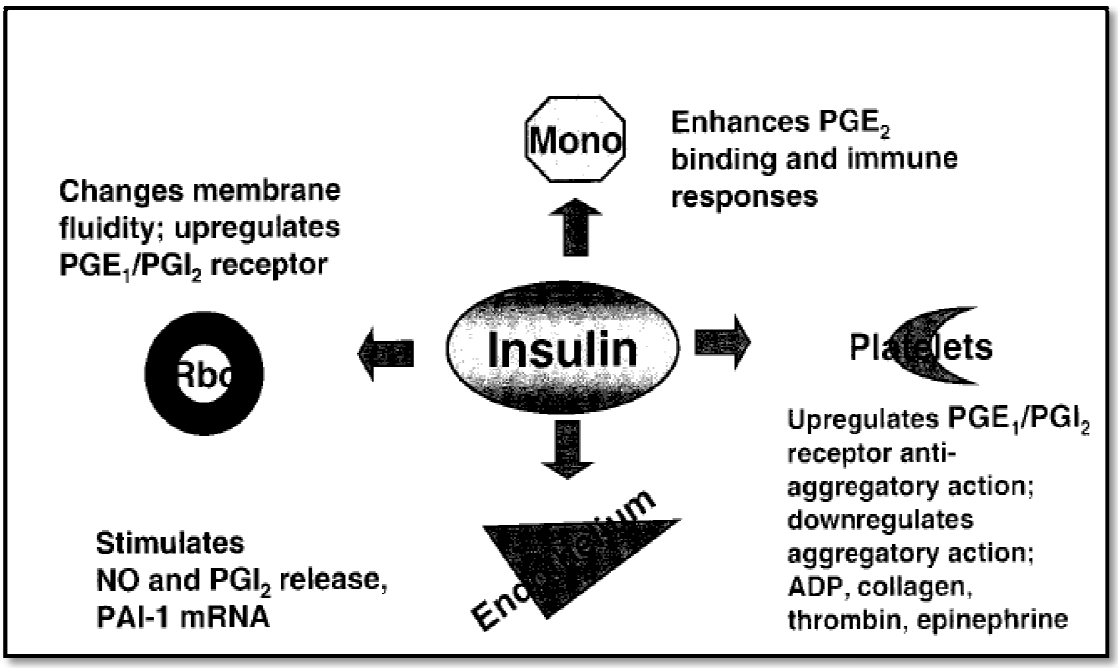


Figure 4 :Targeting sites of insulin[27]

PATHOGENESIS OF COMPLICATIONS OF DIABETES MELLITUS:

Type 2 diabetes mellitus (DM) patients have increased risk of premature cerebral, coronary, and peripheral vascular disease that together constitute the leading cause of death in these patients. Many factors contribute to the prothrombotic condition in patients with DM, such as:increased coagulation, impaired fibrinolysis, endothelial dysfunction and platelet hyperreactivity. The latter is of particular interest, since platelets play a key role in the formation, development and sustainment of thrombi, which are platelet-driven processes.[28]

Platelets of patients with DM are characterised by dysregulation of several signalling pathways and have been proven to be hyperreactive with intensified adhesion, activation and aggregation. Such a hyperreactive platelet phenotype may contribute to the higher proportion of DM patients with thus inadequate response to antiplatelet agents compared with non-DM subjects. Multiple mechanisms caused by cellular and metabolic derangements have been suggested to play a role in the

increased platelet reactivity observed in patients with DM. These mechanisms can be grouped together into the following aetiopathogenic categories:

a. Hyperglycemia
b. Insulin deficiency and resistance
c. Associated metabolic conditions and
d. Other cellular abnormalities [29]

a) Hyperglycemia

Hyperglycemia is the diagnostic hallmark finding in diabetes mellitus and is associated with macrovascular disease even in the prediabetic stage. Hyperglycemia, particularly postprandial, plays an independent and significant role in the DM-associated development of prothrombotic state as well as the cardiovascular disease.[30]

In patients with DM, the hyperglycemia can lead to increased platelet reactivity and platelet activation, evident by increased markers such as soluble P selectin and CD40-ligand. Both acute and chronic hyperglycemia causes in vivo activation of protein kinase C (PKC), a transduction pathway mediator for many proaggregatory platelet agonists. Platelets from patients with DM, unlike those from healthy individuals, also manifest short-term activation of the calcium-sensitive PKCβ isoenzyme by acute hyperglycemia even in vitro, in the absence of additional stimuli, indicating an inherent diabetes-related dysregulation of this pathway.[31]

Recurrent episodes of hyperglycemia leads to the nonenzymatic interaction between the carbonyl group of the reducing sugar and the primary amino group of a protein leading to a cascade of reactions, which results in a heterogeneous group of compounds known as advanced glycation end products (AGEs). Some of these AGE cause phosphatidylserine to come on the external surface of platelet membrane that

leads to surface clotting factor activation and so directly enhance the thrombogenic state.[32] (Figure 5)

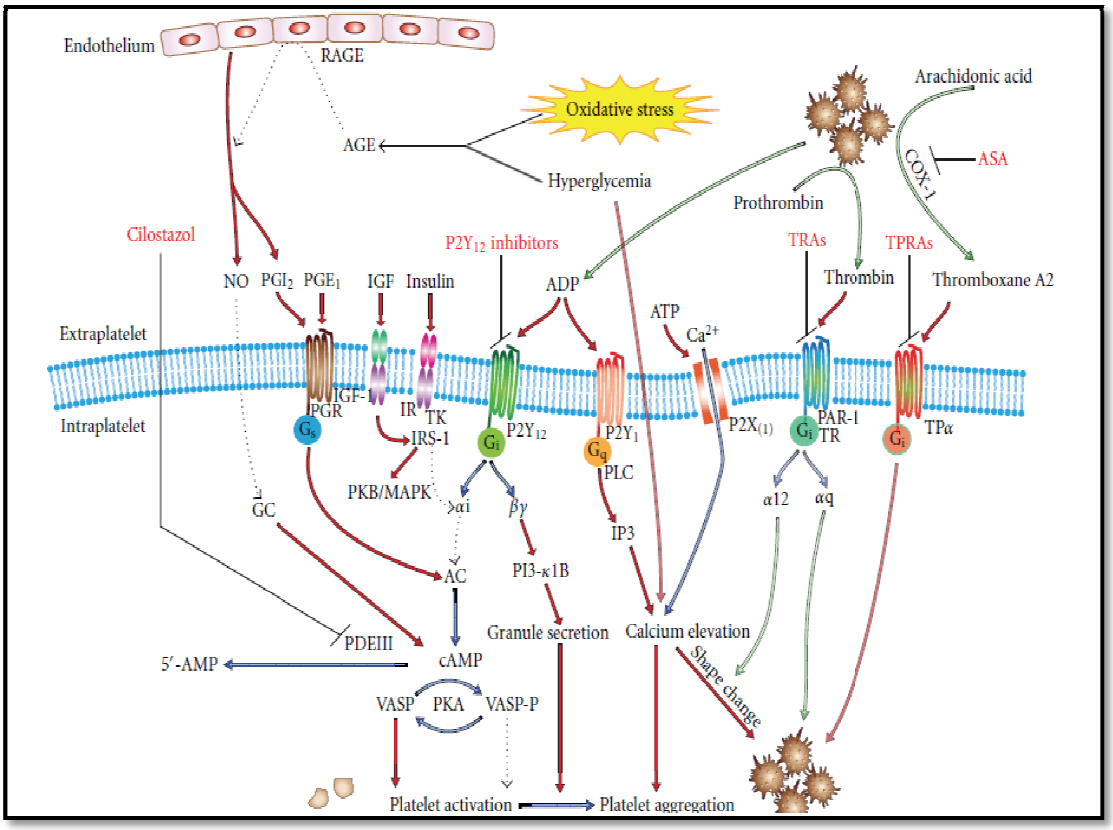


Figure 5: Pathways involved in platelet hyperreactivity in DM patients. AGE: advanced glycation end products,RAGE: AGE receptors, PKA/B/C: protein kinase A/B/C,MAPK: p38 mitogen-activated protein kinase, TK: tyrosine kinase, NO: nitric oxide, GC: guanylate cyclase, PAR-1 TR: protease activated receptor; thrombin receptor, PI-3: phosphoinositol-3 kinase TRA: thrombin receptor antagonist, TP*α*: thromboxane receptor, TPRA: thromboxane receptor antagonist.[32]

b) Insulin deficiency and resistance:

Type 2 DM accounts for 90%–95% of all DM cases and is characterized by reduced tissue sensitivity to insulin. Most people who are destined to develop type2 diabetes exhibit insulin resistance and consequent hyperinsulinemia for 10-20 years before manifesting diabetes. In the prediabetic stage the insulin resistance is initially met by a compensatory increase in insulin production by pancreatic *β*-cells which maintains euglycemic state. In susceptible individuals, due to increased demand, the pancreatic *β*-cells, undergo apoptosis leading to a relative and eventually absolute

deficiency of insulin. Both insulin resistance and insulin deficiency can alter platelet reactivity.

Deficient insulin action is the cardinal factor for development of DM and clearly contributes to platelet dysfunction. Both insulin receptors and insulin-like growth factor-1 receptors are expressed in platelets. Insulin increases surface expression of adenylate cyclase-linked prostacyclin (PGI2) receptor and induces the release of plasminogen activator. However, insulin resistance expression is relatively low as the majority of its subunits heterodimerise with those of IGF-1 receptor to form insulin/IGF-1 receptor. IGF-1 is present in alpha granules of platelets and are expressed on the platelet surface, which may contribute to the amplification of platelet response. Stimulation of platelets by IGF-1 results in dose-dependent phosphorylation of the IGF receptor and in tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and IRS-2, stimulating their subsequent binding with the p85 subunit of phosphoinositide-3 kinase.This leads to phosphorylation of protein kinase B, which is involved in several cellular responses to insulin and IGF-1, including modulation of platelet reactivity.[33]

Insulin-mediated signalling pathways, can be classified as IRS-dependent and independent factors. Among IRS-dependent factors, insulin resistance causes an increase in intracellular calcium concentration as a result of increase in calcium mobilization from intracellular storage pools, which has been correlated with the reduction in membrane fluidity. In addition to alterations in platelet calcium homeostasis, intracellular magnesium concentrations are also reduced, consistent with an increase in platelet hyperaggregability and adhesiveness. Magnesium supplementation can reduce these abnormal platelet functions in people with diabetes.[34]

Among IRS-independent pathways, the role of reduced platelet sensitivity to NO and PGI2 has been noted. Both molecules are released by the endothelium and retard platelet activation leading to an impaired response which is associated with increased platelet reactivity. Diabetic subjects produce less NO and prostacyclin, which normally inhibit platelet-endothelium interactions and promote endothelium mediated vasodilation. The concentration of NO synthase in platelets from patients with type 1 and type 2 diabetes is less than half that measured in platelets from nondiabetic individuals .[35] (Figure 6)

Modesti *et al*[36] reported that platelets from individuals with both type 1 and type 2 diabetes have normal PGI2 receptor numbers. Therefore, the defect is likely to be downstream of the receptors. Altered responses to PGI2 may also be manifested through G-protein malfunction, which occurs due to downstream of the receptor. Livingstone *et al*[37] observed that there was a decrease in the level of Gi in the membranes of platelets from type 2 diabetic patients. This decrease correlated with a decreased stimulation of adenylate cyclase in response to activation of the PGI2 receptor by prostaglandin E1.

Bono *et al*[38] studied 10 nondiabetic lean subjects and 10 obese insulin-resistant subjects and concluded that insulin (100 mU/ ml) enhanced cAMP responses to PGI2 in platelets in lean nondiabetic subjects but not in obese insulin-resistant subjects. Moreover, platelets from diabetic subjects contain reduced antioxidant levels, which tend to be associated with increased aggregability and low platelet vitamin C levels.

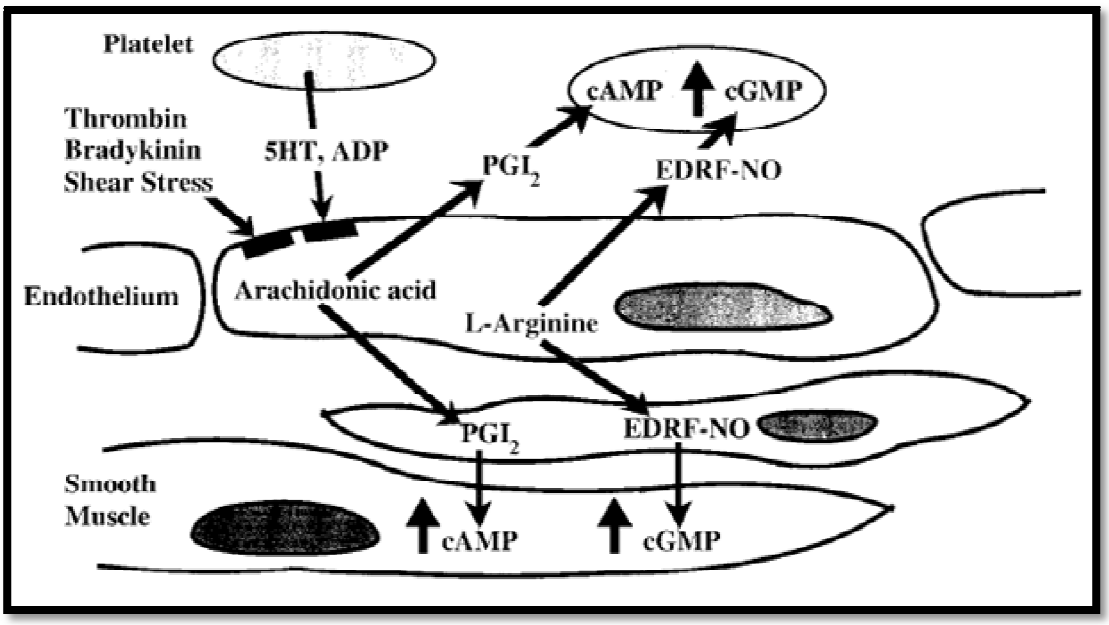


Figure 6 :Synthesis of action of Prostacyclin and Nitrous Oxide in blood vessels[35]

c) Associated metabolic conditions:

It can be : Obesity

Dyslipidemia

Systemic inflammation

Oxidative stress

Obesity: It is a common feature in type-2 DM patients, and may be associated with some degree of insulin resistance, which has relevant implications for platelet reactivity. Nevertheless, other factors present in obese subjects may account for platelet dysfunction, which include:

a. Elevated platelet count and high mean platelet volume, which is related with platelet reactivity and has prognostic implications in atherothrombotic processes such as stroke and acute coronary syndrome.
b. Higher serum leptin concentration, which is associated with increased platelet aggregability

c. Greater cytosolic calcium concentration which also boosts platelet reactivity; and

d. Increased oxidative stress.

Overall, these obesity related metabolic abnormalities ultimately leads to enhanced platelet reactivity. [39]

Dyslipidemia:

Hypertriglyceridemia and low levels of high-density lipoprotein (HDL), are almost invariably found in patients with impaired glucose homeostasis. Hypertriglyceridemia can result in increased triglyceride-rich very low density lipoprotein (VLDL) that potentiates platelet activity, an effect mediated partly through apolipoprotein E and an interaction with the platelet LDL receptor. Additionally, the interaction of lipids and glucose resulting in the formation of glycated low-density lipoprotein (LDL) leads to impaired nitric oxide production and increased intraplatelet calcium concentration, further contributing to platelet hyperreactivity. Interestingly, administration of reconstituted HDL to DM patients can promote cholesterol efflux from platelet membranes which inhibits aggregation.[40]

Oxidative stress and inflammation:

Patients with DM have increased oxidative stress and inflammation compared with healthy subjects. DM is associated with an overproduction of reactive oxygen and nitrogen species and free radicals, such as hydrogen peroxide and superoxide anion, that can directly lead to platelet activation. One mechanism by which superoxide may enhance platelet reactivity is by increasing intraplatelet release of calcium after activation. In addition, superoxide attenuates the biologic activity of NO which would increase platelet reactivity. Oxidative stress also impairs endothelial

function and thereby reduces NO and prostacyclin production. Accordingly, oxidative stress can also promotes greater platelet reactivity through direct effects on platelets.[41]

Inflammation, which is associated with endothelial dysfunction, alters the levels of proteins involved in platelet activation, such as increasing levels of the Fcγ-RIIA receptor that mediates enhanced activation in response to collagen. Both oxidative stress and inflammation are also associated with accelerated turnover of platelets in diabetes mellitus patients compared with healthy individuals, as indicated by the finding of immature, reticulated circulating platelets. As platelet size correlates with activity, these large platelets are inherently hyperreactive and less responsive to antiplatelet drugs like aspirin and clopidogrel.[42]

d) Other cellular abnormalities:

Other platelet anomalies in DM patients that can lead to global hyperreactivity status includes:

- Dysregulation of calcium metabolism,
- Upregulation of P2Y12 signalling pathway and
- Accelerated platelet turnover.

Impaired regulation of calcium metabolism a major feature of platelets in DM patients, contributes to increased intracellular calcium levels. Factors that have been proposed to play a role in impaired calcium homeostasis are:

a. Excessive influx of calcium through the sodium/calcium exchanger
b. Changes in the activity of calcium ATPases
c. Impaired sensitivity to insulin, which decreases sarcoplasmic endoplasmic reticulum calcium-ATPase and
d. Augmented oxidative stress, which enhances calcium signalling due to increased formation of superoxide anions and reduced nitric oxide production.

Altered calcium homeostasis eventually results in an augmented concentration of cytosolic calcium, which leads to enhanced platelet reactivity and aggregation.[43]

Upregulation of platelet adenosine diphosphate P2Y12 receptor signaling pathway has been noted in diabetic platelets especially in type-2 DM. This suppresses cAMP concentration and, in addition to a lower responsiveness to insulin, leads to increased adhesion, aggregation, and procoagulant activity. Another noted abnormality is the increased expression of platelet surface proteins such as P-selectin and glycoproteins Ib and IIb/IIIa, which are integrins that mediate adhesion. [44]

An accelerated platelet turnover is quantified by the presence of a higher number of reticulated platelets , observed in patients with DM. Reticulated platelets are larger and more sensitive, resulting in platelet hyperreactivity and lower response to antiplatelet therapies.[45]

RISK FACTORS OF DIABETES MELLITUS:

The risk factors for diabetes are multifactorial. According to WHO risk factors can be divided into modifiable or behavioural risk factors and metabolic or physiological risk factors. Presence of any one risk factor places an individual in a high risk category of developing diabetes.[46]

TABLE 2 : RISK FACTORS OF DIABETES MELLITUS

MODIFIABLE	NON MODIFIABLE
Overweight and obesity (central and total)	Ethnicity
Sedentary lifestyle	Family history of Type 2 diabetes
Previously identified glucose intolerance (IGT and/or IFG)	Age
Metabolic syndrome: Hypertension Decreased HDL cholesterol Increased trigylcerides	Gender
Dietary factors	History of gestational diabetes
Intrauterine environment	Polycystic ovary syndrome
Inflammation	

MODIFIABLE RISK FACTORS:

SEDENTARY LIFESTYLE:

Sedentary lifestyle is associated with increased risk of diabetes mellitus. There is an evidence that regular physical exercise increases insulin sensitivity, improves lipid levels, lowers blood pressure and thus lowers the risk of cardiovascular disease.[47]

PREVIOUSLY IDENTIFIED GLUCOSE INTOLERANCE (IGT) :

Measurement of plasma glucose will not only detect cases of IFG or IGT, but also cases of undiagnosed diabetes. If fasting plasma glucose is ≥ 6.1–6.9 mmol/l or 110–125 mg/dl then an oral glucose tolerance test (OGTT) is recommended. The presence of IGT and IFG gives a considerably increased risk of developing Type 2 diabetes.[48]

OBESITY:

Obesity is defined as excessive amount of body fat or adipose tissue in relation to lean body mass. WHO criteria define overweight as a BMI ≥ 30 kg/m^2.Grade II - Obesity was associated with 7-12 fold increase in incidence of type 2 diabetes. Obese men (Body weight 125% or more than ideal according to metropolitan life insurance standard) had a risk of developing diabetes 1.5 times higher than those with body weight less than 110% of ideal weight. Waist circumference of ≥ 88 cm for women or ≥92 cm for men are at more risk for developing diabetes.[49]

METABOLIC SYNDROME:

The term metabolic syndrome refers to the clustering of risk factors that include abdominal obesity, dyslipidemia, hyperglycemia and hypertension and is associated with a five fold elevated risk of diabetes mellitus. According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have central obesity plus any two of the following four factors[50]:

Raised triglycerides ⟶ ≥150 mg/dl (1.7 mmol/l) or specific treatment for this lipid abnormality

Reduced HDL cholesterol ⟶ < 40 mg/dl (1.03 mmol/l) in males
< 50 mg/dl (1.29 mmol/l) in females
or specific treatment for this lipid abnormality

Raised blood pressure ⟶ systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension

Raised fasting plasma glucose ⟶ FPG) ≥ 100 mg/dl (5.6 mmol/l), or previously diagnosed type 2 diabetes. If above 5.6 mmol/L or 100 mg/dl, OGTT is strongly recommended but is not necessary to define presence of the syndrome.

