Study The Correlation Between Mean Platelet Volume And Hba1c In Type 2 Diabetes Mellitus With Special Reference To Microvascular Complications

by

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ABSTRACT

Need For Study:

Diabetes mellitus is the most common endocrine disease characterized by metabolic abnormalities, hyperglycemia and by long term complications. Large platelet are younger, more active and aggregable, have dense granules, secrete more proaggregatory molecules. Platelet activation contributes to trigger thrombus formation and causing microcapillary embolization. Platelet is directly regulated by insulin via functional insulin receptor found on human platelets. Insulin inhibits platelet interaction with collagen and attenuates the platelet aggregation effect of agonist in healthy platelets. MPV, a determinant of platelet function, is a newly emerging risk factor for atherothrombosis. Increased in HbA1c concentration is directly proportional to increased MPV. MPV can emerge as an important, simple, effortless, and cost-effective tool for monitoring and for early recognition of patients that could possibly benefit from preventive treatment.

Methods:

It is a comparative study conducted in 98 patients admitted in Sri B.M Patil Medical College and Hospital, who are already diagnosed to have Type 2 DM with more than five years duration and 98 non-diabetic subjects without known microvascular complication. MPV is measured in cases and control groups. Patients were selected on the basis of inclusion and exclusion criteria and statistically analyzed. The study was conducted between December 2017 to july 2019.

Result:

In our study, MPV was significantly higher in diabetics with HbA1c levels ≥ 6.5% than in diabetics with HbA1c levels <6.5%. Among the 98 study group participants total 92 patients had one or more microvascular complications. Of the microvascular complication evident in the form of retinopathy, nephropathy and neuropathy, diabetic neuropathy was the most common, followed by diabetic nephropathy.

Conclusion:

Changes in MPV are seen to be statistically associated with diabetes and its complications. They are easily available, simple, convenient, noninvasive, and easy to interpret method to determine platelet dysfunction and in turn predict the presence of microvascular complications.

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INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disease characterized by metabolic abnormalities, hyperglycemia, and by severe complications.

Large platelets are less in age, more active and aggregable, have denser granules, release large quantity of pro-aggregatory molecules. Platelet activation contributes to the pathology by initiating steps involved in development of thrombus and leading to microcapillary embolization with discharge of constrictive, oxidants and mitogenicfactorssuch as PDGF, VEGF, that enhance the development of local vascular lesions such as neovascularization of lens in diabetic patients with retinal abnormalities. Platelet is controlled by insulin via a by means of functional insulin receptor present on human platelets. Insulin inhibits platelet interaction with collagen and attenuates the platelet aggregation effect of agonist in healthy individuals. Platelet activation is responsible for the pathogenesis by stimulating thrombus development and resulting in micro capillary embolization.

MPV, affects the platelet function, is considered as having role in atherothrombosis. In many studies it has been found that increased in HbA1c concentration is directly proportional to increased MPV. High number of patients with with type 2 DM suffer from avoidable macrovascular and microvascular complications. It is necessary to have There is a need to cultivate risk factor lowering activities to lower the effects of diabetic complications. Assessment of MPV can be carried out easily at the time of haematological analysis by automated hematology analyzers. Thus, MPV can emerge as a critical, easy, and cheap investigation for monitoring and early recognition of patients with diabetic complications to achieve better treatment outcomes.

AIMS AND OBJECTIVES

1. To study correlation between mean platelet volume (MPV) and HbA1c in type 2 diabetes mellitus.
2. Determine if the MPV in the diabetic patients is higher compared to the non-diabetics.
3. To see if there is a variation in MPV in diabetics with and without microvascular complications.