INFLAMMATION:

There is a growing evidence that low grade systemic inflammation enhances the risk of type 2 diabetes mellitus. C-Reactive protein, a marker of inflammation is independently associated with the development of diabetes.[51]

INTRAUTERINE ENVIRONMENT:

The familial predisposition to type 2 diabetes mellitus is mediated by both genetic and intrauterine environmental factors. Maternal inheritance is attributed to mutation in the gene present on mitochondrial DNA which is transmitted by an affected mother to her progeny. However studies have also shown that intrauterine hyperglycemia is not only associated with increased perinatal morbidity and mortality, but also with increased lifelong risks of the exposed offspring for obesity, diabetes, metabolic and cardiovascular diseases.[52]

NON MODIFIABLE RISK FACTORS:

AGE AND GENDER:

The prevalence of Type 2 diabetes increases markedly with age. Dambal *et al*[53] study states that diabetes mellitus is most commonly seen in elderly age group between 40- 50 years. The age of onset has moved down into younger adults and even adolescents in recent decades, due to imbalance between energy intake and expenditure.

GENETIC FACTORS

Type 2 diabetes is associated with a strong genetic predisposition. The magnitude of the differences between ethnic groups when exposed to similar environments implies a significant genetic contribution.[54]

ETHNICITY:

Type 2 diabetes is two- three times more commonly seen in African and Africa-Caribbean people and five to six times more common in people of South Asian descent.[54]

FAMILY HISTORY:

The risk of diabetes mellitus increases with the number of affected first degree relatives.[54]

PREVIOUS GESTATIONAL DIABETES

With gestational diabetes, glucose tolerance usually returns to normal following delivery; however, these women have a substantially higher risk of developing Type 2 diabetes in later life.[55]

POLYCYSTIC OVARIAN DISEASE (PCOD):

Insulin resistance and the compensatory hyperinsulinemia are central features of PCOD. There is high incidence of insulin resistance, accompanied by compensatory hyperinsulinemia in PCOD women, and therefore presents with a seven-fold increased risk for developing type 2 diabetes mellitus compared to that of unaffected women.[56]

ADDITIONAL RISK FACTORS

SMOKING:

Smoking has been identified as a possible risk factor for insulin resistance a precursor for diabetes. Smoking has also been shown to deteriorate glucose metabolism which may lead to the onset of type 2 diabetes.[57]

HYPERHOMOCYSTEINAEMIA:

The European Union Concerted Action Project, "homocysteinaemia and vascular disease", indicated that an elevated level of homocysteine in poorly

controlled type-2 diabetes mellitus is related to increased risk of atherosclerosis and cardiovascular disease.[58]

SERUM FERRITIN:

Increased serum ferritin, reflecting body iron overload, is often associated with measures of insulin resistance, and with development of glucose intolerance, type 2 diabetes, and its micro as well as macrovascular complications.[59]

HYPOVITAMINOSIS D:

Hypovitaminosis D has recently emerged as one of the factors contributing to the development of both type 1 and type 2 diabetes mellitus and its microvascular complications like retinopathy.[60]

PLATELETS: BASIC STRUCTURE OF PLATELETS:

Human platelets are anucleate discoid cells that circulate in the bloodstream and participate in hemostasis. Platelets are heterogeneous in size, with dimensions of 0.5 × 3.0 μm. Platelet plasma membrane have a smooth surface except for periodic invaginations that delineate the entrances to the open canalicular system (OCS) , a complex network of interwinding membrane tubes that permeate the platelet's cytoplasm. Although the surface of the platelet plasma membrane appears featureless in most micrographs the lipid bilayer of the resting platelet contains a large concentration of transmembrane receptors. Some of the major receptors found on the surface of resting platelets include the glycoprotein receptor for von Willebrand factor (VWF); the major serpentine receptors for ADP, thrombin, epinephrine, and thromboxane A2; the Fc receptor Fcγ RIIA; and the β3 and β1 integrin receptors for fibrinogen and collagen.[61]

The anatomy of platelets is divided into three major regions:

The peripheral zone consists of the external and internal membrane systems that provide the exposed surface of the platelets and walls of the tortuous channels making up to the surface-connected open canalicular system. An exterior coat or glycocalyx rich in glycoproteins, constitutes the outermost layer of the peripheral zone. Its chemical constitutes provide receptors for stimuli triggering platelet activation and the substrates for adhesion-aggregation reactions. The middle layer of the peripheral zone is a typical unit membrane which is rich in asymmetrically distributed phospholipids that provides an essential surface for interaction with coagulation proteins. The area lying just inside the unit membrane represents the third component of the peripheral zone. It is closely linked to the unit membrane and translates signals received on the outside surface into chemical messages and physical alterations required for platelet activation.

The internal membrane system includes the open canalicular system, even though it is continuous with, and part of, the external membrane system. Channels of the dense tubular system (DTS) and the membrane complexes (MC) formed by elements of the OCS and DTS are internal membrane systems, but they function with peripheral zone and are considered part of the same.

The sole-gel zone is the matrix of the platelet cytoplasm. It contains several fibre systems in various states of polymerization that support the discoid shape of the unaltered platelets and provide a contractile system involved in shape change, pseudopod extension, internal contraction and secretion. Elements of the contractile system, constitute approximately 30-50% of the total platelet protein and appear to be major components. Sole-gel matrix is also comprised of masses as well as discrete particles of glycogen.

The organelle zone consists of granules, electron dense bodies, peroxisomes, lysosomes, glycosomes and mitochondria randomly distributed in the cytoplasm. It serves in metabolic processes and for the storage of enzymes, non metabolic adenine nucleotides, serotonin, a variety of protein consituents and calcium destined for secretion.[62] (Figure 7)

TABLE 3 : PLATELETS ULTRASTRUCTURE AND FUNCTIONS

Zone and component	**Function**
Peripheral zone	
Glycocalyx-proteins, phospholipids, mucopolysaccharides	Adhesion & aggregation
Phospholipid bilayer Phospholipids	Source of arachidonic acid
Integral proteins Glycoproteins Ib/IC, IIb/IIIa Enzymes	Adhesion aggregation & activation
Structural zone	
Microtubules Cytoskeletal network Cytoplasmic network-actin, myosin Actin binding protein	

Organelle zone	
Granules	Non protein mediators
Dense bodies	Protein mediators
Alpha granules	Enzymes
Lysosomes	Breakdown H_2O_2
Microperoxisomes	
Membrane systems	
Open canalicular system	Secretion of granule contents
Dense tubular system	Calcium storage sites

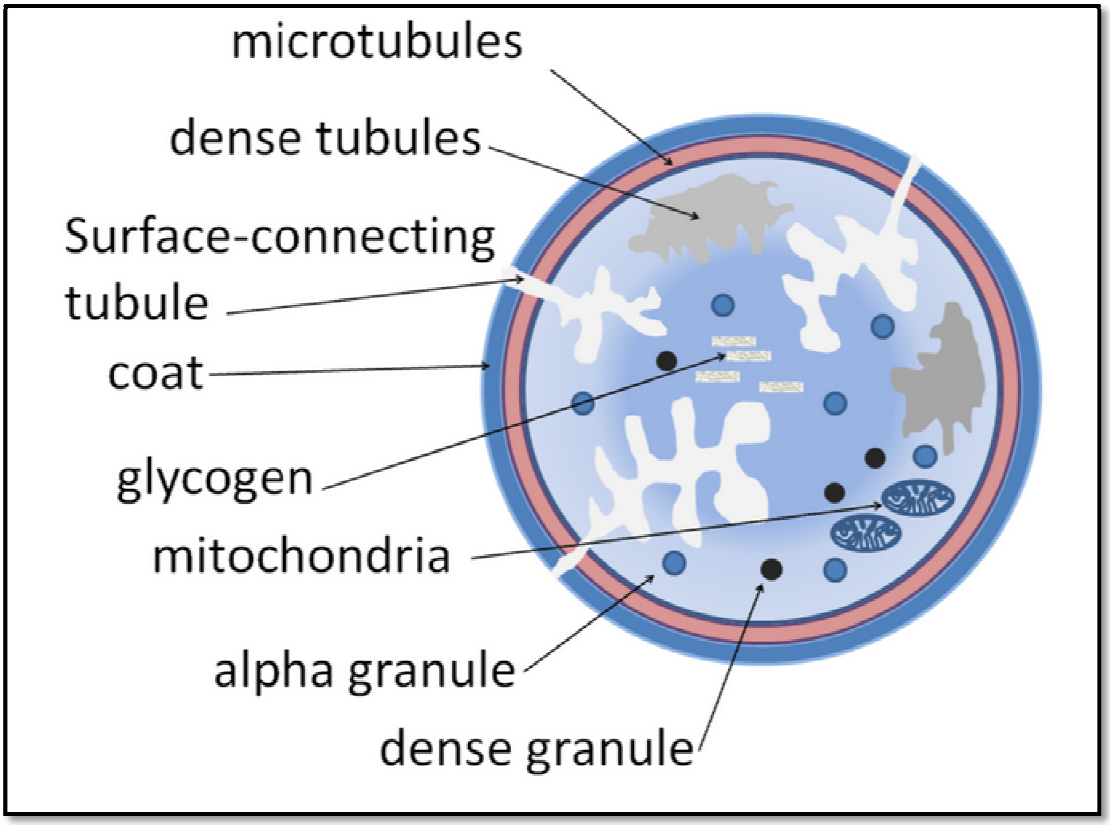


Figure 7 : Diagrammatic representation of platelet structure[62]

ROLE OF PLATELETS IN HOMEOSTASIS:

Platelet adhere to the injured blood vessel to prevent blood loss through a discrete series of steps which involves platelet adhesion, platelet activation-aggregation and platelet release.

PLATELET ADHESION:

Following vessel wall injury, platelets rapidly adhere to exposed elements in the sub endothelial matrix; initiating the first step in the haemostatic response leading to the formation of a platelet plug. This primary adhesion is mediated by the synergistic function of several receptors on the platelet surface. Collagen is the main component of the sub endothelium matrix and is also considered to play the principal role in the adhesion process. The initial arrest of platelets from the flowing blood to collagen is facilitated by the glycoprotein (GP)Ib-IX-V receptor, von Willebrand factor (VWF),α2β1 adhesion and GP Ib-V-IX complex.

PLATELET AGGREGATION:

Once a primary layer of adhesive platelets have covered the exposed sub endothelial matrix, there will be aggregation of platelets which is mainly attributed to the integrin αIIbβ3 (also known as GPIIb/IIIa) receptor. The main ligand of integrin αIIbβ3 is fibrinogen but the receptor also have affinity for multimeric vWF, vitronectin, fibronectin and thrombospondin. The mechanism of aggregation involves two integrin αIIbβ3 receptors on different platelets that bind to the same fibrinogen molecule.

PLATELET ACTIVATION:

Activated state of adhered and aggregated platelets will secrete abundantly ADP and ATP from dense granule which in turn may activate neighboring platelets via ADP and ATP sensitive receptors, thromboxane A2 (TxA2), thrombin, PAR1 and PAR4.

PLATELET RELEASE:

Upon activation platelets release their granular ADP contents, ATP, calcium, serotonin, PDGF, fibronectin, fibrinogen, vWF, epinephrine and TXA2.Weak agonists require (ADP and epinephrine) cyclooxygenase activity to induce secretion whereas strong agonists (collagen and thrombin) induce secretion independent of cyclooxygenase activity.

CLOT FORMATION:

This is the important outcome of homeostasis. The GPIIb-IIIa complex on platelet surface anchor fibrin strands and pull them together to ensure a strong immobilizing fibrin clot.[63]

PRINCIPLE OF AUTOANALYZER: IMPEDANCE MEASUREMENT PRINCIPLE

In impedance measurement principle (resistant measuring principle), cells are passed one after the other through a capillary opening. The passing cell produces an electrical resistance and thus an electronic signal which is propotionate to its volume. Hence, the cells are identified based on their size and get represented in a volume distribution curve. [64]

PLATELET INDICES:

Platelet function tests such as aggregometry reflect the actual haemostatic function of platelets, but are labour intensive and cannot be performed routinely in a hospital. Recent advances in autoanalyzer have made it possible to measure various platelet parameters like mean platelet volume (MPV),platelet distribution width (PDW) and platelet large cell ratio (P-LCR) which can provide important information regarding platelet kinetics.[65] Platelet indices are cost effective, less time consuming, easy to perform and obviate observer bias, by using an automated haematological analyzer. These parameters can be used as early indicators of complications in various diseases and might become significant laboratory tests.[66] (Figure 8)

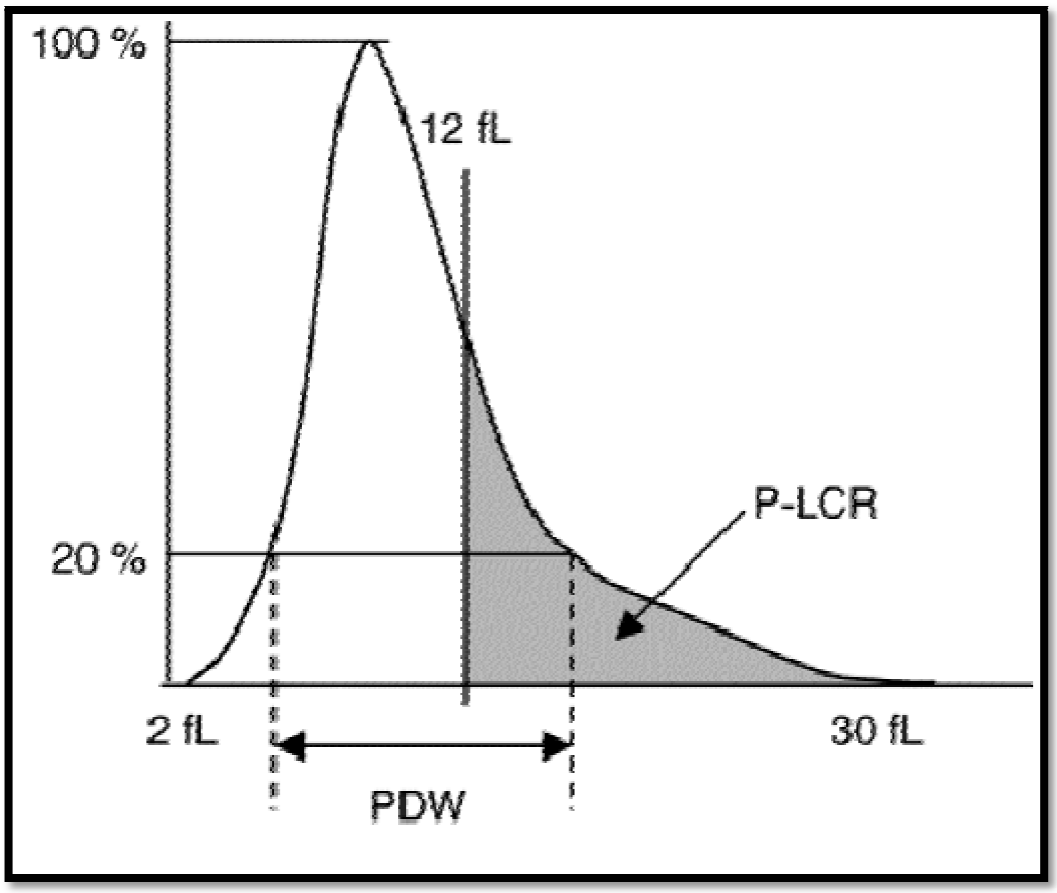


Figure 8: Platelet Histogram[66]

MPV:

MPV is measurement of average size of platelets and is calculated from a log transformation of the platelet volume distribution curve, yielding a geometric mean for this parameter.

It can also be calculated by the following formula:

MPV (fl) = [(platelet (%)/ platelet count ($x10^9$/l)] x 10^5.

Plateletcrit is the ratio of platelet volume to whole blood volume.[67]

The increase of MPV in conditions with increased platelet turn over is probably mediated by cytokines like interleukins 6, 11 and thrombopoietin. Bone marrow megakaryocytes are stimulated by thrombopoietin, and their nucleus becomes hyperlobulated, with much higher DNA content. These stimulated megakaryocytes produce larger and more reactive platelets.[68]

PDW:

Platelet size has been suggested as an indicator of platelet reactivity and increased production of pro-aggregating mediators.PDW is the distribution width on 20% frequency level with the peak taken as 100%.The PDW is useful in differentiating reactive thrombocytosis from the essential type, especially when it is combined mathematically with MPV and platelet count to obtain a discriminant function.[69]

P-LCR:

This is the ratio of large platelets exceeding 12fl discriminator. It is calculated as the ratio of the particle count between 12fl fixed discriminator and Upper Discriminator (UD) to the particle count between Lower Discriminator (LD) and Upper Discriminator.[67]

PLATELET PARAMETERS IN VARIOUS DISEASES:

MPV is normal in aplastic anemia and leukemia. MPV is significantly increased in Idiopathic Thrombocytopenic Purpura, Renal Failure, Congenital Cyanotic Heart Disease, Iron Deficiency Anemia, Acute Post Streptococcal Glomerulonephritis. In Idiopathic Thrombocytopenic Purpura MPV decreases as platelet count increases and comes to normal when platelet count reaches normal range.

Diabetes Mellitus and Rheumatic Heart Disease patients showed a significantly higher mean platelet volume compared to the control group.

In pregnant women with pre-eclampsia significantly higher mean platelet volume was noted compared to the normal spontaneous vaginal delivery, spontaneous premature rupture of membranes and abortion.[70]

Increased MPV is associated with a worse outcome in patients suffering from an acute ischemic cerebrovascular event. Patients with highest quintile of MPV have a two fold risk of suffering from a severe stroke compared with lowest quintile MPV patients.[71]

MPV changes have been observed in Systemic Lupus Erythematosus (SLE)[72] and Rheumatoid Arthritis(RA) in few studies. Lower MPV was observed in patients of Rheumatoid Arthritis compared with osteoarthritis and healthy subjects. This finding was accompanied by increased disease activity, measured by Disease Activity Score 28(DAS 28), platelet count and biomarkers of inflammation which suggest, platelet activation in RA is associated with reactive megakaryocytes as part of active inflammation.[73]

Significant decrease in MPV,PDW levels and increase in PCT levels has been observed in patients with active Ulcerative Colitis and Crohns Disease when compared to the healthy controls. In remission phase of Inflammatory bowel disease while MPV levels were lower, PDW and PCT levels were higher than control group.[74]

Mean platelet volume, platelet distribution width and platelet count are higher in lung cancer patients compared with the healthy control group. Among patients with lung cancer, PDW in small cell lung cancer patients is higher than in non small cell lung cancer patients.[75]

In patients with solid tumor showing metastasis to bone marrow, MPV in patients with marrow metastasis is lower than in patients without metastasis.[76] Increased platelet volume indices contribute to the prothrombotic state in acute ischemic syndromes and that larger platelets may play a specific role in infarction. Because larger platelets are haemostatically more active, the presence of larger platelets is probably a risk factor for developing coronary thrombosis and MI. MPV &

PDW are raised in patients who have suffered STEMI & NSTEMI as compared with patients diagnosed with unstable angina. [77]

EVALUATION OF PLATELET INDICES IN TYPE 2 DIABETES MELLITUS

As explained, larger platelets produced from activated megakaryocytes are younger, more reactive and aggregable and correlates with platelet size and function. They contain denser granules, secrete more serotonin and β-thromboglobulin, than smaller platelets. All these can lead to a pro-coagulant effect and cause thrombotic vascular complications. This suggests a relationship between the platelet function especially MPV, an indicator of the average size and activity of platelets and diabetic vascular complications. Therefore MPV can be used as a useful marker for Type 2 diabetes mellitus complications.[78]

Kodiatte *et al*[79] conducted a cross sectional study on 255 diagnosed cases of Type 2 diabetes mellitus (DM) and 251 non diabetic subjects without coronary artery disease. They concluded that MPV was significantly higher(8.29±0.735fl) in the diabetic patients as compared to the non diabetic group(7.47±0.726fl; $P<0.001$) and the increased platelet size may be one of the factors in the increased risk of atherosclerosis associated with diabetes mellitus and associated vascular complications.