REVIEW OF LITERATURE

Diabetes mellitus

People affected by diabetes mellitus are on rise worldwide. Diabetes mellitus is a disorder affecting the metabolism with increased blood sugar levels. It can cause to complications involving more or less every organs of in the human systems.(1)Diabetes mellitus can be categorized into 2 main varieties: Type 1 Diabetes (T1D) in which there is severe insulin deficiency found commonly in young people and Type 2 Diabetes (T2M)affecting usually adults with insulin resistance.(2)The diabetes cause both microvascular and macrovascular complications, which are key to the multiple organ damage caused by the disease. It is rather difficult to diagnose these damages before the actual functional deterioration of organ is evident. The MPV (Mean Platelet Volume) being a good indicator and independent predictor of vascular health, may be used to overcome these challenges.

The platelet structure and actions are altered with an elevated MPV in T2D individuals. Considerable number of platelets have increased size than normal range. The rise in in MPV takes place because of diabetic condition and remains for significant period.(2)Bigger platelets consist are made up of more dense granules, produce large amount of serotonin and beta thromboglobulin and synthesize more TXA2 compared to smaller platelets. These large platelets are extremely responsive and with high clump forming properties which lead to blood vascular system problems.(2)

Epidemiology

It is projected 382 million individuals globally suffer from diabetes, and this number is anticipated to become 592 million by the year 2035. (3) According to the American Heart Association (AHA), in adultspopulation suffering from T2D there is 2-to-4- folds more risk for cardiovascular morbidity and mortality as compared to non-diabetic adults. Diabetes is deemed to be as "1 of the 7 major controllable risk factors for cardiovascular disease (CVD). (4)Two-third diabetic's deaths that are because of CVD amount to coronary artery disease (40%), stroke (10%) and other forms of heart disease, principally congestive heart failure (15%). Diabetes is becoming an epidemic in India with >62 million population diagnosed with diabetes. It is estimatedthat by 2030, 79.4 million Indian will suffer from diabetes. Type-2 DM is a progressive lifelong disorder with increased overall lmorbidity, mortality and decreased the quality of life. In a developing country like India, there is significant economic crisis in diabetic population, their relatives and community.

Classification

Diabetes mellitus (DM) is a cluster of chronic metabolic disorders that are manifested by raised blood sugar levels due to decreased synthesis of insulin or response to its action or both in the body. The two basic types of diabetes are affecting distinct age groups. The Type 1 DM (T1D) is termed as juvenile diabetes and is mostly due to genetic defect in insulin production and secretion. Whereas the T2D, is adult onset and the principal aetiopathology is insulin resistance at the receptor site. Though clinically it is classified under four distinct entities: (5)

- Type 1 diabetes: it is produced because of autoimmune damage of β-cells resulting in causing complete insulin deficiency

- Type 2 diabetes: results because of continuous deterioration of β-cell insulin production with gradual lack of insulin action
- Gestational diabetes mellitus (GDM): it is detected in the 2nd and 3rdtrimester during gestation that was not present prior to it.
- Diabetes because of specific abnormalities: example, monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), disorder affecting the exocrine part of pancreas (as in case of cystic fibrosis and pancreatitis), and drug- or drug-induced diabetes (because of anti-HIV/AIDS, immunosuppressant, glucocorticoid)

1. Pathophysiology of Diabetes

Genetics

Genetically, both T1D 1 and T2D are characterized as polygenic disorders. The overall disease risk depends on several common types, mainly with small effect size. Disease heritability (h^2) is defined as sibling-relative risk, which is 3 for T2D and 15 for T1D.(6)If one parent has T2D, then the child has about40% lifetime risk of producing T2D. It is even greater if the mother suffers from diabetes. The risk for T1D is ∼5% if a parent has T1D. It is even higher if the father suffers from diabetes. MODY is a monogenic condition with higher h^2 of ∼50.(7)MODY is caused by mutations in any 1 of 13 variants of individual genes. Correct choice of treatment can be made based on genetic diagnosis for example the individuals with MODY because of mutations in KCJN11, sulfonylureasused be used instead of insulin.(8)