Jindal S *et al*[80] conducted a study which included 75 subjects of DM and 50 non selected patients and concluded that MPV, PDW and P-LCR were all significantly higher in diabetic patients compared to the control subjects ($P<0{\cdot}05$) and also PDW is an indicator of microvascular complications in Type 2 DM. Among the diabetics, PDW was higher in those with complications as compared to those without complications.

Zaccardi F *et al*[81] conducted a cross-sectional study on 39 diagnosed cases of Type 2 DM and compared with 39 healthy controls and observed that MPV was significantly higher in Type 2 DM (standardized mean difference, 95% confidence interval: 0.70, 0.50–0.91). PDW was wider in Type 2 DM (0.93, 0.09–1.76).

Zuberi BF *et al*[82] did a comparative study of MPV in DM, Impaired fasting glucose (IFR) and non-diabetic patients. This cross sectional study included sample size of 204 in each group and found that MPV was increased in patients of DM and IFR compared to non diabetics which showed statistically significant intergroup and intragroup differences with a *P* value of <0.001.

Demirtunc R *et al*[83] studied the relationship between glycemic control and platelet activity in Type 2 DM which included 70 patients with type 2 DM and 40 age and sex matched healthy individuals and found increased platelet activity and significantly higher MPV in Type 2 DM patients. Furthermore, platelet activity recovered through improved glycemic control, may prevent the possible role of platelets in cardiovascular events in these patients.

Shah *et al*[84] performed a retrospective analysis of 13,021 participants in the National Health and Nutrition Examination Survey from 1999 to 2004 and concluded that MPV was significantly higher in subjects with diabetes (8.20 vs. 8.06 fl,$P < 0.01$) than non diabetic controls but not in subjects with metabolic syndrome (8.09 vs. 8.07 fl, $P = 0.24$). There was also a significant correlation between MPV and glucose ($P < 0.0001$) and between MPV and hemoglobin A_{1c} ($P < 0.0001$) in subjects with diabetes. These correlations were not 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 ($\geq$9.31 fl). The association

between MPV and diabetes was most apparent in those with the poorest glucose control.

The frequently observed inverse correlation between platelet count and MPV reflects the need to preserve a constant platelet mass. This inverse relationship is often seen in inflammatory disorders, where enhanced thrombopoiesis increases both platelet count and MPV and large amount of reactive large sized platelets migrates to the site of inflammation, where they are consumed. It has been reported that platelet heterogeneity is not related to ageing and changes in platelet volume is determined by megakaryocyte. Circulating platelets contain matrix ribonucleic acid, alpha and dense granules which provide self regulation mechanism by shape change and release of biologically active substances. Rapid (minute-hours) shifts in platelet indices including an increase may take place as a result of the synthesis of prothrombotic and proinflammatory agents in platelets, degranulation of alpha granules and release of highly reactive platelets.[85]

Automated cell counters have made the platelet count and the platelet volume indices (PVI)—mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-LCR)—routinely available in most clinical laboratories. The MPV can reflect changes in either the level of platelet stimulation or the rate of platelet production. The discordance between the different and same cell counter results limits the use of MPV. This can explain partly why hematological laboratories does not display platelet indices. However, there is scope to make better use of the platelet parameters generated and their role in various thrombotic and inflammatory conditions have substantially improved.MPV and PDW has emerged as a reliable marker of platelet function. P-LCR and plateletcrit are yet to be explored fully with respect to its significance.[86]

MATERIALS AND METHODS

Source of data :

A cross sectional hospital based study was carried out on patients fulfilling the inclusion and exclusion criteria attending either outpatient or inpatient department referred to the Department of Pathology in B.L.D.E University's Shri B.M.Patil Medical College, Hospital and Research centre, Vijayapur.

Study period: 1st November 2013 to 30th June 2015

Methods of collection of data

- The study was carried out on patients diagnosed with Type 2 DM.
- All the diabetic and healthy subjects underwent complete clinical and biochemical evaluation.
- MPV, PDW, PLCR in the above target groups were measured using an automated blood cell counter.
- Venous blood samples were collected in di-potassium EDTA tubes.
- The sample was run within two hours of venepuncture using the 6 part differentiated automated Hematoanalyzer (Sysmex XN-1000) and complete blood count analysis of the samples were made including the platelet indices (MPV, PDW, P-LCR).
- The peripheral smear slides of the samples were also made using Leishmann's stain to study platelet morphology and compare with that obtained from the autoanalyser.

Statistical analysis:

- Statistical evaluation was performed using student independent sample two-tailed t-test and pearson correlation test (r value as the coefficient).
- Data was expressed as mean ± standard deviation.
- A *p* value <0.05 was considered statistically significant.

Inclusion criteria:

1. Patients diagnosed with Type2 DM
2. Patients diagnosed with Type2 DM with hypertension and hypercholesterolemia.
3. Age and Sex matched healthy controls.

Exclusion criteria:

1. Hb<13gm%-males

   Hb<12gm%-females
2. Subjects on antiplatelet drugs such as aspirin and clopidogrel.
3. Subjects with any diagnosed malignancy

RESULTS

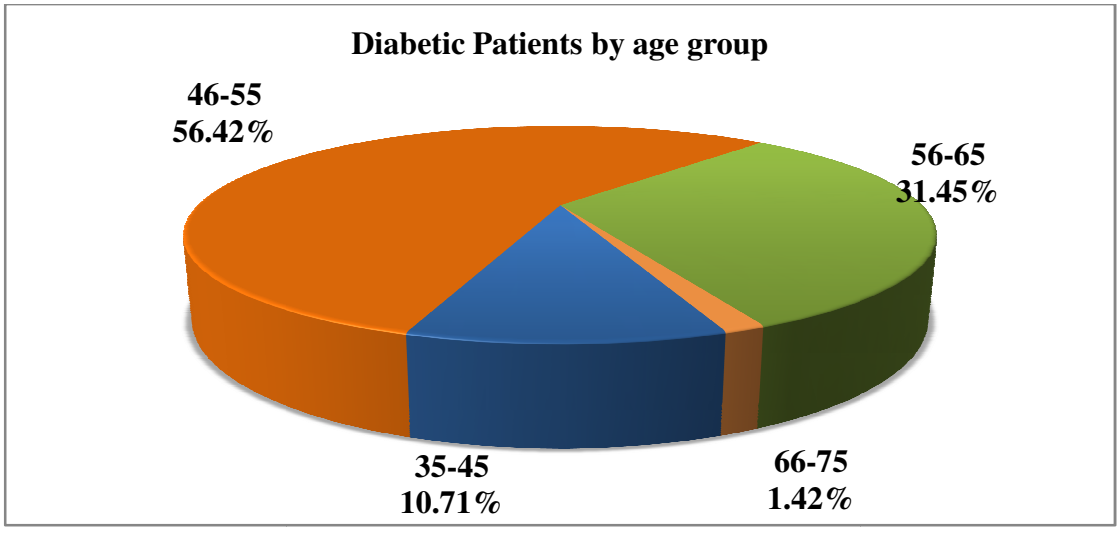
280 Type 2 diabetic cases and 280 age and sex matched controls were included in the present study. Platelet parameters MPV, PDW, P-LCR were compared between diabetic cases and non diabetic controls.

In the present study, the age ranged from 45-70 years. The mean age of patients in our study was 53± 5.7 years. Majority of the patients diagnosed with Type 2 DM belonged to 5th decade of life. The mean duration of diabetes was 4.7±2.5 years.

TABLE 4: DISTRIBUTION OF DIABETES BY AGE

AGE (YEARS)	DIABETIC CASES	
	NUMBER OF PATIENT	PERCENTAGE (%)
35-45	30	10.71
46-55	158	56.42
56-65	88	31.45
66-75	4	1.42
Total	280	100

FIGURE 9 : PIE CHART DISTRIBUTION OF DIABETIC CASES BY AGE



In the present study total number of males including both cases and controls were 315 (56.25%) and number of females were 245 (43.75%)

The total number of males presenting with type 2 DM among cases were 144 (51.43%) and females affected were 136 (48.57%).

TABLE 5: SEX DISTRIBUTION AMONG CASES

SEX (CASES)	FREQUENCY	PERCENT (%)
Male	144	51.43
Female	136	48.57
Total	280	100.00

TABLE 6: SEX DISTRIBUTION AMONG CONTROLS

SEX (CASES)	FREQUENCY	PERCENT (%)
Male	171	61.08
Female	109	38.92
Total	280	100.00

BLOOD SUGAR PARAMETERS

The blood sugar parameters, fasting blood sugar (FBS), random blood sugar (RBS), post prandial blood sugar (PPBS) and HbA1c were investigated among diabetic cases and compared to age and sex matched non diabetic controls.

The mean of FBS was 158.1±33.7mg/dl (*p* value 0.002), RBS was 214.2± 42.1mg/dl (*p* value 0.002), PPBS was235.6±38.5mg/dl (*p* value 0.005) and HbA1c was 7.3±1.1% (*p* value 0.004) in diabetic patients. In non diabetics mean FBS was 81.7 ±5.1 mg/dl, RBS was118.3± 20.6 mg/dl, PPBS was 145.8±9.2 mg/dl and HbA1c was 3.3 ±0.4% respectively. *P* value was highly significant in diabetic patients at 2

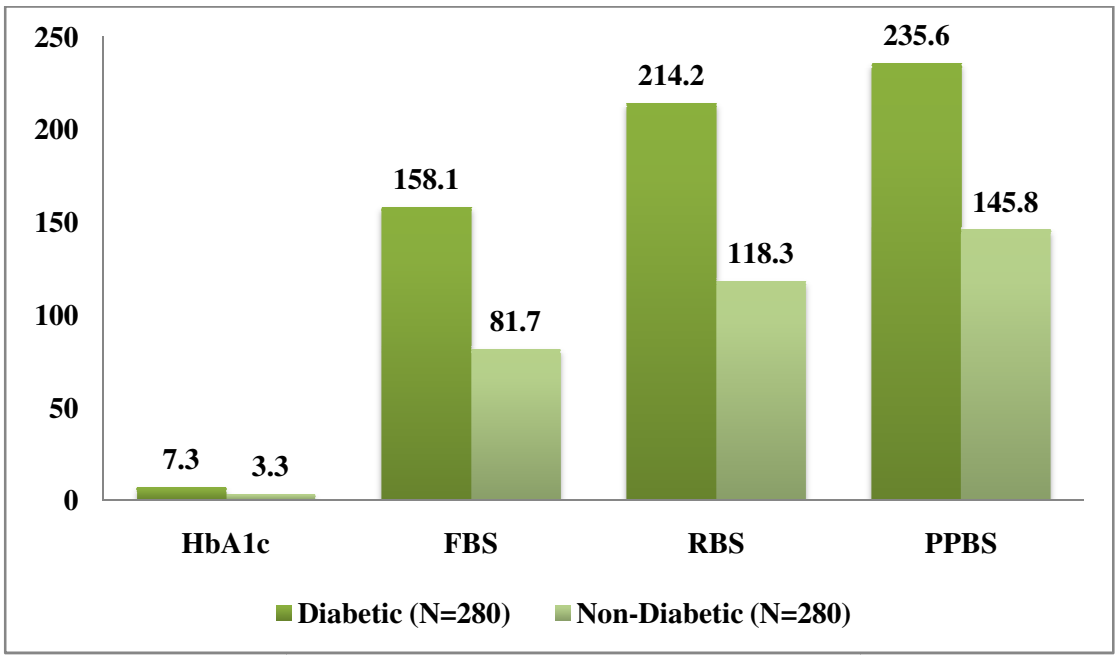
degrees of freedom and 95% confidence interval compared to the age and sex matched healthy controls.

TABLE 7: COMPARISON OF MEAN OF BLOOD SUGAR PARAMETERS BETWEEN DIABETIC CASES AND NON-DIABETIC CONTROLS

PARAMETERS	DIABETIC (N=280)		NON-DIABETIC (N=280)		t TEST
	MEAN	SD	MEAN	SD	*P* VALUE (2 TAILED)
HbA1c (%)	7.3	1.1	3.3	0.4	0.004
FBS (mg/dl)	158.1	33.7	81.7	5.1	0.002
RBS (mg/dl)	214.2	42.1	118.3	20.6	0.002
PPBS (mg/dl)	235.6	38.5	145.8	9.2	0.005

Note: *p* value less than 0.05 is taken as significant

FIGURE 10: BAR GRAPH SHOWING MEAN DISTRIBUTION OF BLOOD SUGAR PARAMETERS BETWEEN DIABETIC CASES AND NON-DIABETIC CONTROLS



HEMATOLOGICAL PARAMETERS

PLATELET INDICES

The platelet indices mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) were studied among diabetic patients and were compared with age and sex matched healthy controls.

The MPV, PDW and P-LCR were noted. The mean MPV in diabetic cases were 11.3± 1.0fl compared to the non diabetics where it was 9±0.6fl with *p* value 0.004 .Mean PDW and P-LCR in diabetic patients were 14.2±2.5 fl and 35.0±8.1% compared to the non diabetic controls where it was 10.7± 0.7fl and 23.0± 2.4% respectively with *p* value of PDW as 0.003 and P-LCR as 0.002

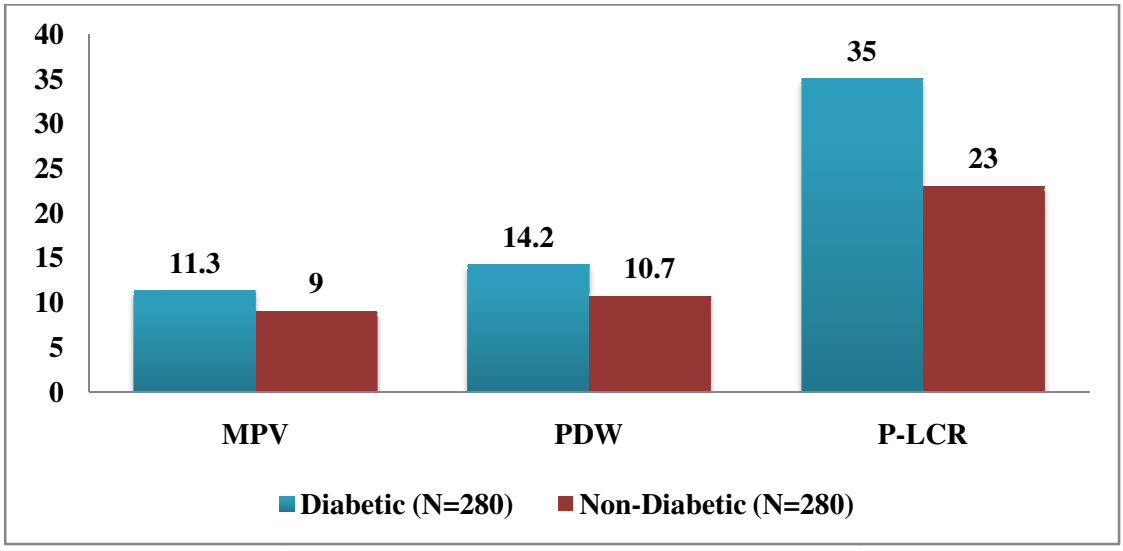
We found that *p* value of MPV,PDW and P-LCR to be highly significant in diabetic patients with *p* value <0.05 at 2 degrees of freedom and 95 % confidence level in comparison to non diabetic controls.

TABLE 8: COMPARISON OF MEAN OF PLATELET INDICES BETWEEN DIABETIC CASES AND NON-DIABETIC CONTROLS

PARAMETERS	DIABETIC (N=280)		NON-DIABETIC (N=280)		t TEST
	MEAN	SD	MEAN	SD	*P* VALUE (2-TAILED)
MPV (fl)	11.3	1.0	9.0	0.6	0.004
PDW (fl)	14.2	2.5	10.7	0.7	0.003
P-LCR (%)	35.0	8.1	23.0	2.4	0.002

Note: *p* value less than 0.05 is taken as significant

FIGURE 11 : BAR GRAPH REPRSENATION OF MEAN DISTRIBUTION OF PLATELET INDICES AMONG DIABETIC CASES AND NON-DIABETIC CONTROLS



COMPARISON OF PLATELET INDICES BETWEEN DIABETIC AND NON DIABETIC MALE PATIENTS

In the present study the number of male patients in diabetics cases were 144 and 171 in non diabetics. The mean age of presentation in diabetic cases was 53.8±5.8 years. The mean of MPV was 11.4 ± 1.1 fl, PDW was 14.3±2.7 fl and P-LCR was 35.5±8.4 % in male diabetic cases. In non diabetic male controls the mean of MPV was 9.0±0.6fl, PDW was 10.8±0.7 fl and P-LCR was 23.4 ±2.4%.

2-tailed t test was used for calculation of *p* value. In our study, *p* value of MPV was 0.005, PDW was 0.003 and P-LCR was 0.004 which was statistically significant compared to healthy male controls, when *p* value <0.05 was taken as significant at 95% confidence level.

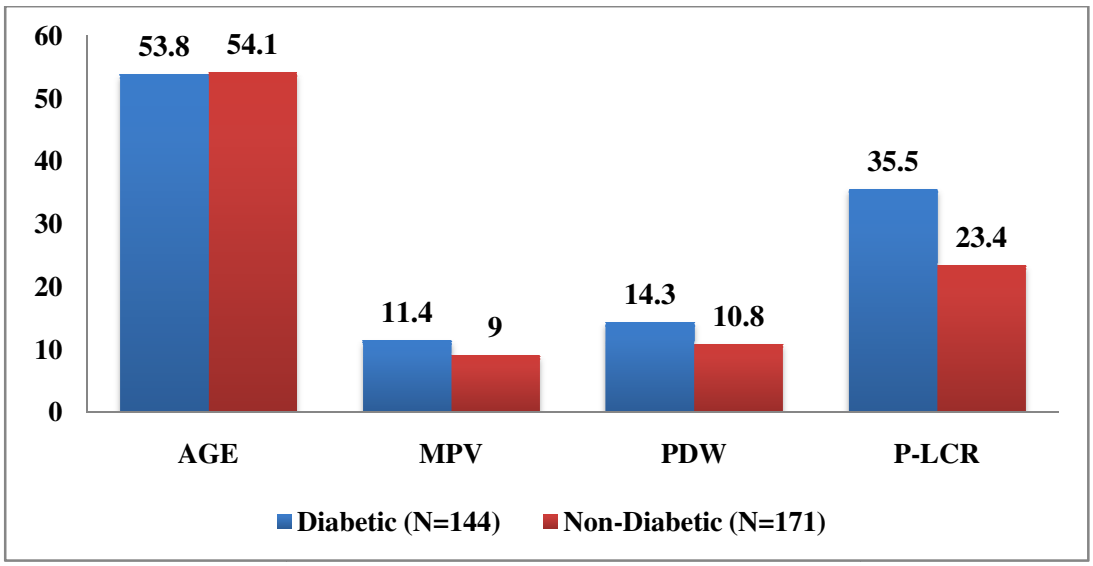
TABLE 9: COMPARISON OF MEAN OF AGE AND PLATELET INDICES AMONG MALE DIABETIC CASES AND CONTROLS

PARAMETERS	MALE DIABETICS (N=144)	MALE NON-DIABETICS (N=171)	t TEST

	MEAN	SD	MEAN	SD	*P* VALUE (2-TAILED)
AGE (years)	53.8	5.8	54.1	5.2	0.710
MPV (fl)	11.4	1.1	9.0	0.6	0.005
PDW (fl)	14.3	2.7	10.8	0.7	0.003
P-LCR (%)	35.5	8.4	23.4	2.4	0.004

Note: *p* value less than 0.05 is taken as significant

FIGURE12: BAR GRAPH SHOWING MEAN DISTRIBUTION OF AGE AND PLATELET INDICES AMONG MALE DIABETIC CASES AND CONTROLS



COMPARISON OF PLATELET PARAMETERS BETWEEN DIABETIC AND NON DIABETIC FEMALE PATIENTS

The number of female patients in diabetics cases in the present study were 136 and 109 in non diabetics. The mean age of diabetic patients was 52.2±5.6 years. The mean of platelet parameters MPV, PDW and P-LCR in female diabetic female patients were 11.3 ± 1.0 fl, 14.0±2.3 fl and 34.5±7.6 % compared to non diabetic female controls where the mean of MPV was 8.9±0.5fl, PDW was 10.6±0.7 fl and P-LCR was 22.6 ±2.2%.

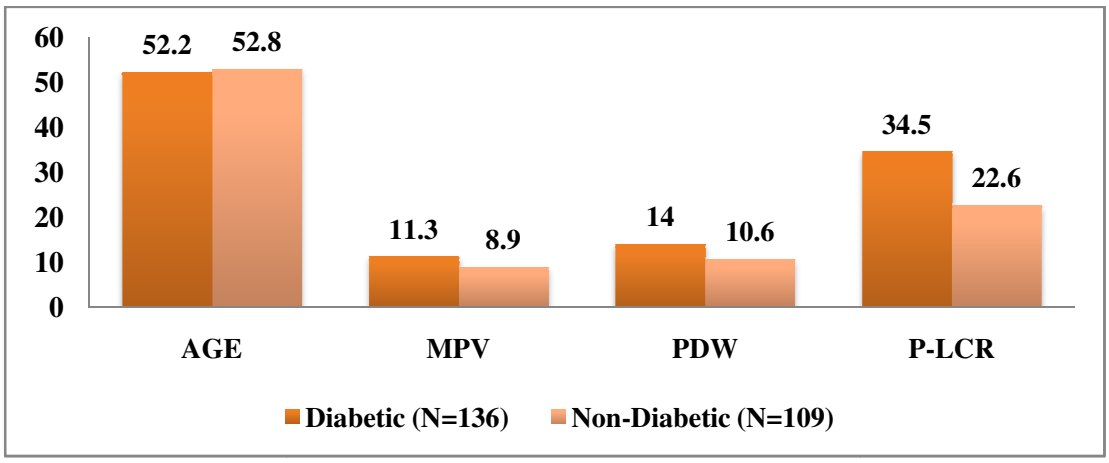
Our study showed that *p* value of MPV & PDW was 0.004 and P-LCR was 0.005 which were significantly higher in female diabetic patients compared to non diabetic female controls when *p* value <0.05 is taken as significant at 2 degrees of freedom and 95% confidence level.

TABLE 10: COMPARISON OF MEAN OF AGE AND PLATELET INDICES BETWEEN FEMALE DIABETIC CASES AND CONTROLS

PARAMETERS	FEMALE DIABETICS (N=136)		FEMALE NON-DIABETICS (N=109)		t TEST
	MEAN	SD	MEAN	SD	*P* VALUE (2-TAILED)
AGE (years)	52.2	5.6	52.8	5.5	0.380
MPV (fl)	11.3	1.0	8.9	0.5	0.004
PDW (fl)	14.0	2.3	10.6	0.7	0.004
P-LCR (%)	34.5	7.6	22.6	2.2	0.005

Note: *p* value less than 0.05 is taken as significant

FIGURE 13: BAR GRAPH REPRESENTATION OF MEAN DISTRIBUTION OF AGE AND PLATELET INDICES BETWEEN DIABETIC AND NON-DIABETIC FEMALE PATIENTS



CORRELATION OF MEAN PLATELET VOUME (MPV) WITH VARIOUS PARAMETERS

Our study showed positive Pearson correlation of MPV with HbA1c (r=0.048), FBS(r=0.055), RBS (r=0.011), PPBS (r=0.029), duration of diabetes (0.126) and complications (r=0.095). A statistical significance was found between MPV with blood sugar parameters and complications. Although MPV showed a positive correlation with duration of diabetes but it was not statistically significant with *p* value as 0.538**.**

TABLE 11 : PEARSON CORRELATION COEFFICIENT BETWEEN MPV AND SELECTED CHARACTERISTICS AMONG DIABETIC CASES

CHARACTERISTICS		**CORRELATION COEFFICIENT (*r* VALUE)**	***P* VALUE**
MPV (fl)	**HbA1c (%)**	0.048	<0.001
MPV (fl)	**FBS (mg/dl)**	0.055	<0.01
MPV (fl)	**RBS (mg/dl)**	0.011	<0.001
MPV (fl)	**PPBS (mg/dl)**	0.029	<0.05
MPV (fl)	**DURATION OF DIABETES(years)**	0.126	0.538
MPV (fl)	**COMPLICATIONS**	0.095	<0.05

CORRELATION OF PLATELET DISTRIBUTION WIDTH (PDW) WITH VARIOUS PARAMETERS

A positive Pearson correlation of PDW with HbA1c (r=0.071), FBS (r=0.098), RBS (r=0.049), PPBS (r=0.075), duration of diabetes (r=0.161) and complications (r=0.096) were observed in our study. However, no statistical significant difference was seen with PDW and duration of diabetes. (*p* value=0.553).

TABLE 12: PEARSON CORRELATION COEFFICIENT BETWEEN PDW AND SELECTED CHARACTERISTICS AMONG DIABETIC PATIENTS

CHARACTERISTICS		CORRELATION COEFFICIENT (*r* VALUE)	*P* VALUE
PDW (fl)	**HbA1c (%)**	0.071	<0.001
PDW (fl)	**FBS (mg/dl)**	0.098	<0.001
PDW (fl)	**RBS (mg/dl)**	0.049	<0.05
PDW (fl)	**PPBS (mg/dl)**	0.075	<0.02
PDW (fl)	**DURATION OF DIABETES(Years)**	0.161	0.553
PDW (fl)	**COMPLICATIONS**	0.096	<0.02

CORRELATION OF PLATELET LARGE CELL RATIO (P-LCR) WITH VARIOUS PARAMETERS

In diabetic group, a positive statistical Pearson correlation was observed between P-LCR and HbA1c (r=0.064, *P*<0.001), FBS (r=0.091, *P* <0.001), RBS (r=0.021, *P*<0.02), PPBS (r=0.052, *P* <0.05) and complications (r=0.094, *P* <0.001). Although a positive Pearson correlation was observed between P-LCR and duration of diabetes (r=0.140) but independent student t test did not show any statistical significance (*P*=0.148).

TABLE 13: PEARSON CORRELATION COEFFICIENT BETWEEN P-LCR AND SELECTED CHARACTERISTICS AMONG DIABETIC PATIENTS

CHARACTERISTICS		CORRELATION COEFFICIENT (*r* VALUE)	*P* VALUE
P-LCR (%)	**HbA1c (%)**	0.064	<0.001
P-LCR (%)	**FBS (mg/dl)**	0.091	<0.001
P-LCR (%)	**RBS (mg/dl)**	0.021	<0.02
P-LCR (%)	**PPBS (mg/dl)**	0.052	<0.05
P-LCR (%)	**DURATION OF DIABETES(Years)**	0.140	0.148
P-LCR (%)	**COMPLICATIONS**	0.094	<0.001

COMPARISON OF DIABETIC STUDY POPULATION BETWEEN GROUP A (HbA1c LEVEL <6.5%) AND GROUP B (HbA1c LEVEL ≥ 6.5%)

Diabetic patients were divided into two groups after baseline evaluation according to their HbA1c level. Group A consisted of patients with HbA1c <6.5% and group B comprised of patients with HbA1c level ≥ 6.5%. The latest HbA1c cut off for diabetic range according to American Diabetic Association 2010 criteria is ≥6.5%.

Out of 280 Type 2 DM cases, there were 60 patients (21.43%) in group A (mean HbA1c <6.5 %) and 220 patients (78.57%) in group B. The mean age in group A is 50.2±5.6 years where as in group B the mean age is 53.8±5.5 years. In the present study, mean FBS level in group A is 137.9±12.4 mg/dl while that of group B was 163.7±35.5 mg/dl (*p* value 0.002), mean RBS level in group A was 183.5±17.6 mg/dl while in group B was 222.6±42.9 mg/dl (*p* value 0.004). The mean PPBS level in group A was 212.1±24.7mg/dl while that in group B was 242.1±39.1mg/dl (*p* value 0.001)

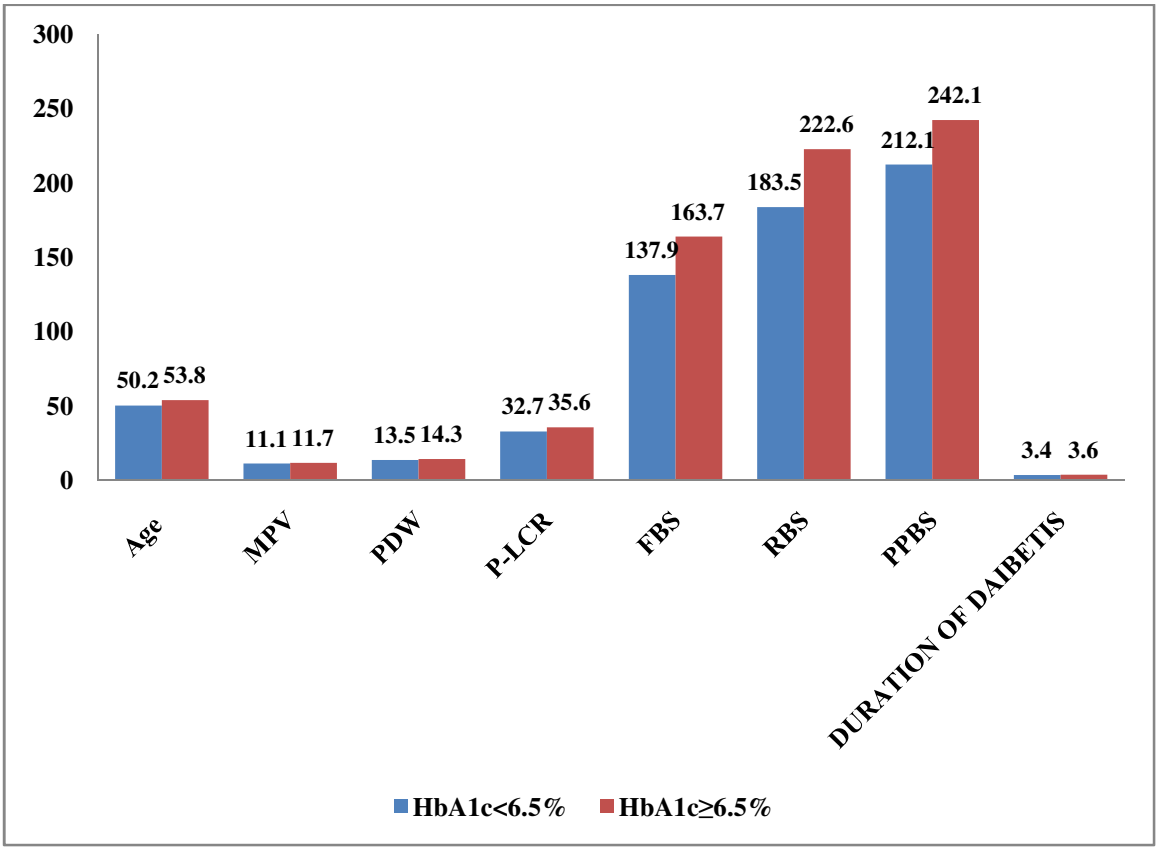
We also compared platelet indices (MPV, PDW and P-LCR) between group A and group B. The mean MPV in group B was 11.7±1.0 fl and in group A it was 11.1±1.2fl, which was statistically significant (*p* value <0.05).The mean PDW in group B was 14.3±2.4 fl which was significantly higher than group A with mean PDW being 13.5±2.7fl.The mean P-LCR in group B was 35.6±7.7% while that of group A was 32.7±9.1 %,which showed statistical significant difference (*p* value <0.05). Duration of diabetes was also compared between two groups and it was concluded that independent t test was not statistically significant with *p* value as 0.746.

TABLE 14: COMPARISON OF MEAN OF VARIOUS PARAMETERS BETWEEN HbA1c LEVEL <6.5% AND HbA1c LEVEL ≥ 6.5% AMONG DIABETIC PATIENTS

PARAMETERS	GROUP A HbA1c<6.5% (N=60)		GROUP B HbA1c ≥6.5% (N=220)		t TEST
	MEAN	SD	MEAN	SD	*P* VALUE (2-TAILED)
AGE (years)	50.2	5.6	53.8	5.5	0.001
MPV (fl)	11.1	1.2	11.7	1.0	0.024
PDW (fl)	13.5	2.7	14.3	2.4	0.022
P-LCR (%)	32.7	9.1	35.6	7.7	0.013
FBS (mg/dl)	137.9	12.4	163.7	35.5	0.002
RBS (mg/dl)	183.5	17.6	222.6	42.9	0.004
PPBS (mg/dl)	212.1	24.7	242.1	39.1	0.001
DURATION OF DIABETES(Years)	3.4	1.6	3.6	1.7	0.746

Note: *p* value less than 0.05 is taken as significant

FIGURE 14: BAR GRAPH REPRESENTATION OF MEAN DISTRIBUTION OF VARIOUS PARAMETERS BETWEEN HbA1c LEVEL <6.5% AND ≥6.5% AMONG DIABETIC CASES



COMPARISON OF MEAN OF PARAMETERS AMONG DIABETIC CASES WITH AND WITHOUT COMPLICATIONS

Out of 280 patients in the present study, 117 (41.79 %) patients had complications such as diabetic foot, hypertension, coronary artery disease, diabetic retinopathy, diabetic nephropathy, autonomic neuropathy, peripheral neuropathy, peripheral vascular disease, hypercholesterolemia and hypertriglyceridemia and 163(58.21%) cases did not present with complications. The number of patients with diabetic complications were 69 (47.90%) out of 144 male diabetic patients and 48 (35.30%) out of 136 female diabetic patients.

TABLE 15: DISTRIBUTION OF COMPLICATIONS BY GENDER

GENDER		COMPLICATIONS		TOTAL
		ABSENT	PRESENT	
MALE	**NUMBER**	75	69	144
	%	52.10%	47.90%	100.00%
FEMALE	**NUMBER**	88	48	136
	%	64.70%	35.30%	100.00%
TOTAL	**NUMBER**	163	117	280
	%	58.21%	41.79%	100.00%

We also divided the patients suffering from complications based on HbA1c levels. The number of patients with diabetic complications and HbA1c levels <6.5% was 9 (15.00%) and with HbA1c level ≥ 6.5% and complications was 108 (49.10%). *P* value was significantly higher (*p* value 0.008) in patients with diabetic complications and HbA1c level ≥ 6.5%.

TABLE 16: DISTRIBUTION OF COMPLICATIONS BY HbA1c LEVEL

HbA1c		COMPLICATIONS			CHI SQUARE *P* VALUE
		ABSENT	PRESENT	TOTAL	
<6.5%	**NUMBER**	51	9	60	0.008
	%	85.00%	15.00%	100.00%	
≥ 6.5%	**NUMBER**	112	108	220	
	%	50.90%	49.10%	100.00%	
TOTAL	**NUMBER**	163	117	280	
	%	58.20%	41.80%	100.00%	

Note: *p* value less than 0.05 is taken as significant

Platelet indices were also compared between diabetic patients with and without complications. It was concluded in the present study that the mean of MPV was higher in diabetics (11.5±1.4 fl) with complications compared to diabetic patients without complications (11.3±1.0 fl). The mean of PDW and P-LCR in diabetic cases with complications (14.4±2.7fl and 35.9±8.3%) were higher compared to diabetics without complications (13.9±2.1fl and 34.4±7.8%).

Although the mean of platelet parameters were higher in diabetic cases but independent t test did not show statistical significance between diabetic cases with complications compared to diabetics without complications (*p* value of MPV=0.105, PDW=0.099 and P-LCR=0.104).

In the present study, the mean levels of HbA1c, FBS, RBS and PPBS were higher in diabetic cases with complications (mean of HbA1c=7.8±1.1%, mean of FBS=168.9±36.3mg/dl, mean of RBS=227.9±43.1 mg/dl and mean of PPBS= 266.8±43.0mg/dl) compared with diabetic patients without complications (mean of HbA1c=7.0±1.0%, mean of FBS= 149.6±24.2mg/dl, mean of RBS= 200.9±30.6 mg/dl, mean of PPBS=225.3±26.9mg/dl). Independent t test showed a statistically significant p value between diabetic cases with and without complications (p value <0.05).

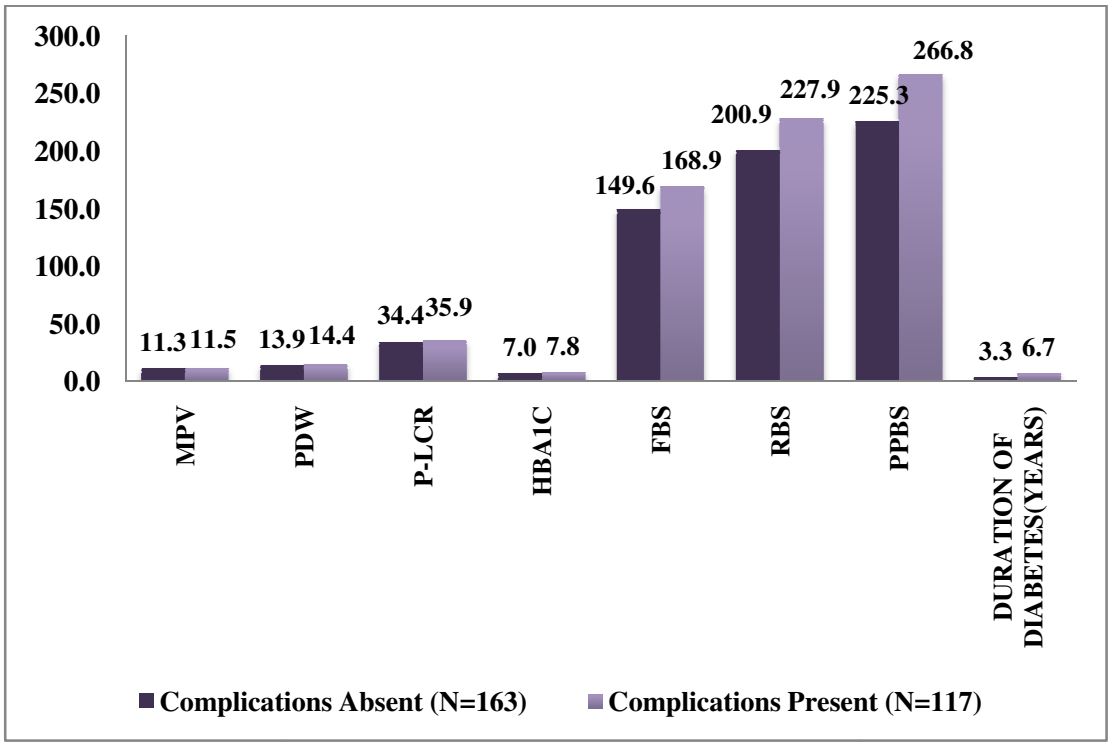
The mean of duration of diabetes was higher in diabetic patients (6.7±2.3 years) with complications when compared with diabetic cases without complications (3.3±1.3years).It was found that *p* value for mean duration of diabetes was statistically significant in diabetic patients with complications when compared with diabetic cases without complications (*p* value< 0.05)

TABLE 17: COMPARISON OF MEAN OF VARIOUS PARAMETERS AMONG DIABETIC PATIENTS WITH & WITHOUT COMPLICATIONS

PARAMETERS	COMPLICATIONS ABSENT (N=163)		COMPLICATIONS PRESENT (N=117)		t TEST
	MEAN	SD	MEAN	SD	*P* VALUE (2-TAILED)
MPV (fl)	11.3	1.0	11.5	1.4	0.105
PDW (fl)	13.9	2.1	14.4	2.7	0.099
P-LCR (%)	34.4	7.8	35.9	8.3	0.104
HbA1c (%)	7.0	1.0	7.8	1.1	0.005
FBS (mg/dl)	149.6	24.2	168.9	36.3	0.004
RBS (mg/dl)	200.9	30.6	227.9	43.1	0.007
PPBS (mg/dl)	225.3	26.9	266.8	43.0	0.002
DURATION OF DIABETES (Years)	3.3	1.3	6.7	2.3	0.025

Note: *p* value less than 0.05 is taken as significant

FIGURE 15: BAR GRAPH SHOWING COMPARISON OF MEAN OF VARIOUS PARAMETERS AMONG DIABETIC PATIENTS WITH & WITHOUT COMPLICATIONS



DISCUSSION

Diabetes mellitus is a major global health problem.[78] India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the " diabetes capital of the world". It is one of the major causes of morbidity and mortality affecting youth and middle aged people in India.[10] The aeitology of the diabetes mellitus is undoubtedly multifactorial. Platelets from patients with type 2 diabetes mellitus have increased reactivity and baseline activation which are likely to play a key role in formation, development and sustainment of vascular complications.[29]

The present study included 280 patients diagnosed with Type 2 DM. 280 age and sex matched healthy controls were also included in this study. Patients with Hb<13gm% in males and <12gm% in femlaes, on antiplatelet drugs such as aspirin and clopidogrel and any diagnosed malignancy were excluded as studies have shown persisiting high platelet indices values in these cases. Out of 280 cases, 60 patients had HbA1c levels <6.5% and 220 cases had HbA1C level ≥ 6.5% .In the present study, out of 280 diabetic cases, 117 patients presented with vascular complications and 163 cases did not present with any complications.