Type 1 Diabetes

A higher prevalence of T1Dis found in relatives of index case which indicates a higher genetic risk. The genetic risk is linked with with the proband correlates.(9)Altered gene in one main locus, human leukocyte antigen (HLA).(9)It is

responsible for 50–60% risk due to genetic factor. It caused HLA protein binding to antigenic peptides and antigen presentation to T cells.(10)Minor effects are observed by 50 additional individual genes.(11) These gene types alter immune response and tolerance,(12)alter the response to viruses,(13)affect the interaction to environmental stimuli and endocrine function. The importance of role genetic mechanisms on the stimulating islet autoimmunity and disease prognosis are being evaluated among relatives.(14) Overall, about 80% heritability of T1Dscan be explained by different gene variant. The disease activity and risk changes over time which can be explained by epigenetic, gene expression, and regulatory RNA characteristics.(8)

Therefore the genetic variations are critical to distinguish individuals with higher risk. They are also helpful in anticipating the decline in the rates of C-peptide and effect of different treatment modalities. (15) In future superior knowledge of inheritance characteristics may help in developing newer drug new targets for individualized treatment.(8)

Type 2 Diabetes

The diabetes also can be classified genetic basis depending on subset of genetic variants that are linked with both T2D and T1D.(16),(17)About >130 genetic types linked with T2D, glucose levels, or insulin levels are identified in Genome-wide association studies. However, these variants explain <15% of disease heritability.(18)Among the many possibilities which explain the majority of T2D heritability, include heterogeneity of disease profile, epigenetics and interaction between genes. Majority of the type 2 variants are found in noncoding genomic regions. Certain variants for example as those in *KCNQ1*, demonstrate dominant parent-of-origin impact.(19)The off-springs born to mothers with *KCNQ1*, inherit decreased amount functional β-cells. This is responsible for decreased insulin

production and release when exposed glucose challenge. Identification of genetic variants that offer protection from T2D could be another area of research and development of new therapy.(8)

Research in the area of utility of predictive value of known genetic variants as compared to the conventional risk factors such as obesity, blood glucose level, family history, glucose has been very less T2D.There is a need to develop molecular genetic tools and decreasing costs for next-generation sequencing to improve the knowledge of genetics in diabetes. But currently apart from distinguishing T1D and T2D there has been scare number of specific variants that identify small subgroups of patients (MODY).(8)

Environmental impact

Environmental factors have a critical activity in the pathogenesis of both types of diabetes, as the genetic do explain the increasing the prevalence. Several common environmental elements are involved in diabetes such as pollution, dietary ingredients, endocrine disruptors and altered gut microbiome composition. High BMI and insulin resistance may also have a role in development of T1D. On the other hand in T2D, association of autoimmunity and environmental factors (e.g., food, infection) may be responsible.(8)

Type 1 Diabetes

Recent evidence indicated that environmental factors have significant impact on causality of T1D.For example factors such as different rates of T1D in twins, difference in prevalence rate in different regions worldwide, the increase in the new cases around the globe, integration of local disease incidence rates in immigrants from low- to high-incidence countries may have link in the development of T1D. Research data indicates environmental elements interact with genetic factors in both stimulating

autoimmune response and development of T1D. Moreover in majority of patients with the greater-risk HLA haplotypes T1D may not occur. The time at environmental triggers exposure occurs is also critical. In childhood-onset T1D exposure to certain environments triggers in early life may stimulate onset of islet autoantibodies which indicates that environmental element may be responsible contributors(8) The onset of disease at different age makes it difficult to study this the mechanisms of environmental exposures.

Certain infections such as enterovirus and alteration in gut flora explain the environmental associations linked to TID.(20), (21)The time at which the diet such as cereal and nutrients such as gluten are exposed could affect β-cell autoimmunity. Vitamin D deficiency also has been linked to T1D.The risk factors during perinatal period and harmful doses of nitrosamine compound might be responsible for the development of diabetes.(8) Environmental toxin could be one of the contributor for T1D. The research on lacking and controversial.

Type 2 Diabetes Mellitus

In T2D, the production of required insulin by β-cells is insufficient to meet the demand, especially in relation to increased insulin resistance. Autoimmunity against the beta cells of islet also plays a role in small number of cases.(22)One of the main risk factor forT2Dis increased BMI in addition to with complex genetic and environmental elements.