AGE:

The prevalence of type 2 diabetes increases markedly with age. The onset of diabetes among Indians is about a decade earlier than their western counterparts.[87] In the present study, the age ranged from 45-70 years. The mean age of patients in our study was 53±5.7years.Majority of the patients diagnosed with Type 2 DM belonged to 5th decades of life. This is in accordance with other studies. [78,79,80,83]

TABLE 18 : COMPARISON OF MEAN AGE OF DIABETES WITH OTHER STUDIES

PUBLICATION	CASES	MEAN AGE OF DIABETES (years)
Hekimsoy *et al*[78] (2015)	145	56.66
Kodiatte *et al*[79] (2012)	255	51.5
Jindal S *et al*[80] (2011)	75	52.13
Demirtunc *et al*[83] (2007)	70	55.1
Present study	280	53

PLATELET INDICES:

Platelet indices (MPV, PDW & P-LCR) were analysed in patients with Type 2 DM and compared with healthy controls.

MEAN PLATELET VOLUME:

The MPV value evaluated in our study was 11.3±1.0 fl in diabetic group and 9±0.6 fl in the control group. We found that MPV was significantly higher ($p<0.05$) in patients diagnosed with Type 2 DM when compared with age and sex matched healthy controls. This is in agreement with the results of similar studies done by other researchers.[78,79,80,82,83,88,90,91,92]

TABLE 19 : COMPARISON OF MPV WITH OTHER STUDIES

PUBLICATION	CASES	MPV (fl)	CONTROLS	MPV(fl)	*P* VALUE
Hekimsoy *et al*[78] (2004)	145	10.62	100	9.15	<0.001
Kodiatte *et al*[79] (2012)	255	8.29	251	7.47	<0.001
Jindal *et al*[80] (2011)	75	12.08	50	11.42	<0.05
Zuberi *et al*[82] (2008)	204	9.34	204	8.63	0.000
Demirtunc *et al*[83] (2007)	70	8.7	40	8.2	0.002
Mowafy *et al*[88] (2015)	30	10.45	15	9.99	<0.05
Akinsegun *et al*[89] (2014)	100	8.69	100	8.91	0.593
Ozder A and Eker H[90] (2014)	201	10.66	201	10.04	<0.001
Ulutas *et al*[91](2014)	65	8.3	40	7.1	<0.001
Papanas *et al*[92] (2004)	265	14.2	151	7.1	0.01
Present study	280	11.3	280	9	<0.05

MPV is an indicator of the average size and activity of platelets. Larger platelets are younger, more reactive and aggregable. Hence, they contain denser granules, secrete more serotonin and β-thromboglobulin, and produce more thromboxane A2 than smaller platelets which can produce a pro-coagulant effect and cause thrombotic vascular complications. This suggests a relationship between the platelet function, especially MPV and diabetic vascular complications, thus indicating changes in MPV reflect the state of thrombogenesis. Activated platelets respond to activated leukocytes and endothelial cells via adhesion molecules linking inflammation and thrombosis.[78] Therefore, high MPV is emerging as a new risk factor for the vascular complications of DM of which atherothrombosis plays a major role. Thus, DM has been considered as a "prothrombotic state" with increased platelet reactivity.[79]

The value of MPV evaluated in our study was closest to the studies done by Hekimsoy *et al*[78] and Mowafy *et al*[88].

Hekimsoy *et al*[78] have shown a correlation between higher MPV and fasting blood glucose and glycosylated hemoglobin in their prospective study. Kodiatte *et al*[79] study showed that there might be small bleeds due to the rupture of atherothrombotic plaques leading to increased platelet recruitment, hyper reactivity, and bone marrow stimulation and thus higher MPV in diabetes mellitus. Jindal *et al*[80] study concluded MPV to be significantly higher in diabetic cases as compared to the non diabetic cases ($p < 0.05$). Zuberi *et al*[82] showed in their study that MPV is significantly associated with diabetic and impaired fasting glucose patient and could increase the risk of thrombotic complications.

Demirtunc *et al*[83] suggested in his study that platelet dysfunction begins at the early stages of diabetes even before symptoms appear and vascular pathology and thus MPV can be used as a cost effective and easy method to evaluate platelet function and activity. However they also concluded that improved glycemic control decreases MPV and may prevent possible role of platelets in thrombotic events in type 2 DM.

Mowafy *et al*[88] concluded that MPV was significantly higher in diabetic patients (10.45±1.05fl) than with control group (9.9±0.63 fl) and can be used as an easy laboratory test to predict the occurrence of early vascular complications. Ozder and Eker stated that MPV is significantly increased in diabetic group and is indicative of worsening glycemic control. [90]

Ulutas *et al*[91] concluded in their study that although MPV was significantly higher in diabetic cases, but glycemic control reduces the platelet activity and it may prevent or delay vascular complications in patient with type 2 DM.

Papanas *et al*[92] showed in their study that MPV was significantly higher in type 2 diabetes patients (14.2±2.2fl) than in age-and sex matched healthy controls (7.1±1.2fl).They measured MPV in two automated blood cell counter Sysmex SF 3000 and Cell-Dyn 3700 and concluded that higher MPV was a constant finding in their study, and did not depend on the type of blood cell counter used.

However, Akinsegun *et al*[89] study showed that MPV was lower in diabetic cases (8.69±0.67fl) as compared to the controls (8.91±0.80 fl) and no statistically significant difference existed between the mean platelet volume in diabetics and healthy controls which is in contrast to our study where MPV was significantly higher in diabetic cases ($p<0.05$).

COMPARISON BETWEEN MPV AND GLYCOSYLATED HEMOGLOBIN (HbA1c) <6.5% AND ≥ 6.5% WITH OTHER STUDIES

The MPV was significantly higher (11.7±1.0fl) in group B cases with HbA1c ≥ 6.5% as compared to Group A cases (11.1±1.2fl) with HbA1c <6.5% in our study. This is in agreement with the results of studies done by other researchers.[79,83,90,91, 93]

TABLE 20 : COMPARISON BETWEEN MPV AND GLYCOSYLATED HEMOGLOBIN (HbA1c) WITH OTHER STUDIES

PUBLICATION	HbA1c <6.5% CASES (GROUP A)	MPV (fl)	HbA1c ≥ 6.5% CASES (GROUP B)	MPV (fl)	*P* VALUE
Kodiate *et al*[79] (2013)	34	7.95	221	8.35	0.003
Demirtunc *et al*[83] (2009)	35	8.4	35	9.0	0.01
Ozder A and Eker H[90] (2014)	45	10.17	156	10.80	0.001
Ulutas *et al*[91] (2014)	33	7.5	32	8.3	0.039
Demirtas *et al*[93] (2015)	82	8.9	225	9.4	0.018
Present study	60	11.1	220	11.7	0.024

In our study, there were high number of diabetics with HbA1c levels ≥ 6.5% which is similar to the observation in the study done by Kodiatte *et al*[79] .This might have been due to poor dietary practices and lack of knowledge regarding the diet and exercise regimens that ought to be followed in diabetics.

The value of MPV observed in our study among group A (HbA1c <6.5%) and group B (HbA1c ≥6.5%) was closest to the study done by Ozder A and Eker H. [90] They concluded that as glycemic control improves, HbA1c and MPV tends to decrease. Therefore, it may be concluded that glycemic control improves platelet activity and function and may prevent or delay possible diabetic vascular complications. Demirtunc *et al*[83] concluded in his study that although MPV was higher in Group B (HbA1c ≥6.5%) patients when compared with Group A (HbA1c <6.5%) cases but there is a significant decrease in MPV in Group B patients compared to baseline MPV when Group B patients achieved glycemic control that is, HbA1c level <6.5% at the end of the follow up period of 3 months.

Demirtas *et al*[93] concluded in their study that levels of MPV were significantly different between the regulated (HbA1c <6.5%) and unregulated (HbA1c≥6.5%) diabetic patients and levels of these parameters were tend to increase in unregulated patients.

COMPARISON BETWEEN MPV WITH AND WITHOUT COMPLICATIONS WITH OTHER STUDIES:

Our study showed that MPV was higher in diabetic patients with complications (11.5±1.4 fl) when compared to diabetic patients without complications (11.1±1.0fl) but was not statistically significant**.**

TABLE 21: COMPARISON OF MPV AND COMPLICATIONS WITH OTHER STUDIES

PUBLICATION	DIABETIC PATIENTS WITH COMPLICATIONS	MPV (fl)	DIABETIC PATIENTS WITHOUT COMPLICATIONS	MPV (fl)	*P* VALUE
Hekimsoy *et al*[78] (2004)	76	10.56	69	10.3	0.42
Kodiate *et al*[79] (2013)	159	8.35	96	8.2	0.145
Jindal *et al*[80] (2011)	50	12.25	25	11.77	0.212
Mowafy *et al*[88] (2015)	15	10.45	15	9.87	0.272
Papanas *et al*[92] (2004)	167	15.8	98	10.9	0.043
Demirtas *et al* [93] (2015)	67	9.54	240	9.20	0.006
Present study	117	11.5	163	11.1	0.105

Our observations concluded that higher values of MPV was observed in diabetic subjects with micro and macrovascular complications but were not

statistically significant. Higher values of MPV were also seen in studies done by Kodiate *et al*[79] and Mowafy *et al*[88] which were not statistically significant, similar to our study. However Papanas *et al*[92] and Demirtas *et al*[93] concluded significantly higher MPV in diabetics with complications than in diabetics without complications in their study. This suggested a role for the increased platelet activity in the pathogenesis of vascular complications.

On the other hand, in the studies done by Hekimsoy *et al*[78] and Jindal *et al*[80] MPV was not significantly different in subjects with diabetic complications from that of diabetic patients without complications. Their possible explanation was centered on the rapid consumption of activated platelets in diabetics with complications.

PLATELET DISTRIBUTION WIDTH:

The PDW was significantly higher in the patients diagnosed with Type 2 DM (14.2±2.5 fl) as compared to the control group (10.7±0.7 fl).

TABLE 22 : COMPARISON OF PLATELET DISTRIBUTION WIDTH WITH OTHER STUDIES

PUBLICATION	CASES	PDW (fl)	CONTROLS	PDW (fl)	*P* VALUE
Jindal *et al*[80] (2011)	75	17.25	50	15.34	0.002
Mowafy *et al*[88] (2015)	30	16.55	15	15.84	<0.05
Demirtas *et al*[93] (2015)	307	16.4	187	15.4	<0.001
Jabeen *et al*[94] (2013)	170	15.02	92	14.12	0.003
Dalamaga *et al*[95] (2010)	30	16.4	30	13.0	<0.001
Present study	280	14.2	280	10.7	0.003

Our observations showed that PDW was significantly elevated among the type 2 diabetic cases as compared to the healthy controls. Similar results were noted in other studies done by Demirtas *et al*[93], Jabeen *et al*[94] and Dalamaga *et al*[95] with significantly higher PDW levels among diabetic cases. Jabeen *et al*[94] observed highly significant PDW value in diabetic cases in comparison to control subjects in their study which is because of the fact that, in addition to thrombopoietin (a chief hormonal regulator of platelet production) nitric oxide which is generated during oxidative stress in diabetes, can also stimulate platelet production.

Dalamaga *et al*[95] in their study observed that MPV and PDW are associated with glycemic indices in diabetic patients but not in diabetic myelodysplastic patients with normal platelet counts. This suggests other factors inherent to bone marrow dysplasia, platelet turnover and biochemistry, or vascular environment affect platelet morphology in diabetic myelodysplastic patients which may be an early marker for myelodysplasia. These findings support change in platelet morphology as a risk marker for elevated macrovascular disease.

Vagdatli E *et al*[86] in their study on puerperas in different trimester, patients with established platelet activation like diabetes mellitus and those with phlebothrombosis and healthy people concluded that PDW seemed to be more specific indicator of platelet activation than MPV, since it was not elevated during single platelet distension caused by platelet swelling. The combined use of PDW and MPV could predict activation of coagulation more efficiently.

COMPARISON BETWEEN PDW WITH AND WITHOUT COMPLICATIONS WITH OTHER STUDIES

Higher PDW value was observed in diabetics with complications (14.4±2.7fl) than in diabetics without complications(13.9±2.1fl) which was not statistically significant in our study.

TABLE 23 : COMPARISON BETWEEN PDW AND COMPLICATIONS WITH OTHER STUDIES

PUBLICATION	DIABETIC CASES WITH COMPLICATIONS	PDW (fl)	DIABETIC CASES WITHOUT COMPLICATIONS	PDW (fl)	*P* VALUE
Jindal *et al*[80] (2011)	50	18.14	25	15.67	0.006
Mowafy *et al*[88] (2015)	15	16.55	15	14.41	0.391
Present study	117	14.4	163	13.9	0.198

In our study higher levels of PDW was observed in patients of diabetes suffering from complications but was not statistically significant. Higher value of PDW was also observed in studies done by Mowafy *et al*[88] but was not statistically significant similar to our study. However, Jindal *et al*[80] observed a statistically significant higher PDW in diabetic patients with complications when compared to diabetic cases without complications, which is in contrast to our study.

PLATELET LARGE CELL RATIO:

The P-LCR parameter is generated by only a few machines, with the Sysmex analyser being one of them. It is not often quoted in literature, probably because it is relatively a new PVI parameter. The value of P-LCR observed in our study in diabetic group was 35.0±8.1% compared to non diabetic group where it was 23.0±2.4%.

TABLE 24 : COMPARISON OF PLATELET LARGE CELL RATIO WITH OTHER STUDIES

PUBLICATION	DIABETIC CASES	P-LCR (%)	CONTROLS	P-LCR (%)	*P* VALUE
Jindal *et al*[80]	75	42.31	50	36.93	0.004
Ashraf *et al*[96]	125	44.71	97	33.42	0.001
Present study	280	35.0	280	23.0	0.002

Our study concluded that P-LCR was significantly higher (*p* value 0.002) in diabetic cases than non diabetics. This is in agreement with the studies done by Jindal *et al*[80] and Ashraf *et al*[96] which concluded that P-LCR is significantly higher in diabetes compared to non diabetic cases.

Ashraf *et al*[96] study reflected a sequential disease burden measurement index of progressive diabetes visible by platelet parameters to distinguish controlled, controllable and uncontrolled diabetic population from non-diabetic population. They also evaluated biochemical parameters like HDL and triglycerides for possible link between platelet reactivity and these parameters, and observed that elevated triglycerides and decreased HDL levels are the best predictors of cardiovascular disease in patient with type 2 diabetes. Thus, larger platelets are more reactive and contribute to vaso-occlusive events in diabetic patient. Hence, P-LCR may serve to identify larger more active platelets during development of thromboembolic ischemic events in diabetic cases.

COMPARISON BETWEEN P-LCR WITH AND WITHOUT COMPLICATIONS WITH OTHER STUDIES

Higher P-LCR value was observed in diabetics with complications (35.9±8.3%) than in diabetics without complications (34.4±7.8%) which was not statistically significant in our study.

TABLE 25 : COMPARISON BETWEEN P-LCR AND COMPLICATIONS WITH OTHER STUDIES

PUBLICATION	DIABETIC CASES WITH COMPLICATIONS	P-LCR (%)	DIABETIC CASES WITHOUT COMPLICATIONS	P-LCR (%)	*P* VALUE
Jindal *et al*[80] (2011)	50	43.89	25	39.52	0.096
Present study	117	35.9	163	34.4	0.104

We observed in our study that higher levels of P-LCR was observed in patients of diabetes with complications but was not statistically significant. However, Jindal *et al*[80] observed a statistically significant higher P-LCR in diabetic patients with complications compared to diabetic cases without complications, which is in contrast to our study.

Our data suggest that increased platelet volume indices contributes to the prothrombotic state in diabetes mellitus and larger platelets may play a specific role in prothrombotic state. Because larger platelets are hemostatically more active, the presence of larger platelets, probably is a risk factor for developing vascular complications in diabetes. Platelets with larger platelets can be easily identified during routine hematological analysis because PVI are generated as by product of the automated blood counts. Thus, in conclusion PVI provides an important, simple, effortless and cost effective tool which can be useful in predicting an impending thrombotic state and vascular complications of diabetes.

CONCLUSION

The study was undertaken to determine whether an association exists between platelet indices-mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (P-LCR) in type 2 diabetes mellitus.

These indices are useful means of identifying larger and more active platelets which are risk factors for developing prothrombotic state in diabetes mellitus. P-selectin, GP IIb/IIIa, β-thromboglobulin are used as markers of platelet activation but these methods are costly and time consuming and need specialized equipment. Thus, they are unlikely to be useful in estimating the risk in large number of patients.

On the other hand, such patients can easily be identified during routine hematological analysis which are cost effective and easy method to evaluate platelet function, and possibly prevent the vascular complications and benefit from preventive anti-platelet treatment.

Our study observed that the platelet indices- mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR), relatively simple and inexpensive useful markers, were significantly elevated in patients of type 2 diabetes mellitus referred to our hospital. Therefore, it can be concluded that increased platelet size as measured by the platelet parameters may be one of the factors in the increased acceleration of atherothrombotic, micro- and macrovascular complications associated with diabetes mellitus.

SUMMARY

- This study was undertaken in Shri B.M.Patil Medical College, Vijayapur, Karnataka to study efficacy of platelet indices in Type 2 Diabetes Mellitus.
- A total of 560 cases were studied and divided further into two groups of 280 patients each, who were patients diagnosed with Type 2 Diabetes Mellitus and age and sex matched healthy controls.
- Majority of the patients diagnosed with Type 2 Diabetes Mellitus belonged to the 5th decade of life and mean duration of diabetes was 4.7±2.5 years.
- The total number of males in Type 2 DM group were 144 (51.43%) and females were 136 (48.57%) compared to non diabetic group where males were 171 (61.08%) and females were 109 (38.92%).
- In our study, the number of patients with diabetic complications were 117 (41.79 %) and 163(58.21%) diabetic cases did not present with any complications.
- In the present study, diabetic cases were divided in two groups. Group A comprised of 60 diabetic patients (21.43%) with HbA1c level <6.5% and Group B comprised of 220 (78.57%) diabetic cases with HbA1c level ≥ 6.5%.
- The mean of FBS was 158.1±33.7mg/dl (*p* value 0.002), RBS was 214.2± 42.1mg/dl (*p* value 0.002), PPBS was 235.6±38.5mg/dl (*p* value 0.005) and HbA1c level was 7.3±1.1% (*p* value 0.004) in diabetic patients which was significantly higher than non diabetics.
- The mean MPV in diabetic cases was 11.3± 1.0fl compared to the non-diabetics where it was 9±0.6fl, which was statistically significant (*p* value 0.004).

- The PDW was significantly higher in diabetic cases which was 14.2±2.5fl than non diabetics with PDW as 10.7± 0.7 fl (*p* value 0.003).
- The P-LCR recorded in our study (35±8.1%) was higher in comparison to the non diabetic group (23±2.4%), which was statistically significant (*p* value 0.002).
- In our study, mean MPV in group B (HbA1c ≥6.5%) was 11.7±1.0fl and in group A (HbA1c <6.5%) mean MPV was 11.1±1.2 fl, which was statistically significant (*p* value <0.05).
- In the present study, mean PDW and P-LCR in group B was 14.3±2.4fl and 35.6±7.7 %, which was significantly higher than group A with mean PDW being 13.5±2.7fl and P-LCR being 32.7±9.1% (*p* value <0.05).
- In our study, the mean MPV(11.5±1.4fl), PDW (14.4±2.7fl) and P-LCR (35.9±8.3%) in diabetic cases with complications were higher in comparison to diabetic cases without complications where mean MPV was 11.3±1.0fl, PDW was 13.9±2.1fl and P-LCR was 34.4±7.8%. The *p* value for MPV,PDW & P-LCR were however not statistically significant.

LIMITATIONS OF THE STUDY

- Follow up of the cases was not possible to determine the prognostic significance of our findings.
- Patients with qualitative disorders and reactive causes for raised platelets were not assessed that constitute a minor role.
- Platelet function tests could not be conducted on the sample to substantiate our findings further.