The ectopic position of lipids in the liver and muscleis also responsible for development of Insulin resistance. Lipids may also get deposited in the pancreas. This leads to deterioration in β-cell activity, inflammation in the ilets, and ultimately death of β-cell. T2D develops at variable threshold limits of BMI/body fat components in different subjects. In susceptible individuals, a personal “fat threshold” exists at which

there is deposition of ectopic fat. This causes worsening of insulin resistance and resulting in decompensation of β-cells. It is for Asians and Asian Americans have lower threshold BMI development of D2M.(23)

Loss of weight loss helps in improving insulin sensitivity in hepatic tissue and myocytes. This could additionally lower fat deposition in pancreas.(24)In prediabetes and recent-onset T2D deficiencies in insulin production could be reversible to some extent with decreased caloric intake and reduction in BMI.(25)But similar reversal does not occur in chronic diabetes with weight reduction even significant weight as a result of bariatric surgery.(8)

Abnormal sleep time,that is less or more is linked with occurrence increased weight and DM. Obstructive sleep apnea causes decreased sleep duration and quality. It is linked with metabolic syndrome and T2D. The current "24-hour culture" has the decreased the sleep time, thereby may resulting in increased risk of T2Ds.(8)

Natural History and Prognosis

Although most individual diabetic patient has different pathophysiology, but the common factor is increased blood sugar because of β-cell damage or abnormal function.

As the duration increase these a continuous increase in blood sugar level as a result of decrease insulin production and action. The natural history associated with β-cell mass and activities is key to classifying the disease. The understanding of these causative factors will guide where and how best intervention is possible to prevent disease progression or delay the complications.(8)

β-Cell Mass and Function

The two types of DM, have distinct modality. T1D develops because of immunological response mediated death of β-cells whereas T2D is mainly because of defect in secretion of insulin in response to blood sugar. In both T2D and T1D, the stress response induced by hyperglycemia is responsible for apoptosis of β-cell.

Alteration in β-cell phenotype because of high blood sugar levels demonstrates a differentiation of β-cells critical to the natural history and severity of diabetes. Thus inadequate number or decrease in functionof β-cells is responsible forhigh blood glucose levels and the diabetes related. The β-cell status and activity is critical in classifying diabetes subtypes.(8)

Treatment of diabetes with lifestyle modification or medication helps in slowing the progress from prediabetes stage to actual DM. Moreover, health advantage of early treatment has been evidenced with decrease in retinopathy and cardiovascular morbidity and all-cause death.(26) This dataindicates that early diagnosis of prediabetes and maintaining blood sugar levels within normal range may alter natural history of DM.(27), (8)

Autoimmunity

Investigations such as detection of glutamic acid decarboxylase (GAD), autoantibodies against insulin in the circulation can be carried out even before the clinical diagnosis of T1D. The protein tyrosine phosphatase IA-2, and/or zinc transporter are other two investigation that can be done before diagnosis of T1D. Subjects having single antibody can have reversion on the contrary it is not seen in those with multiple antibodies. (28)At present, there are lack useful biomarkers and mean of imaging procedures to assess autoantibody flare-ups and reversible so as to

monitor the progress of T1D. The presence of in children or with relatives who have T1D is associated with a within 10 years.(29)

There is about 75% risk of having clinical DM in younger patients with HLA risk genotypes or ≥ 2 autoantibodies against islet. As the numbers of autoantibodies increase there is proportional increase in the risk of T1D.(29)

T1D is based on the presence of positive test for minimum 2 autoantibodies. The identification of autoantibodies against islet suggests there is an immune B- and T-cell interaction to β-cell antigens. This leads lead to decrease in β-cell mass and action. The occurrence of glucose intolerance,is phase before the development of clinical presentation of DM.(8)

The detection of autoimmunity in T1D has useful prognostic value, however it is of no help in the prevention of treatment of this condition. HLA is responsible for strong vulnerability for the formation of ≥ 2 islet autoantibodies. Data indicates that in children, first 2 years of life are critical period for so as to prevent the β-cell autoimmunity response.(28),(8)

It is observed that in about5% of subjects with T2D autoantibodies against GAD can be detected. GAD antibody–positive patients with T2D have lesser BMI and residual β-cell action in comparison to GAD antibody–negative patients. Further, their genetic profile is more like of that of T1D patients. In adults with early need of insulin injection therapy, suggest that autoimmune response could be a form of T1Ddiabetes which has slow progresssion in later years of life.(8)

Complications

Several vital organ systems of the body can be adversely affected by diabetes.As times passes diabetes may produce serious complications, categorized into microvascular or macrovascular. Microvascular problems involve nervous tissue

injury (neuropathy), renal tissue injury (nephropathy) and ocular injury (retinopathy). Macrovascular pathology lead to heart and blood vessel disorder, stroke, and peripheral vessels disorder. The peripheral vessels disease can cause non-healing ulcers which can lead gangrene formation, and finally may result in amputation of the involved body part.(30)

a. Microvascular

i. Neuropathy Long standing diabetes leads to common complication diabetic peripheral neuropathy (DPN) which is projected to impact 30% to 50% of subjects. The main risk factor for DPN is uncontrolled blood sugar level. Several independent risk element involves are how old the the patient, early onset of disease, uncontrolled blood pressure, smoking, raised triglycerides, higher BMI, alcohol intake, and more height.(30)

The most frequent form of DPN is chronic sensorimotor distal symmetric polyneuropathy which can result in loss of sensation, loss of muscle strength, and increase pain perception. Generally, typical polyneuropathy is slow to occur with sensory impairment, along with burning and loss of sensation in the feet(numbness) that is undetected for long duration. Neuropathic pain is encountered in about 11% to 32% of patients suffering from polyneuropathy which may be severe when present.(30)

Diabetic peripheral neuropathy may result in several disabilities and loss of function.Patients with DNP have greater risk ulceration of feet which eventually may require amputation of lower limb. They have a more hospitals visits annually, disability causing work loss because of immobility.Other impending problems of DPN also leads to other health related issues like falls, fractures and sedentary life style.(30)

In patients with DPN it was found that increased from age-adjusted hospital discharge rate changes from 4.7 per 1,000 to 6.8 in 7 years. Discharge rates estimates were greater for younger males as compared to both women and older men.(30)

ii. **Nephropathy** Diabetic nephropathy (DN) is defined as persistent proteinuria (> 500 mg of protein or 300 mg of albumin in 24 hours) in patients not having infection of urinary tract or other disorder leading to proteinuria. In T1D patients the nephropathy develops reasonably late; whereas in T2D patients, it can found during diagnosis.(30)

The incidence of diabetic nephropathy in T2D patients is low during the initial 10 to 15 years of onset of DM and it rapidly aggravates to a maximum by about 18 years of duration, and then declines.High prevalence of nephropathy at diabetes is because the actual onset of T2D generally precede its clinical diagnosis by many years giving rise to complication due to late diagnosis.Diabetic nephropathy is responsible for 44% of new patients of end-stage renal disease (ERSD) in 20002. About 153,730 patients with ESRD because of DM had either got a kidney transplant or were on dialysis for long time.(30)

The cause of DN is not clearly known. Many risk elements are responsible for the pathogenesis of DN, some of which are modified while some are unmodifiable.