BIBLIOGRAPHY

1. Alberti KG, Zimmet PZ. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: Report of a WHO Consultation. Part 1. Diagnosis and classification of Diabetes mellitus. Geneva. Diabetic Med 1998;15:539–3.
2. Colwell J, Nesto R. The platelet in diabetes. Diabetes Care 2003;26: 2181-8.
3. Keating FK, Sobel BE, Schneider DJ. Effects of increased concentrations of glucose on platelet reactivity in healthy subjects and in patients with and without diabetes. Am J Cardiol 2003; 92:1362–5.
4. Mishra J, Shah P, Sanil R. Hematological disorders from The Kota Tribes of the Nilgris, India. Asian J Biochem Pharm Res 2012;2:156-62.
5. Masanori S, Tomohoro N, Koji N, Mutsuhiro K, Norinao H. Correlation between Mean Platelet Volume and fasting plasma glucose levels in prediabetic and normoglycemic individuals. Cardiovas Diabetol 2013;12: 12-14.
6. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R et al. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus2. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160–7.
7. International Expert Committee. International Expert Committee report on the role of the HbA1c assay in the diagnosis of diabetes. Diabetes Care 2009;32:1327– 34.
8. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res 2007;125:217-30.

9. Kusagur S, Gururaj K. J, Negalur N.Prevalence of Diabetic Retinopathy in Type II Diabetes in Relation to Risk Factors: A Hospital Based Study. J Evol Med Dent Sci 2014;3:513-21.

10. Gaikwad A, Kanitkar S, Kalyan M, Tamakuwala K, Agarwal R, Bhimavarapu B. Prevalence of type 2 diabetes mellitus in candidates contesting for municipal corporation elections in an urban industrialized town. Indian J Basic Appl Med Res 2014;3:412-18.

11. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–97.

12. American Diabetes Association.Diagnosis and Classification of diabetes Mellitus. Diabetes Care 2012;35:66-71.

13. Metzger B, Gabbe S, Persson B, Buchanan T, Catalano P, Damm P et al. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010;33:676–82.

14. Costa A, Bescós M, Velho G, Chevre J, Vidal J, Sesmilo G et al. Genetic and clinical characterisation of maturity-onset diabetes of the young in Spanish families. Eur J Endocrinol 2000;142:380-6.

15. Musso C,Cochran E,Ann Moran S,Skarulis M,Arioglu E,Taylor S et al.Clinical Course of Genetic Diseases of the Insulin Receptor Type A and Rabson-Mendenhall Syndromes:A 30-Year Prospective.Medicine 2004;83:209–22.

16. Kuzuya T, Matsuda A. Classification of diabetes on the basis of etiologies versus degree of insulin deficiency. Diabetes Care 1997; 20: 219–20.

17. Tuomi T, Carlsoon A, Li H, Isomaa B, Miettinen A, Nilsson A et al. Clinical and genetic characteristics of type 2 Diabetes with and without GAD antibodies. Diabetes 1999;48:150-7.

18. Chan J, Malik V, Jia W, Kadowaki T, Yajnik C, Yoon KH et al. Diabetes in Asia epidemiology risk factors and pathophysiology. J Am Med Assoc 2009;301:2129-40.

19. Gill RG, Haskins K. Molecular mechanisms underlying diabetes and other autoimmune diseases. Immunol Today 1993;14:49-51.

20. Poulsen M, Spinas GA, Prowse SJ, Hansen BS, Jorgensen DW, Bendtzen K et al.Islet cytotoxicity of interleukin-l. Influence of culture conditions and islet donor characteristics. Diabetes 1987;36:641-7.

21. Ozougwu JC, Obimba KC, Belonwu CD,Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol 2013;4:46-57.

22. Abdul-Ghani MA, Matsuda M, Jani R. The relationship between fasting hyperglycemia and insulin secretion in subjects with normal or impaired glucose tolerance. Am J Physiol Endocrinol Metab 2008;295:401–6.

23. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 2003;46:3-19.

24. Greenberg AS, McDaniel ML. Identifying the links between obesity, insulin resistance and β-cell function : potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. Eur J Clin Invest 2002;32: 24-34.

25. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–70.

26. Kohei K. Pathophysiology of Type 2 Diabetes and Its Treatment Policy. Jpn Med Assoc J 2010; 53:41–6.

27. Vinik A, Erbas T, Park S,Nolan R,Pittenger G.Platelet Dysfunction in Type 2 Diabetes.Diabetes Care 2004;24:1476-85.

28. Jokl R, Colwell JA: Arterial thrombosis and atherosclerosis in diabetes. Diabetes Metab Rev 1997;5:1–15.

29. Stratmann B,Tschoepe D. Pathobiology and cell interactions of platelets in diabetes. Diab Vasc Dis Res 2005;2:16–23.

30. Kakouros N,Rade J, Kourliouros A,Resar J.Platelet Function in Patients with DiabetesMellitus:From a Theoretical to a Practical Perspective. Int J Endocrinol 2011;12:1-13.

31. Vaidyula V,Rao A,Mozzoli M,Ho C,Cheung P,Boden G. Effects of hyperglycemia and hyperinsulinemia on circulating tissue factor procoagulant activity and platelet CD40 ligand. Diabetes 2006;55:202–8.

32. Wang Y, Beck W,Marshall S,Hoenich S,Thompson M.Advanced glycation end products elicit externalization of phosphatidylserine in a subpopulation of platelets via 5-HT2A/2C receptors. Am J of Physiol 2007;293:C328–C336.

33. Hers I. Insulin-like growth factor-1 potentiates platelet activation via the IRS/PI3Kalpha pathway. Blood 2007;110:4243–52.

34. Gawaz M, Ott I, Reininger AJ, Neumann FJ. Effects of magnesium on platelet aggregation and adhesion: magnesium modulates surface expression of glycoproteins on platelets in vitro and ex vivo. Thromb Haemost 1994;72:912–8.

35. Martina V, Bruno GA, Trucco F, Zumpano E, Tagliabue M, Di Bisceglie C. Platelet NOS activity is reduced in patients with IDDM and NIDDM. Thromb Haemost 1998;79:520–2.

36. Modesti PA, Fortini A, Gensini GF, Vanni D, Prisco D, Abbate R. Human prostacyclin platelet receptors in diabetes mellitus. Thromb Res 1991;63:541–8.

37. Livingstone C, McLellan AR, McGregor M, Wilson A, Connell JM, Small M. Altered G-protein expression and adenylate cyclase activity in platelets of noninsulin-dependent diabetic (NIDDM) male subjects. Biochem Biophys Acta 1991;1096:127–33.

38. Bono MD, O'Connell CJ, Nolan RD. In obesity, platelets are resistant to the inhibitory effects of insulin and prostacyclin. Diabetes 1996;45:65-9.

39. Anfossi G, Russo I and Trovati M. Platelet dysfunction in central obesity. Nutr Metab Cardiovasc Dis 2009;19:440–9.

40. Ferretti G, Rabini R,Bacchetti T. Glycated low density lipoproteins modify platelet properties: a compositional and functional study. J Clin Endocrinol Metab 2002;87:2180–4.

41. Freedman J.Oxidative stress and Platelets. Arterioscler Thromb Vasc Biol 2008;28:S11-S16.

42. Guthikonda S,Alviar L,Vaduganathan A. Role of reticulated platelets and platelet size heterogeneity on platelet activity after dual antiplatelet therapy with aspirin and clopidogrel in patients with stable coronary artery disease. J Am Coll Cardiol 2008;52:743–9.

43. Randriamboavonjy V, Fleming I. Insulin,Insulin Resistance, and Platelet Signalling in Diabetes. Diabetes Care 2009;32:528-30.

44. Matsuno H, Tokuda H, Ishisaki A, Zhou Y, Kitajima Y,Kozawa O. P2Y12 receptors play a significant role in the development of platelet microaggregation in patients with diabetes. J Clin Endocrinol Metab 2005; 90:920–7.

45. Guthikonda S, Lev EI, Patel R, DeLao T, Bergeron AL,Dong JF et al. Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. J Thromb Haemost 2007; 5:490–6.

46. Alberti G,Zimmet P,Shaw. International Diabetes Federation: a consensus on Type 2 diabetes prevention. Diabetic Medicine 2007;24:451–63.

47. Hu FB, Leitzmann MF, Stampfer MJ, Colditz GA, Willet WC, Rimm EB. Physical activity and television watching in relation to risk for type 2 diabetes mellitus in men. Arch Intern Med 2001;161:1542–8.

48. Saristo T, Peltonen M, Lindström J, Saarikoski L, Sundvall J, Eriksson J et al. Cross-sectional evaluation of the Finnish Diabetes Risk Score: a tool to identify undetected type 2 diabetes, abnormal glucose tolerance and metabolic syndrome. Diabetes Vasc Dis Res 2005;2:67–72.

49. Jain S,Gupta R,Gupta D,Jain M. A study on body mass index and its correlation with type 2 diabetes. Int J Res Med Sci 2014;2:1638-41.

50. Alberti G, Zimmet P, Shaw J. Metabolic syndrome—a new worldwide definition. A consensus statement from the International Diabetes Federation. Diabet Med 2006;23:469–80.

51. Bandyopadhyay R,Paul R,Basu A,Chakraborty P,Mitra S. Study of C Reactive Protein in Type 2 Diabetes and its Relation with Various Complications from Eastern India. J Appl Pharm Sci 2013;3:156-9.

52. Seshiah V,Balaji V,Balaji S. Scope for prevention of diabetes –focus intrauterine milieu interieur. J Assoc Physicians India 2008;56:109-13.

53. Manuel D, Schultz S. Health-related quality of life and health adjusted life expectancy of people with diabetes mellitus in Ontario, Canada, 1997. Diabetes Care 2004;27:407–14.

54. De Fronso RA, Ferrannini E, Keen H, Zimmet P. International Textbook of Diabetes Mellitus.3rd ed. Milan:John Wiley & Sons; 2004:1345–70.

55. Hu F, Manson J, Stampfer M. Diet, lifestyle and the risk of type 2 diabetes mellitus in women. New Engl J Med 2001;345:790–7.

56. Manneras HL, Leonhardt H, Kullberg J. Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. J Clin Endocrinol Metab 2011;96:304-11.

57. Eliasson B. Cigarette smoking and diabetes. Prog Cardiovasc Dis 2003;45:405-13.

58. Ramachandran L, Negi NS, Gupta B. Prevalence of hyperhomocysteinaemia in type-2 diabetes mellitus and its correlation with its complication. J Ind Acad Clin Med 2012;13:277-81.

59. Boke U,Kora S. Serum Ferritin Levels In Type II Diabetes Mellitus. J. App Med Sci 2013;1:472-5.

60. Ahmadieh H, Azar S, Lakkis N, Arabi A. Hypovitaminosis D in Patients with Type 2 Diabetes Mellitus:A Relation to Disease Control and Complications.Int J Endocrinol 2013;2:1-7.

61. Italiano JE, Gresele P, Fuster V,Lopez JA. The structure and production of blood platelets In:Platelets in Hematologic and Cardiovascular disorders.1st ed. New York:Cambridge University Press; 2008:1-20.

62. French JE. Blood Platelets: Morphological Studies on their Properties and Life Cycle. Br J Haematol 2008;13:595–603.

63. Broos K, Feys H, De Meyer S, Vanhoorelbeke K,Deckmyn H. Platelets at work in primary hemostasis. Blood Rev 2011;25:155–67.

64. Butarello M,Plebani M. Automated blood Cell Counts-State of the art. Am J clin Pathol 2008;130:104-16.

65. Lewis SM, Bain BJ, Bates I. Basic Hematological techniques In: Dacie and Lewis Practical Hematology.11th ed. China: Churchill Living Stone; 2006:26-56.

66. Kaito K, Otsubo H, Yoshida M, Tanno J, Kurihara E, Matsumoto K et al. Platelet size width,platelet large cell ratio, and mean platelet volume have sufficient sensitivity and specificity in the diagnosis of immune thrombocytopenia. Br J Haematol 2004;128:698-702.

67. Tejinder S. Automation in Cell Counts,Hemoglobin separation, Immunophenotyping and Coagulation In: Atlas and Text of Hematology. 3rd ed: Delhi: Avichal Press; 2014:53-79.

68. Corash L, Chen HY, Levin J. Regulation of thrombopoiesis: effect of the degree of thrombocytopenia on megakaryocyte ploidy and platelet volume. Blood 1987;70:177-85.

69. Osselaer JC, Jamrt J, Scheiff JM. Platelet distribution width for differential diagnosis of thrombocytosis. Clin Chem 1997;43:1072-6.

70. Kim KY, Kim KF, Kim KH. Mean platelet volume in the normal state and in various clinical disorders.Yonsei Med J 1986;27:219-26.

71. Greisenegger S, Endler G, Hsieh K, Tentschert S, Mannhalter C, Lalouschek W. Is Elevated Mean Platelet Volume Associated With a Worse Outcome in Patients With Acute Ischemic Cerebrovascular Events? J Am Heart Assoc 2004;35:1688-91.

72. Kanonidou C, Arampatzi S, Nikolaidou A, Tsavdaridou V, Diza E. Study On platelet indices in patients with autoimmune diseases. Rheumatol Int 2010;31:129-34.

73. Gasparyan AY, Kalinoglou AS, Mikhailidis DP, Toms TE, Douglas KJ. Platelet Function in Rheumatoid Arthritis:Arthritic and Cardiovascular Implications. Rheumatol Int 2010;31:153-64.

74. Ozturk ZA, Dag MS, Kuyumcu ME, Cam H,Yesil Y. Could platelet indices be new biomarkers for inflammatory bowel disease? Eur Rev Med Pharmacol Sci 2013;17:334-41.

75. Karagoz B, Alacacioglu A, Bilgi O, Demirci M, Ozgun A. Plaetlet count and platelet distribution width increase in lung cancer patients. Anatol J Clin Invest 2009;2:32-4.

76. Aksoy S, KilicKap S, Hayran M, Harputluoglu H, Koca E, Dede DS et al. Platelet size has diagnostic predictive value for bone marrow metastasis in patients with solid tumors. Int J Lab Hematol 2008;30:214-9.

77. Manchanda J,Potekar R,Badiger S,Tiwari A. The study of platelet indices in acute coronary syndromes. Ann Pathol Lab Med 2015;02:A31-A35.

78. Hekimsoy Z, Payzin B, Ornek T, Kandoğan G. Mean platelet volume in Type 2 diabetic patients. J Diabet Complications 2004;18:173-6.

79. Kodiatte TA, Manikyam UK, Rao SB, Jagadish TM, Reddy M, Lingaiah HK. Mean Platelet Volume in type 2 Diabetes Mellitus. J Lab Physicians 2012; l4:5-9.

80. Jindal S, Gupta S, Gupta R, Kakkar A, Singh HV, Gupta K, Singh S. Platelet indices in diabetes mellitus: indicators of diabetic microvascular complications. Hematology 2011;16:86-90.

81. Zaccardi F, Rocca B, Pitocco D, Tanese L, Rizzi A, Ghirlanda G. Platelet mean volume, distribution width, and count in type 2 diabetes, impaired fasting glucose, and metabolic syndrome: a meta-analysis. Diabetes Metab Res Rev 2015;31:402–10.

82. Zuberi BF, Akhtar N, Afsar S. Comparison of mean platelet volume in patients with diabetes mellitus, impaired fasting glucose and non-diabetic subjects. Singapore Med J 2008;49:114–6.

83. Demirtunc R, Duman D, Basar M, Bilgi M, Teomete M, Garip T. The relationship between glycemic control and platelet activity in type 2 diabetes mellitus. J Diabet Complications 2009;23:89-94.

84. Shah B, Sha D, Dawei X. The Relationship between Diabetes, Metabolic syndrome and Platelet Activity as measured by Mean platelet volume. Diabetic Care 2012;35:1074-8.

85. Jagroop IA, Clatworthy I, Lewin J, Mikhailidis DP. Shape change in human platelets: measurement with a channelyzer and visualization by electron microscopy. Platelets 2000;11:28-32.

86. Vagdatli E, Gounari E, Katsibourlia E, Tsikopoulou F, Labrianou I. Platelet distribution width:a simple,practical and specific marker of activation of coagulation. Hippokratia 2010;14:28-32.

87. Lt Gen Mehta SR, Col Kashyap AS, Lt Col Das S. Diabetes Mellitus in India: The Modern Scourge. Armed Forces Med J India 2009;65:50-4.

88. Mowafy N, Metwaly E, Hashish B, Bazeed M. A study of the value of some platelet parameters in patients with type 2 diabetes mellitus. Al-Azhar Assiut Med J 2015;13:13-8.

89. Akinsegun A, Olusola D, Sarah J,Olajumoke O, Adewumi A,Majeed O et al. Mean platelet volume and platelet counts in type 2 Diabetes: Mellitus on treatment and non-diabetic mellitus controls in Lagos, Nigeria. Pan African Med J 2014;18:1-5.

90. Ozder A, Eker H. Investigation of mean platelet volume in patients with type 2 diabetes mellitus and in subjects withimpaired fasting glucose: a cost-effectivetool in primary health care. Int J Clin Exp Med 2014;7: 2292-7.

91. Ulutas K, Dokuyucu R, Sefil F, Yengil E, Sumbul A, Rizaoglu H et al. Evaluation of mean platelet volume in patients with type 2 diabetes

mellitus and blood glucose regulation: a marker for atherosclerosis. Int J Clin Exp Med 2014;7:955-61.

92. Papanas N, Symeonidis G, Maltezos E, Mavridis G, Karavageli E, Vosnakidis T et al. Mean platelet volume in patients with type 2 diabetes mellitus. Platelets 2004;15:475-8.

93. Demirtas L, Degirmenci H, Akbas E, Ozcicek A, Timuroglu A, Gure A et al. Association of hematological indicies with diabetes, impaired glucose regulation and microvascular complications of diabetes. Int J Clin Exp Med 2015;8:11420-7.

94. Jabeen F, Rizvi H, Aziz F, Wasti A. Hyperglycemic induced variations in Hematological Indices in Type 2 Diabetics. Int J of Adv Res 2013;1: 322-34.

95. Dalamaga M, Karmaniolas K, Lekkab A, Antonakosa G, Thrasyvoulides A, Papadavid E et al. Platelet markers correlate with glycemic indices in diabetic, but not diabetic myelodysplastic patients with normal platelet count. Dis Markers 2010;29:55–61.

96. Ashraf S, Ranjan R, Singh S, Singh H, Kudesia M, Sharma R. Diabetes Disease Burden by Platelet Indices As Possible Biomarkers in Evaluation of Initial Vascular Risks in Grading Diabetes Mellitus: Correlation of Platelet Dysfunction Indices With Hematopoietic and Biochemical Biomarkers in Diabetes mellitus. Open J Biochem. Forthcoming 2015.

ANNEXURE-I

Institutional Ethical Committee
BIJAPUR-586 103.
OUTWARD
No. 50/13
Date 14-11-13
BLDE U's Shri B.M. Patil Medical College,

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Study of platelet indices in type 2 diabetes Mellitus patients"

— x — x —

Name of P.G. student Dr. Kumari Shilpi,
Department of Pathology

Name of Guide/Co-investigator Dr R.M. Patekar.
Professor of Pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-II

SAMPLE INFORMED CONSENT FORM

TITLE OF THE PROJECT	**:**	STUDY OF PLATELET INDICES IN TYPE 2 DIABETES MELLITUS PATIENTS.
PRINCIPAL INVESTIGATOR	**:**	Dr. KUMARI SHILPI P.G. DEPARTMENT OF PATHOLOGY
P.G.GUIDE	**:**	Dr. R.M.POTEKAR M.D, PROFESSSOR DEPARTMENT OF PATHOLOGY

PURPOSE OF RESEARCH: I have been informed that this study is done to know the efficacy of platelet analysis in assessing the prognosis of Diabetes mellitus.

PROCEDURE: I understand that my blood sample will be drawn from my forearm using a 5ml syringe in an EDTA vacutainer and given for complete blood count analysis.

RISK AND DISCOMFORTS: I understand that, there is no risk involved in the procedures performed.

BENEFITS: I understand that my participation in the study will help to know the prognosis of diabetes mellitus.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If the data is used for publications the identity of patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any time. I also understand that **Dr**. **KUMARI SHILPI** may terminate my participation in the study after she has explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

I have explained the patient the purpose of the study, the procedure required and possible risk and benefit to the best of my ability in the vernacular language.

_____ _____

Dr. KUMARI SHILPI Date

(Investigator)

STUDY SUBJECT CONSENT STATEMENT:

I confirm that **Dr. KUMARI SHILPI** has explained to me the purpose of research, the study procedure, that I will undergo and the possible discomforts as well as benefits that I may experience in my own language. I have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give consent to participate as a subject in this research project.

_____ _____

(Participant) Date

_____ _____

(Witness to signature) Date

ANNEXURE III

PROFORMA FOR THE STUDY OF PLATELET INDICES

NAME	:	OP/IP No.	:
AGE	:		
SEX	:	D.O.A	:
RELIGION	:	D.O.D	:
OCCUPATION	:		
RESIDENCE	:		
Presenting Complaints	:		
Past history	:	Hypertension, History of drug intake, Diabetes Mellitus.	
Personal history	:		
Family history	:		
Treatment history	:		

General physical examination:

Pallor	present/absent
Icterus	present/absent
Clubbing	present/absent
Lymphadenopathy	present/absent
Edema	present/absent
Built	poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

Haematological investigations:

Parameters	
WBC	
RBC	
HGB	
HCT	
MCV	
MCH	
MCHC	
PLATELETS	
LYMPHOCYTES(%)	
EOSINOPHILS (%)	
NEUTROPHILS(%)	
MONOCYTES (%)	
RDW	
PDW	
MPV	
P-LCR	

Peripheral Smear Examination:

RBC:

WBC:

PLATELETS:

IMPRESSION:

Biochemical Investigations:

i. RBS

ii. FBS

iii. PPBS

iv. HbA_1C

KEY TO MASTER CHART

MPV	Mean Platelet Volume
PDW	Platelet Distribution Width
P-LCR	Platelet Large Cell Ratio
PLT	Platelets
FBS	Fasting Blood Sugar
RBS	Random Blood Sugar
PPBS	Post Prandial Blood Sugar
HbA1c	Glycosylated hemoglobin
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
Hb	Hemoglobin
DUR	Duration
YRS	Years
COMP	Complications
0	No Complications
1	Complications

MASTER CHART

S.NO.	NAME	AGE	SEX	MPV	PDW	P-LCR	PLT	Hb	MCV	MCH	MCHC	Hb A1c	FBS	RBS	PPBS	DUR. OF DIABETES (YRS)	COMP.
1	ANAND	51	M	10.9	11.7	30.9	3.32	13	83.5	27.1	32.4	7.5	149	210	228	5	0
2	SIDAPPA	49	M	8.7	9.1	15.5	3.02	13.1	71.4	20.1	28.1	6.2	138	190	236	3	0
3	BHIMAPPA	55	M	8.8	8.4	14.8	6.2	13.6	73.4	23.4	31.9	8.3	224	280	326	4	1
4	DAVALSAB	50	M	10	11	22.1	3.83	15.6	80	27.6	34.7	6.2	130	190	200	5	0
5	RAMAPPA	52	M	11.7	14.7	40	2.86	15	79.9	27.6	34.8	6.5	130	200	208	3	1
6	RAJU	47	M	10.8	13	25.7	2.96	15.7	85.9	28.8	33.5	9.2	160	300	288	6	1
7	MAHADEV	55	M	11.1	13.4	34.6	2.79	13	85.1	28	32.9	7.8	140	200	265	5	0
8	PADMA	55	F	10.4	12.5	28.4	1.8	13	90.3	30	33.3	7.5	160	210	230	6	0
9	RAMAGONDA	57	M	11.8	14.8	34	2.52	15.2	79.4	26.3	33.3	9.8	144	190	207	6	1
10	MUKUND	48	M	13.1	14	40.5	3.51	14.5	85.4	28.3	33.2	9.6	196	240	380	5	1
11	SIDDRAMAPPA	54	M	10.6	12.8	29.5	1.89	13	95.6	31.1	32.5	6.8	130	200	180	5	0
12	GEETHA	52	F	11.6	14.9	40	2.41	12	80.4	25.4	32	7.2	143	210	200	3	1

13	SHRISHAIL	50	M	10.8	11.9	27.8	2.43	12.8	79.2	23.7	30	10	236	270	280	4	0
14	DEVERAMMA	55	F	9.5	9.5	20.1	2.64	12	78.7	26.3	33.4	5.8	140	210	230	4	0
15	DRAKSHARI	49	F	11.5	14.5	34.4	3.09	15.8	95.5	30.7	32.2	6.9	134	160	250	6	1
16	RAMAPPA	57	M	11.3	15.2	35.5	3.53	15.4	82.9	28.4	34.2	9.8	136	210	240	5	1
17	M.BHARIKUTTI	58	M	12.2	15.2	32.4	2.16	15	85.2	29.6	34.8	8.4	130	190	200	3	0
18	SADHAKA	53	F	9.7	10.6	23	2.74	12.8	82.7	28.9	35	8.6	146	220	190	3	0
19	SIDRAM	50	M	11.8	14.5	32.6	3.26	14.4	85.8	26.9	31.4	7.2	140	200	210	3	1
20	VEERAPPA	58	M	11.7	14.8	35.4	3.41	15.7	80.6	26.7	33.1	8.2	180	208	292	6	1
21	Y.KAMBALI	56	M	11.2	14	36.7	2.1	15	81.9	26.9	32.9	9.2	220	320	348	7	1
22	GANAPATHI	61	M	9.3	9.7	19.7	3.38	14.7	75.4	24.5	32.5	6.8	195	260	311	5	1
23	BHARATI	48	F	12.1	14.5	38.8	3.41	13	77.9	25.8	33.2	7	148	210	218	2	0
24	LALEETHA	48	F	9.5	9.6	20.2	4.93	12.1	72.2	22.8	31.6	8.4	200	290	409	9	1
25	SAROJINI	47	F	12.6	14.3	32.6	4.09	13.2	76.4	23.9	30	6.5	140	190	200	5	0
26	SHAKUNTALA	50	F	11.5	14.7	38.8	2.17	14	87.9	29.3	33.3	8.8	130	180	226	3	0
27	REKHA	53	F	11.8	14.6	44.3	4.09	13.2	85.3	27.9	32.8	8.4	210	280	290	5	1
28	SHARADA	51	F	11.4	14.5	35.8	5.71	13.3	82	25.5	31.1	8.6	180	200	220	4	0
29	KAMALABAI	55	F	11.8	14.1	34.4	2.22	13.6	92.4	30.2	32.7	8.4	160	200	210	3	1
30	SHARANAPPA	57	M	12.5	16.3	44.6	2.16	13.3	81.2	26.1	32.1	9.2	142	190	200	4	1

31	SANGAPPA	54	M	11.1	14	38.1	3.93	15.1	83.7	28	33.5	7.8	130	196	213	3	0
32	SIDESHWAR	52	M	11	13.9	32.2	2.93	13.8	77.9	26.7	34.2	8.2	160	200	208	5	0
33	JANUBAI	53	F	11.1	14.1	34.1	3.12	12	72.8	25.8	32.2	8.8	168	190	263	6	1
34	SANGAMMA	51	F	11	14.2	34.4	3.45	12.5	80.5	26.8	33.2	7.8	172	194	205	5	0
35	ANNAPURNA	50	F	10.8	13.9	30.1	3.19	12.3	75.7	24.4	32.1	8	148	190	300	10	1
36	MAHADEVI	58	F	12.8	18	46	1.9	13	83.4	27.3	32.7	7.8	144	180	271	8	1
37	VITTAL	52	M	11.6	15.4	39.6	2.88	15.1	85.6	29.4	34.3	9.2	240	231	354	7	1
38	YALLAWWA	53	F	11.3	13.8	39.7	3.72	12.2	81.9	26	31.7	6.8	136	200	231	8	0
39	B.S.BIRADAR	55	M	11.9	14.9	40.2	2.01	13.1	88.3	28.4	32.2	8.2	200	280	257	6	1
40	GURUBAI	49	F	9	9.6	16.8	3.07	13.3	86.3	27.7	32.1	5.8	160	190	219	5	0
41	NINGANAGOUDA	60	M	11.8	15.1	39.9	2.17	14.8	87.1	29.3	33.6	7.8	198	300	288	9	1
42	RATNABAI	63	F	12.3	17.9	38.2	3.69	12	72.4	26.2	32.8	9.2	216	240	292	8	1
43	MAHADEVAMMA	52	F	11.9	16	38	2.44	12.8	88.4	28.4	31.7	6.8	160	210	215	4	0
44	CHANDANA	53	F	11.7	14.3	32.2	2.51	12	81.1	27.6	34	6.2	128	190	226	5	0
45	HUSSEINIBI	57	F	11.9	15.8	42	2.55	12.1	76.7	23.5	31.1	6.4	160	210	251	5	1
46	KULLAGOUDA	54	M	11.6	15.6	36.6	1.78	14.1	79.6	26.1	32.8	6.5	148	180	210	4	0
47	B.S.MASALI	55	M	11	13.8	34.6	2.35	13	85.2	28.6	33.6	6.8	130	200	264	6	1
48	GURUSIDAPPA	58	M	11.2	14.9	48.6	1.96	13.8	95.4	30.7	32.1	7.2	160	210	256	7	1

49	LAXMIBAI	57	F	12.2	15.9	42.2	1.84	12	84.4	27.4	32.5	6.6	140	200	219	8	0
50	SUNANDA	56	F	11.4	15.7	34.2	2.21	12.1	75.7	25.7	32.6	8.4	224	280	292	6	1
51	KAMALA	54	F	12.5	15.6	41.1	4.13	12.2	85.4	26.6	31.1	7.6	180	216	243	2	0
52	SUJATA	52	F	9.4	10.8	21.3	5.07	12.5	77.1	26.8	30	7.4	130	210	221	4	0
53	SARASWATI	49	F	11.2	14.6	36.4	2.79	13.3	82.2	26.9	32.7	8.6	196	218	258	2	0
54	J.P.KAPPARAD	50	M	12.4	14.8	31.6	4.17	13.8	79.7	25.9	33.3	6.2	148	180	259	6	1
55	RAMSINGH	55	M	11.2	14.2	42.4	2.6	14.7	90.7	31.8	35.1	8.8	202	220	278	8	1
56	SUSALABAI	56	F	10.3	12.1	27.7	2.99	13.2	78.5	26.3	33.5	6.1	160	200	221	4	0
57	TUKARAM	52	M	9.7	10.5	22.7	2.84	15.5	79.1	26.7	33.8	5.5	136	180	237	4	0
58	SARASWATI	53	F	12.1	15.2	37.3	4.02	14.4	82.5	27.6	33.5	6.5	130	196	254	5	1
59	M.F.PADAGAN	51	M	9.5	10	20.1	4.47	12.7	85.3	27.8	32.6	8.6	220	290	316	5	1
60	MUTTUSWAMI	49	M	11.7	15.8	32.8	2.96	15	84.1	30.5	36.2	8.8	241	320	225	4	0
61	SHIRINGAPPA	50	M	12.2	15.7	38.2	2.94	14.1	73.4	23.4	31.9	7.8	190	210	299	7	1
62	NEELAKANTAPPA	54	M	9.8	10.8	23.2	2.35	14.3	88.2	29.1	32.9	5.8	120	190	227	3	0
63	PARANGONNDAPA	59	M	13	15.9	38.5	2.09	13.6	86.4	30.4	35.1	6.8	140	200	278	10	1
64	CHANNA BATOYYA	50	M	11.9	14.6	44	2.45	15.6	84.5	27.8	32.9	8.2	190	210	229	2	0
65	SHANTABAI	47	F	9.4	9.9	19.7	3.66	12.3	79.5	24.4	30.7	9.8	245	300	315	7	1
66	MINAXI	48	F	10.1	11.5	26	4.04	12.7	81.4	24.7	30.4	6.8	152	186	231	5	1

67	SUJATHA	55	F	11.7	15	37.9	2.23	13.1	90.2	30.5	33.8	7	150	194	242	2	0
68	VACHALABAI	56	F	9.1	9.2	17.2	3	12	85.3	25.3	32.1	8.6	222	241	273	10	1
69	RAKMABAI	55	F	9.7	9.8	21.2	1.5	12	87.2	29.9	34.2	6.5	130	184	234	2	0
70	PUSHPA	52	F	12.2	16.3	43	2.92	12.8	80.5	26	32.2	8.2	184	242	269	4	0
71	SUJALA	51	F	10.3	12.3	28.8	2.03	12.1	92.8	31.1	33.5	6.8	146	194	236	4	0
72	VJAYALAKSHMI	52	F	9.6	10.4	22.4	2.94	12.3	86.3	26.7	32.8	6.6	132	188	216	2	0
73	S.M.JAGUR	51	M	11.8	13.2	30.4	2.65	14.3	80.5	27	33	7.1	152	210	238	5	1
74	SHANTAMMA	54	F	10.2	11.4	26.9	1.89	12	91.2	30.5	33.5	5.6	120	186	300	6	1
75	GURUSABAPPA	55	M	9.2	8.8	16.9	3.8	14.2	75.9	25.3	33.3	7.2	180	220	260	7	1
76	YAMUNADEVI	57	F	11.3	14.1	35	1.8	12	84.9	28.6	33.7	6.9	176	212	241	2	0
77	SUNDRAMA	58	F	10.1	11.1	25.1	3.11	12.7	86.5	29	33.5	5.8	124	176	234	3	0
78	CHANDRAKANTH	51	M	11.2	14.2	33.7	2.47	13.2	82.5	27.7	34.4	7.6	162	202	277	7	1
79	SHARANAMMA	58	F	11.1	14.8	34.2	2.54	12	81.5	28	34.3	6.9	186	226	244	8	1
80	BHAGAMMA	51	F	11.6	14.9	37.5	1.63	12.1	84.1	28.1	33.4	6.8	148	196	257	3	0
81	VAISHNAVI	53	F	11.3	15.3	36.3	1.5	12.4	84.7	28.2	33.3	7.6	152	202	246	2	0
82	TAZEN	50	M	11.7	14.3	37.6	3.22	13.1	72	23.5	32.7	8.4	168	212	251	4	0
83	SHIVANI	47	F	10.2	11.3	26.4	2.03	12	87.5	29	33.2	6	122	182	248	2	0
84	SANGEETA	47	F	11.2	13.1	33.7	3.18	12.2	84.7	28	34.3	6.4	138	198	231	1	0

85	SHEHNAAZ	53	F	9.4	10.9	20.1	1.5	12.4	82	26.4	32.2	7.4	164	218	250	3	0
86	RAMIYA	51	F	10.6	11.3	28.9	3.34	13.1	91.1	31.3	34.4	7.4	150	198	219	2	0
87	RANJEET	54	M	11.8	14.3	32.9	1.68	17.4	86.6	28.3	32.7	6.5	146	188	252	2	0
88	RAMANAGOUDA	56	M	11.1	14.5	33.4	3.32	13	83.2	28.5	34.2	6.6	140	190	267	7	1
89	SUNITABAI	52	F	12.7	16.6	46.9	2.95	12.2	85.6	28.8	33.6	8.8	180	242	254	8	1
90	MALLAMMA	51	F	12.7	17.3	47	2.8	12.4	85.7	28.7	33.5	7.8	176	212	278	10	1
91	SUKHADEVI	57	F	12.6	16.4	45.6	2.64	13.3	85.5	28.6	33.4	6.9	160	210	256	6	1
92	BHAGIRATHI	54	F	12.6	17.3	44.8	2.22	13.3	85.6	28.5	33.3	9.8	234	324	236	3	0
93	GANGABAI	57	F	11.2	13.5	35.2	2.9	12.5	80.5	24.6	31.8	9.4	200	302	258	4	0
94	SAIRABANU	58	F	11.8	15.2	32.6	3.27	13.2	83.8	27.8	33.2	8.2	178	238	261	6	1
95	MADHU	54	F	10.3	11.6	27.4	3.2	13.3	83.5	28.1	33.6	6.5	134	178	256	2	0
96	BHIMARAY	55	M	11.7	16.7	38.8	1.65	18	85.5	28.9	33.8	9.2	261	344	300	5	1
97	BHAGAPPA	51	M	11.8	14.3	33	1.34	17	80.1	26.4	33	6.4	132	182	262	3	0
98	VIJENDRA	52	M	11	14	33.9	2.1	15	83.8	26.8	32	7	156	200	257	4	0
99	KALYANI	53	F	10.4	11.7	27.7	3.26	15.1	85.2	29.8	35	6.2	130	188	264	5	0
100	IRANNA	55	M	12.4	16.2	37.2	1.9	14.3	85.2	29.1	34.1	7.2	164	234	276	5	1
101	MADHAVI	52	F	11.2	14.9	35	2.91	16	84.8	26.7	31.9	8.2	188	244	266	5	0
102	RAGHAVENDRA	56	M	11	14	33.1	2.86	16	83.6	26.9	32.2	8	180	238	235	3	0

103	HIMANSHU	47	M	11.6	14.6	34.2	2.28	14.7	93.6	32.2	34.4	9.2	198	256	277	3	0
104	HEMANTH	50	M	11.7	15	41.3	2.37	14.1	93.5	32.6	34.9	8.4	164	244	269	5	1
105	RAJU	51	M	11.5	14.9	36.3	2.58	15.1	88.3	30	34	8.6	166	258	261	2	0
106	LALITA	49	F	9	12	19.5	1.5	15.2	74.4	21.7	30.1	6.5	132	202	272	2	0
107	GEETA	48	F	11.6	15.3	39.2	2.75	12	77.7	20.5	32.3	8.8	186	216	234	1	0
108	SHIVAMMA	53	F	11	13.4	32.4	1.79	13.5	88.5	29.3	33.2	8.6	170	210	245	3	0
109	SAVITA	56	F	12.2	17.8	41.9	2.76	12	72	23.2	31.4	9.4	200	280	284	9	1
110	KASHINATH	56	M	10.1	11.1	24.7	3.32	12.9	83.2	28.5	34.2	10	128	234	300	5	1
111	SUNITABAI	55	F	12.7	16.6	46.9	2.95	12.6	85.6	28.8	33.6	6.2	164	200	284	3	0
112	MALLAPPA	55	M	12.7	17.3	47	2.8	12	85.7	28.7	33.5	6.7	128	200	216	6	1
113	DEVAKI	50	F	12.6	16.4	45.6	2.64	12.8	85.5	28.6	33.4	6.8	134	194	234	3	0
114	KAVERY	45	F	12.8	17.3	44.8	2.22	12.4	85.6	28.5	33.3	7.2	128	210	276	3	0
115	DUNDAMMA	58	F	11.2	13.5	35.2	2.9	12	77.5	24.6	31.8	6.9	156	234	300	5	1
116	MAHANANDA	44	F	10.4	11.8	27.7	3.27	13.2	83.8	27.8	33.2	5.8	144	196	198	2	0
117	PARUBAI	49	F	10.3	11.6	27.4	3.2	13.3	83.5	28.1	33.6	6	140	188	200	1	0
118	GAURIDEVI	47	F	11.7	16.7	38.8	1.5	15.4	85.5	28.9	33.8	6.8	154	222	294	8	1
119	MAHESHWARI	50	F	10.5	9	28.7	1.8	12.4	93	32.1	33.6	6.8	150	198	186	3	1
120	GATEWWA	58	F	11.8	14.1	38	1.9	12.1	82	27.9	34	7	184	212	220	3	1

121	ASHOK	60	M	12.2	13.7	35.3	2.26	15.3	81	28.2	34.9	7.2	200	234	300	10	1
122	DRANAPPA	54	M	11.5	13.6	35.4	1.84	13.9	89.2	29	32.5	7	156	222	254	6	1
123	MALLASIDDA	50	M	12.2	13.2	34.1	2.23	15.1	81.1	28	34.5	6	144	188	202	2	0
124	DUNDAPPA	56	M	11.4	12.2	35.2	1.89	13.8	88.8	28.5	32.1	6.4	138	180	176	4	1
125	SHAKUNTALABAI	55	F	10.2	11.4	26.9	1.89	12.5	91.2	30.5	33.5	5.9	128	178	188	4	1
126	SHANTEWWA	60	F	11.3	13.7	35.2	2.95	15.4	82.1	27.5	33.5	6.8	168	216	224	8	1
127	BASAVRAJ	56	M	12.2	15.5	43	3.33	12.7	97.6	30.5	31.2	6.2	140	196	200	2	0
128	YANKAMMA	62	F	11.4	12.7	35.8	1.77	11.7	81.2	27.5	33.8	6.9	164	212	312	8	1
129	BABU	50	M	9.6	12	23.7	1.84	10.2	64.4	18.9	29.3	5.8	146	188	200	2	0
130	KIRAN	39	F	9.2	9.9	18.4	2.65	13	91.2	29.1	31.9	6	130	144	168	1	0
131	SOUDATTI	52	F	11.1	13.1	32.1	1.85	13.5	88.5	29.4	33.3	6.8	134	198	212	3	1
132	BAGAWWA	43	F	11.5	14.5	38.5	2.78	12.3	84.2	20.6	30.5	6	126	168	170	1	0
133	NINGAPPA	55	M	10.6	11.9	29	2.08	17.8	88.5	30	33.9	6.2	138	200	212	1	0
134	MADIWALLAMMA	58	F	10.9	12.5	31.9	2.38	12.1	79.4	27.8	35	6.8	160	200	218	5	1
135	NAGARJUN	48	M	11.9	14.6	41.2	2.87	13	81.3	25	30.8	7	148	198	220	3	0
136	BASAMMA	60	F	11.8	14.9	40.2	3.25	12.6	99	30.6	30.9	8.3	186	242	300	10	1
137	SHANTA	46	F	11.2	13.8	34.9	2.61	15.6	86.1	28.6	33.3	6.5	132	168	198	2	0
138	GEETABAI	42	F	10.8	13	31.6	2.14	15.9	92.6	31.9	34.5	6	134	160	198	2	0