Alteration of metabolism is one of the major modifiable risk element in the pathogenesis of DN. Both in T1D and T2Dtight metabolic control results in a significant lowering of the risk of microalbinuria and development of proteinuria of persistent nature. The implication of tight metabolic control on

progress is more effective severe in diabetic individuals with decreased microalbuminuria. Hypertension pressure also raises the risk of development of renal failure in diabetics. However, currently it is not known whether high blood pressure at diabetic onset is responsible for producing DN. Different risk factors include increased BMI, smoking, anemia, obesity and genetic elements.(30)

The patients with T2D having diabetic nephropathy are more prone to higher of several different diabetic related problems account to renal-retinal syndrome. These patients have higher probability of developing ischemic heart disease and stroke in comparison to diabetics without nephropathy. Thus DM patients with DN are at risk of mortality due to macrovascular diseases.(30)

In the past few decades, the incidence of DP declined due to improvements in disease management that promote tight regulation of blood sugar level as well as better control of hypertension.

iii. **Retinopathy** In diabetics, diabetic retinopathy (DR) is a major microvascular complication which is responsible for more than 10,000 new cases of loss of vision annually. Incidence of retinopathy is associated with chronic high blood sugar levels. Although diabetic retinopathy develops slowly, few data indicate its onset as early as 7 years ahead of identification of T2D. Women with DM were found to have visual impairment relatively more than their male counterparts. A steady decline in prevalence rate is observed in women since 2001, but they have stayed fairly constant among men. The prevalence among racial groups had been comparable during 1997–2005. In T2Dpatients' duration of diabetes is a critical factor in predicting vision loss. About 90% of blindness caused by retinopathy in diabetic patients can be prevent with early

diagnosis and treatment.Therefore, yearly dilated ocular examination should be carried out in every diabetic patient (30)

b. Macrovascular

i. Cardiovascular disease and stroke Cardiovascular disease (CVD)amounts to almost 65% of all-cause mortality in diabetic patients. Coronary artery disease and is responsible for the high morbidity related to DM.In diabetic patients mortality rates due to cardiovascular disorder and incidence of stroke are 2 to 4 times higher as compared to non-diabetic individuals. More than 70% of diabetics suffer from increased blood pressure or are taking anti-hypertensive medications.In diabetic patients, the contribution of high blood sugar level to the pathogenesis of cardiovascular complications need to be investigated.

Risk factors causing CVD disease among diabetic patients are similar to those for non-diabtic population. It is also comparable to smokers, hypertensives and those with dyslipidemia.These risk factors contribute to adverse effects and complication among diabetic individuals as in comparison to non-diabetics. Evidence on trends in diabetic CVD outcomes are known for the duration of 1950s to 2003 in different groups of people and regions. Over time, there has been remarkable lowering in the incidence of CVD.With advancement of newer medicines to manage hyperglycemia, hypertension and dyslipidemia there was significant fall in these conditions. It appears, however, that the success has little improvement since the late 1990s.(30)

ii. Peripheral vascular disease Peripheral arterial disease (PAD)or peripheral vascular disease (PVD), results due to reduction in the lumen of blood vessels that transport blood to peripheral organs such as the limbs, stomach, and kidneys. The risk for PAD in diabetic population increases with age of onset,

duration of diabetes, and nerve involvement. Research data on PAD, suggests the hospital discharge rates for PAD as the basic diagnosis have lowered gradually from year 1996. (30)

Several elements found in CVD, like C-reactive protein and homocysteine levels contribute to , higher risk for PAD. Typically 2 types of complaints are experience by patient: intermittent claudication and pain at rest. PAD is one of the main risk factor leading to requirement of amputation of lower limb.(30)

4. Platelets

Platelets are specialized cells primarily involved in regulation of hemostasis to stop bleeding with secondary roles in angiogensis and innate immunity. They get activated in response to bleeding from blood vessel injury leading to formation of clump and blood clot thereby preventing blood loss. They are anucleate, tiny blood cell fragments, discoid in shape, about 2-3 μm in diameter. The blood platelet count in human adults is about trillion platelets with average life span of 8 to 9 days.