139	SIDDARAM	54	M	11.4	14.2	36.1	2.7	15.7	86.3	28.8	33.3	6.8	142	188	208	3	0
140	JAYAPRAKASH	60	M	10.9	12.6	31.7	2.01	15.9	92.8	31.9	34.3	7.2	140	224	268	5	1
141	SADANNA	55	M	11.1	13.3	33.7	2.7	15.6	86.4	28.7	33.2	7	138	198	234	3	0
142	SHANTA KUMBHAR	56	F	12	15.7	40.4	2.88	13	88.9	28.8	32.3	7.6	146	212	246	2	0
143	BASAVRAJ DESAI	50	M	11.1	13.8	33.7	2.19	13.6	105.2	28.8	27.1	8	168	212	268	7	1
144	SIDDALINGAWWA	70	M	12.6	21.8	44.1	1.35	12.4	86	26.9	31.3	9.1	168	278	308	15	1
145	MAHADEVAPPA	48	M	11.6	17.7	39.7	1.5	12.3	86.5	26.6	30.7	6.8	134	168	188	2	0
146	SIDAPPA	52	M	11.3	14.2	35.2	2.95	15.3	82	27.2	33.2	6.4	138	168	178	4	0
147	LALABI	40	F	10.3	12.1	27.8	3.12	12	80.4	25.7	30.4	6	134	158	180	2	0
148	KANNAPPA	56	M	9.6	10.4	21.7	2.14	13.9	88.3	28	31.7	6.3	148	198	212	4	0
149	SUCHITRA	44	F	11.8	14.1	37.3	2.48	12.7	90.4	30.5	33.8	6.8	152	188	200	2	0
150	SULOCHANA	55	F	11.6	13.7	36.3	2.42	12.7	90.6	30.6	33.8	6.2	148	198	212	8	1
151	WAREPPA	65	M	11.1	13.8	34.7	2.08	15.4	80.8	27.1	33.5	7.8	168	206	254	6	1
152	NEELAMMA	42	F	13	17	49.4	2.01	12	83.8	26.9	32.1	5.8	136	140	198	1	0
153	PUTLABAI	54	F	11.1	13.8	34.8	3.36	14	82.8	26.7	32.3	7	154	198	234	2	0
154	MUKUNDRA	48	M	8.6	8.9	14.4	3.19	15.2	81.4	27.2	33.4	6.9	142	184	206	1	0
155	SUDHAKINI	58	F	9.3	9.5	19.4	2.77	12.8	83.5	29.6	35.4	6.3	152	198	214	4	0
156	IQBAL	54	M	9.1	10.4	18.4	3.1	14.2	85.6	29.6	34.5	7	138	200	216	3	0

157	HANAMMANTH	56	M	12.8	15	43	4.28	12.1	82	26.1	31.6	7.5	146	210	230	5	0
158	ABDULLAH	62	M	12.6	17	45.9	3.94	13.2	86.6	25.2	29.1	7.2	142	190	236	10	1
159	CHANABASAMMA	56	F	12.8	18.1	46.2	1.76	13.1	98	31.5	29.2	8.3	224	280	326	4	0
160	BHIMANAGOUDA	48	F	11.2	13.7	33.5	1.87	13.6	94.3	28.3	30.7	6.2	130	170	200	3	0
161	BHIMSHANKAR	52	M	10.7	12.6	31.6	3.37	12.9	81.3	28.7	35.3	6.5	130	198	208	7	1
162	PRASHANT	48	M	13.2	20.2	48.2	1.8	12.4	91.1	26.6	29.2	7	132	167	198	3	0
163	PARVATI	52	F	10.9	14.7	33.3	1.56	12.5	81.4	25.8	31.6	7.8	140	200	265	8	1
164	PRAVEEN	54	M	11.1	12.8	34.3	1.93	13	86.2	29	33.6	7.5	160	210	230	4	0
165	CHANDRASHEKHAR	58	M	11.3	12.7	33.9	1.62	13.1	85.3	29.5	34.6	8.4	144	190	207	6	1
166	MUKANDAPPA	60	M	9.6	10.2	21.6	4.47	14.1	100	34.8	31.8	9.6	196	262	380	5	1
167	KALPANA	41	F	12.1	13.7	31.2	2.52	13.4	89.1	29.3	32.9	6.8	130	198	180	5	0
168	BARASAPPA	63	M	11.5	13.4	37.1	1.93	13	86.3	29.1	33.3	7.2	143	210	234	5	1
169	SUDHAMMA	52	F	12.7	19.6	47.8	1.9	12.4	83.4	27.3	32.7	10	236	270	280	4	0
170	ZARINA KANDAGAL	55	F	11.9	14.6	38.6	2.9	12	86	26.7	31.1	5.8	140	158	200	4	0
171	KUSUM CHOUGALE	56	F	11.3	13.2	34.8	2.74	12.2	91.9	28.4	30.9	6.6	134	160	188	6	0
172	SHAVARAMMA	60	F	12.3	15.4	44	2.13	14.9	86.4	26.8	31	9.8	136	210	268	9	1
173	SHARADA	44	F	12.2	16	43.1	2.17	14.9	86.5	26.8	31	8.4	130	182	200	3	0
174	VIRUPRAKASH	45	M	12.1	13.6	38.6	1.5	12.2	88.9	28.8	32.4	8.6	146	212	190	3	0

175	BHIMA PATIL	64	M	11.7	14.3	38	1.8	12.2	89.4	28.7	32.1	7.2	140	200	260	7	1
176	DANDIRAM	59	M	10	11.3	23.8	2.43	13.6	81.5	27.6	33.9	8.2	180	216	292	6	1
177	NEELAWWA	75	F	10.3	11.2	27.2	4.8	12	81.7	26.7	31.4	9	220	312	346	7	1
178	GURAPPA	51	M	10.5	11.6	28.2	1.56	12.5	81	25.1	31	6.8	144	198	221	5	1
179	SHIVAPPA	53	M	11	13.2	36.3	1.6	12.1	85.8	27.6	32.2	7	148	186	198	2	0
180	SURAMMA	57	F	12.9	18.8	47.6	2.34	14.8	82.5	27	32.7	8.4	145	184	268	2	0
181	ABHISHEK	49	M	12.4	16.6	45.1	4.08	13.1	86	25	29	6.5	140	186	198	5	0
182	M.BHIMAPPA	59	M	13	16.7	46.5	1.71	13.6	104	31.9	29.6	8.8	130	180	234	3	0
183	SHIVENDRA	43	M	10.8	12.7	32.6	3.46	12.9	80.9	28.3	35	5.8	142	234	212	3	0
184	SOUJANAYYA	44	F	11.9	14.6	39.8	2.1	12.3	83.7	26.1	31.2	8.6	160	184	210	3	0
185	INDRABAI	51	F	12.2	18.8	44	2.64	12.6	84.7	25.6	29.7	8.4	160	200	237	6	1
186	PARASURAM	61	M	10.4	12	28.1	2.54	12.3	81.6	25.6	30.9	9.2	142	190	275	9	1
187	UMESH	41	F	10.7	12.2	29.9	2.49	14.5	82.9	26.9	32.4	7.8	130	196	209	3	0
188	BASANTRA	57	M	11.8	14.8	38.7	1.87	14.1	95.8	29.9	31.2	8.2	160	200	269	5	1
189	SOMASHEKAHR	43	M	12.4	16.1	43.2	1.68	15	89.2	29.5	33	8.8	168	187	241	3	0
190	SANTOSH	47	M	12.5	10.3	27.1	2.54	15	83.8	28.2	33.6	7.8	156	182	205	3	0
191	FAIYAZ	65	M	13.6	22	52.5	1.5	13.5	98	34.2	34.2	8	132	167	246	5	1
192	TIPANNA	55	M	11.9	16.8	40.3	3.11	18.3	93.7	28.7	30.6	7.8	144	180	245	7	1

193	SHRISHARAN	47	M	11.1	13.8	34.4	3.14	13.7	94.4	27	28.6	8	198	312	234	3	0
194	ASHOK	50	M	11.4	14	35.7	1.51	13.9	89.8	28.9	32.2	6.8	136	200	235	5	0
195	C.M.NAETI	58	F	12.1	16.4	42.2	3.28	15.6	89.4	27.5	30.7	8.2	188	218	242	8	1
196	BHEEMSHANKAR	53	M	10	11.5	24.8	3.35	16.8	85	27	31.8	5.8	160	190	215	4	0
197	SHAINAZ	55	F	12.1	16.4	42.7	3.78	12	80.1	21.1	28.3	7.8	186	296	306	9	1
198	BASVANTHRAY	64	M	11.3	13.3	46.3	3.9	13.8	90.2	28.6	31.7	9.2	216	240	312	10	1
199	RUKAMINI	41	F	11.3	13.8	35.5	2.7	14.1	89.5	28	31.3	6.4	160	198	208	4	0
200	LAKAWWA	57	F	12.3	17	41.1	1.25	12	83	27	32.5	6.2	128	184	219	4	0
201	PANCHAYYA	53	F	11	14	33.5	4.32	12.3	80.6	23.3	33	6.4	148	210	246	4	0
202	CHANABASAPPA	59	M	10	10.5	24.8	3.52	12.2	82.3	26.1	32.7	6.5	138	180	211	4	0
203	SIDDARAYA	61	M	11	13.2	34.4	4.36	12	80.7	23.2	32.9	6.8	130	202	218	5	0
204	B.K.KOTI	65	M	12	16.1	42.7	3.64	14.5	89.4	26	29.1	7.2	160	210	264	8	1
205	B.H.MULLIMANI	56	M	11.8	15.3	40.6	2.65	14.2	83.2	25.8	31	6.6	140	194	226	5	1
206	SANGANGOUDA	57	M	10.3	11.7	27	4.72	13.5	84.8	26.4	31.1	8.4	224	280	229	6	1
207	VIDHYAVATI	54	M	12.7	16.4	45.7	1.89	13.5	83	27.2	32.8	7.6	180	208	244	2	0
208	CHANDAWWA	44	F	9.7	10	22.2	3.01	12.1	86.7	28.3	32.7	7.4	130	210	217	4	0
209	SHRIKANTH	48	M	12.7	17.5	45.8	1.82	13.2	82.9	26.8	32.3	8.6	196	218	238	2	0
210	HUVANNA	54	F	12	15.6	42.1	2.72	14.5	84.1	26.5	31.5	8.8	214	301	245	4	0

211	G.TIMMAREDDY	58	M	12.2	15.4	42.8	2.72	14.5	83	26	30.9	7.8	190	210	225	5	1
212	SHOBHA	41	F	10.7	12.5	31	2.64	12	80	25.2	31.5	5.8	120	178	229	2	0
213	KASHIBAI	56	F	10.7	12.3	30.2	2.39	12.1	87.4	25	28.6	6.8	140	189	228	3	0
214	NEELAVATI	50	F	12.1	16.3	43.2	2.85	13.2	78.3	24.7	31.6	8.2	190	212	246	2	0
215	B.S.YADAWAD	57	M	12	17.1	43	2.86	15.6	76.9	25.5	33.3	9.8	383	400	300	9	1
216	PRAKASH	45	M	12	15.1	38.9	1.83	14.9	92.4	30.4	32.9	6.8	152	182	231	4	0
217	SHAILAJA	51	F	9.9	11.4	24.5	3.62	12.8	86.9	27.5	31.6	8.8	202	280	220	3	1
218	VIKAS	40	M	12.8	16.9	47.2	2.31	16	84.2	27.4	32.5	6.1	160	200	228	4	0
219	BALAPPA	58	M	10.3	12.5	27.5	2.06	15.8	87.9	28	31.9	5.5	136	180	239	8	1
220	TUMANNA	60	M	14.4	24	59.9	1.64	12.8	82.8	26.7	32.2	6.5	130	196	218	5	1
221	MD.SALIM	50	M	14.7	22.6	60.8	1.98	13.2	82.8	26.7	32.2	6.2	148	180	254	6	1
222	ROHAN	45	M	12.9	19.1	48.4	2.53	16.1	84.2	27.4	32.5	8.6	220	290	324	10	1
223	ANNAPPA	45	M	11.4	15.5	36.2	1.56	12.7	89.1	28.7	32.2	6.6	130	188	232	2	0
224	RAJASHREE	47	F	10.6	12.9	30.6	1.58	12	80.6	24.8	31.5	7.1	154	195	238	4	0
225	NOORJANA	56	F	11.1	13.2	33.5	1.5	12.5	82.7	24.2	31.4	5.6	120	186	212	4	0
226	SHANKAWWA	54	F	11.4	16	37.5	1.67	12.7	88.8	28.3	31.9	8.8	189	231	268	7	1
227	SUSHILENDRA	57	M	11.4	15.5	37.1	1.58	12.7	88.5	28	31.6	6.9	171	214	230	4	0
228	CHANDRAKANTH	52	M	11.7	14.7	38.8	2.76	15.2	86.3	28.2	32.7	5.8	126	176	236	3	0

229	RAJAK	41	M	10.8	12.9	31	1.51	13.5	88.7	27.8	31.6	7.6	143	202	212	2	0
230	CHANDASAB	55	M	10.5	12.4	29.1	3.2	14.7	94	29.5	31.4	6.9	157	228	245	8	1
231	VEERESH	48	M	10.7	13	30.2	2.14	15	81.6	28.1	34.4	6.3	134	178	234	4	0
232	KASTURI	55	F	11.8	14	38.8	2.3	12	83.4	26.8	34.7	6.5	130	180	230	4	0
233	MALLAMMA	51	F	11.9	15.8	40	2.22	12.4	84.2	26.9	35.2	8.6	168	234	198	3	0
234	MANTESHARI	54	F	12.1	15.6	31.1	2.58	13.2	83.8	27.5	32.5	6.8	157	198	228	7	1
235	SUSHILA	49	F	10.4	11.8	27.2	2.95	12.1	82	26.4	32.1	8.2	184	234	254	4	0
236	S.P.PAWAR	55	M	10.4	12	27.7	2.06	13.7	101.4	36	34	6.8	146	196	212	4	0
237	ABDULSALAM	63	M	10.5	11.5	28.2	2.11	15	81.4	28.2	34.7	6.8	134	184	240	3	0
238	BASVANTHRAY	56	M	10.8	11.9	30.7	3.08	13.6	98.4	30.4	35.2	6.8	148	198	265	7	1
239	BUNDIBAI	49	F	11.5	17.1	36	1.93	14.5	92.1	29.4	31.9	6.5	141	187	234	4	0
240	MAHALAXMI	45	F	10.9	14.2	33.2	1.86	14.8	92.5	29.2	31.6	5.4	126	165	172	2	0
241	DHANALAXMI	51	F	11.9	15.3	37.1	1.87	14.4	92.5	29.4	31.8	5.9	132	171	198	3	0
242	S.S.WAGAMARE	54	M	12	14.9	41.2	3.54	16.2	84.3	28.2	33.4	6.9	168	189	236	10	1
243	VITHPPA	53	M	12.4	16.1	44	1.56	13.9	87.6	27	30.8	6.9	156	189	234	2	0
244	BHIMANNA	57	M	12.3	16.8	43.3	1.67	13.9	87.5	26.9	30.6	7.2	165	234	265	7	1
245	SUNIL	43	M	11.9	18.4	41.3	1.57	14	87.4	27.2	31.2	6.1	167	216	234	1	0
246	SHANTAMMA	56	F	12.2	16.5	41.9	1.78	13.7	87.2	27	30.9	6.7	178	256	289	6	1

247	SHIVAGONDAPPA	61	M	12.2	17.2	43.5	1.76	14	87.4	27	30.9	8.3	154	289	302	10	1
248	JANABAI	53	F	10.9	12.7	32.2	6.2	12.1	92.3	28.3	30.7	5.8	132	199	201	3	0
249	HAWABAI	57	F	11.8	15.8	39.3	3.35	12	80.4	24.5	32.8	7	145	186	212	4	0
250	SALMA SHEIKH	58	F	11.1	13.4	32.9	6.12	12.1	92	28.3	30.8	7.2	167	214	200	4	0
251	MALAKANNA	67	M	11	12.7	33.2	5.89	12	97	28	30.3	9.2	198	298	312	8	1
252	SADANNA	53	M	12	14.4	41.5	3.32	13	83.2	25.4	32.2	6.9	145	199	265	4	0
253	GURUAPPA	63	M	12.5	15.6	44.2	3.22	13	82.2	24.6	33.1	8.6	187	267	312	10	1
254	RAJAGOUDA	51	M	12.7	14.7	45.4	2.25	13.2	84.6	26.1	33.7	7.1	144	198	234	2	0
255	NEELAMMA	43	F	12.2	15.1	42.2	3.41	12	80.3	24.6	33	5.2	123	164	168	3	0
256	DUNDABAI	61	F	11.3	13.8	31.3	3.91	14.4	78.9	26.2	33.2	8.9	167	289	312	10	1
257	RATNABAI	58	F	9.8	11.3	22.2	4.06	13.6	89.5	31.1	34.7	7.5	145	245	256	5	1
258	BASHASAB	49	M	10.2	11.9	27.2	3.94	14.5	79	26.1	26.1	6.5	132	200	234	4	0
259	BASAPPA	51	M	11.5	13.2	28.9	3.95	14	79.2	25	32.7	6.8	136	231	212	2	0
260	BHIMASHA	54	M	10.4	11.7	27.9	3.85	13.9	78.8	26	33	6.5	145	246	252	3	0
261	PARANAPPA	65	M	12	15.9	41.2	3.75	15.8	86.4	28.6	33.1	9	148	298	321	15	1
262	SUNIL	43	M	12.8	18.8	46.6	2.12	16.8	100.7	29.2	29	5.9	120	168	216	1	0
263	BHAGAVANTH	51	M	10.8	12.9	31.3	5.62	14.1	99.8	33.1	33.2	6.8	143	180	202	3	0
264	M.B.PATIL	55	M	13.6	20.6	54.1	1.77	13.6	85.4	26.6	31.1	6.2	140	175	200	2	0

265	RAMAPPA	57	M	12.8	18.8	46.5	2.2	16.7	100.7	28.7	28.5	6.6	138	212	280	4	0
266	PARANAMMA	56	F	10	10.9	24.9	3.06	14.9	94.7	30.2	31.8	7	132	234	289	5	1
267	MAHANANDA D.	59	F	11.9	16.5	41	3.82	15.8	86.5	28.4	32.8	7.3	139	214	287	5	0
268	BANUBAI	67	F	12	15.8	41.4	3.34	15.3	85.3	28.3	32.7	9.4	169	323	344	12	1
269	NINGANGOUDA	55	M	10.5	11.9	29.6	4.69	13.5	91.5	28.9	32.2	7.1	143	265	231	6	0
270	GOURAMMA	57	F	11.3	15.8	38.3	2.62	12.5	86.7	24.5	28.2	6.9	136	258	219	4	0
271	GURALINGAWWA	53	F	10.1	11.4	24.7	3.68	12.6	93.2	31.4	33.7	6.3	128	189	228	6	0
272	SIDDAMMA	57	F	12.2	15.9	41.9	2.99	12.7	86.5	24.6	28.4	6.4	134	168	212	6	1
273	CHANDRAMMA	59	F	11.9	15.5	40.4	2.91	12.8	87.2	24.8	28.9	7	143	198	202	5	0
274	SHININMAR RAO	48	M	11.5	14.4	32.9	2.03	12.9	75.5	22.8	30.1	5.5	126	148	159	4	0
275	REVANSIDAPPA	58	M	11.9	13.8	33.4	2.31	12.7	78.4	22.6	29.9	6	134	159	198	3	0
276	PADMABAI	49	F	11.4	15.4	37.5	2.27	12	81.2	21.9	30.7	6.2	145	178	215	3	0
277	SHIVSHANKAR	53	M	10.9	14.1	33.8	2.27	13.5	87	23.9	32.3	7	143	187	231	4	0
278	S.M.BIRADAR	56	M	10.8	12.6	30.9	2.69	13.9	88.8	30	33.8	7.2	146	213	241	7	1
279	SHANKARAWWA	50	M	11.9	16.5	41	3.64	15	87	28.2	33.3	7.1	144	216	259	5	0
280	RAMAPPA	52	M	12.5	17.8	40	2.71	13.2	86.4	29.5	32.1	7.5	139	200	239	6	1