Platelets develop from large (30-100 μm) nucleated cells called megakaryocytes. Each megakaryocyte which resides in the bone marrow produces between 1-3 thousand platelets during its lifetime.Reserve platelets are present in the spleen, and are released in the circulation when required by splenic contraction induced by the sympathetic nervous system. Old platelets are removed by phagocytosis in the spleen and liver.(31)

a) MPV

Mean platelet volume (MPV) is commonly used for diagnosis or predictive purposes. It is generally estimated as a component of the complete blood cell count (CBC) with the help of automated blood cell counters. It is not very accurate and

reproducible hence has less significance in practice.Moreover, MPV is associated with several pathological situations, hence could be an under-utilized parameter. Currently, MPV utilized for diagnosis of hereditary thrombocytopenic disorders a type of macrothrombocytopenias.(32)Recent data demonstrates an association of MPV with acquired clotting conditions. MVP is also associated with thrombotic disorders and other cardiovascular risk factors high blood pressure, diabetes mellitus and tobacco smoking.(33)To overcome some of the variables affecting MPV measurement by automated cell counters an different procedure that is Mean platelet diameter (MPD) of evaluating platelet size has been created.

i. Natural variation in MPV

In a subject, platelets are of different size and density which is evident in a classical log-normal distribution of platelet size. An inverse relationship between MPV and the platelet count is evident within the normal range which helps in the maintenance of normal haemostatic role.(34) Normal variation in platelet count is complemented by a corresponding variation in MPV. The data on the impact of age and sex on MPV is quite conflicting.(35)

ii. Association within platelet size and function

Certain hereditary syndromes are manifested by enhanced platelet size which lead to decrease in platelet function example as in Bernard Soulier syndrome. When the size of platelets is enhanced they have higher thrombotic activity. They produce increased levels of glycoprotein, P-selectin and IIb–IIIa per unit volume of platelet.(36)Rabbit experimental studies have demonstrated that thromboxane B2 production/unit volume of platelet is higher in bigger platelets produced after one day of thrombocytopenia in comparison to those produced in normal steady state. Thus

platelets produced during thrombocytopenia in addition to having larger mean platelet volume are also more reactive.(37)

In a study on 34 normal subjects, on platelet aggregation in response to ADP, collagen, or epinephrine. It was found that platelet count was inversely proportional to platelet volume ($r = -0.53$, $p < 0.001$).(38) Larger platelets are denser, have large quantity of secretory granules and mitochondria per unit volume of platelets, and are also more reactive.(39)

iii. Factors affecting MPV

Following factors affect MPV:

- Thrombopoietin: it controlsmegakaryocytopoiesis
- Cytokines and growth factors: They interact in platelet development and release of bigger platelets. (40)

It is evident that there is positive correlation between cytokines (thrombopoietin, interleukin- 6 and interleukin-3) and larger platelet production.(41)During thrombocytopenia when the platelet count is low, more cytokines are released and newer platelets are produced which are larger in size.(40)

iv. Genetic factors determining MPV

The variations with respect to number of platelets, MPV and the activity are controlled by genetics. Genome–wide association studies (GWASs) have identified several loci associated with platelet disorders. As a result of these observations and sequencing in further generation, genetic analysis is the basis of diagnosis of macrothrombocytopenia since birth. A comprehensive analysis was conducted by Panova-Noeva et al of known genetic and non-genetic determinants of MPV. They assessed 15010 subject data from the population-based Gutenberg Health Study.(35)Seven single nucleotide variants (SNVs) were determined in women and in

men by genotyping. These variants were associated with higher MPV. These studies on different parameters, consisting of both genetic and non-genetic factors, could explainonly 20% and 19% of the MPV variance in women and men, respectively.(35)

v. Acquired determinants of MPV

Several factors such as smoking, obesity, hypertension and hyperlipidemia are associated with increased MPV. These are also related to thrombosis in arteries and veins. It has been indicated that the increased MPV in these states, without thrombotic events, could be because of abnormalities of arteries and/or subclinical activation of coagulation, thus leading to platelet activation-consumption and ensuing in larger platelets.(42)

vi. MPV and platelet age

The newly produces platelets are reticulated consisting of RNA. They are bigger in size and more reactive than mature platelets. The number of reticulated platelets (RP) indicate the rate of formation of new platelets.(43)Standardization of the techniques for measurement of reticulated platelets is absent hence it is not useful variable to assess platelet function. Immature platelet fraction (IPF) is a variable which could help in expressing the quantity RP. Flow cytometry techniques is used to measure IPF. However the correlation between MPV and IPF is not significant in subjects with a platelet formation abnormalities leading to thrombocytopenia.(44)

vii. MPV and inherited thrombocytopenia

Platelet macrocytosisis used to characterize different types of inherited thrombocytopenias. Determination of platelet size helps in diagnosis of thrombocytopenias. The platelet size measurement is difficult in individuals having inherited thrombocytopenia. Following are different forms thrombocytopenia:

MYH9- related disease (MYH9-RD),

Biallelic and mono-allelic Bernard- Soulier syndrome (BSS)

These forms have very large size or giant size platelets. These platelets are identified by the automated counters, thus giving misleading result of platelet count and MPV.(45), (46)

viii. MPV and acquired thrombotic disorders

Platelets express and produced many substances having role in coagulation, inflammation, thrombosis and atherosclerosis. Large research data have demonstrated raised MPV in subjects with coronary artery disease. MPV has been described as an independent risk factor for ischemic heart disease and stroke.(47), (48)

2.Glycatedhaemoglobin

Hemoglobin is an iron-containing metalloprotein that is involved in oxygen transport by the erythrocytes. It is made up of tetramer of two types of chains:

a. α globin chains and
b. and two β chains ($\alpha_2\beta_2$),

The α globin genes are HbA1 and HbA2. Non–α globin genes are β, γ, δ.(49) Posttranslational modification of HbA leads to formation of some minor hemoglobin constituents such as A1a, A1b, and A1c. A1c is highest in quantity among these minor hemoglobin constituent. In the red blood cells the condensation of hemoglobin and glucose leads to formation of A1c where it is present in high amount. The formation of A1c takes plce at slow pace over a period of 4 months. Higher the quantity of glucose more is the formation of amount of A1c.(49) Hence, in case of consistent raised blood glucose levels the quantity of A1c is in the same proportion. There A1c is an useful parameter in monitoring the patients by to estimating the control of blood sugar lever over period of 120 days.

A significant association between blood sugar level control estimated by A1c and the risk for diabetes-related complications was evident in different clinical trials. Glycylated hemoglobin is commonly uses parameter to estimate the blood glucose control over 3 months (50) which is a weighted mean of glucose levels in the prior 3 months.(51) As a guide for every 1 % change in A1c is associated with an about 30 mg/dL change in estimated average glucose.

Table 1: Correlations Between Estimated Average Glucose, A1c, and Fructosamine(51)

Glucose (mg/dl)	A1c (%)	A1c (mmol/mol)	Fructosamine (μmol)
97	5	31	131
126	6	42	203
154	7	53	273
183	8	64	345
212	9	75	417
240	10	86	487
269	11	97	559
298	12	108	631

A1c, hemoglobin A1c

3. Relationship between MPV and HbA1c

There is variation in platelet structure and function diabetic patients. These variations are probably related the pathological processes and raised vulnerability to vascular diseases. .

It is observed that MPV values are increased in diabetic subjects with uncontrolled blood glucose levels as compared to patients with controlled blood sugar levels. A significant proportion of patients experience microvascular adverse effects

like diabetic retinopathy. This indicates that PV mean could be pivotal in early identification of vascular complications of diabetic patients.(52)Several clinical studies have demonstrated variations in mean platelet volume in T2D patients.

Clinical evidence

Zuberi BF et al compared the MPV in diabetic individuals, impaired fasting glucose (IFG), and controls. The study enrolled total of 612 subjects consisting of DM patient, IFG and controls. It was concluded that MPV there was significant rise in the IFG group in comparison to non-DM group, and which further aggravated in comparison to the DM and IFG groups.(2)

HekimsoyZet al carried out a study to assess MPV in T2D patients compared to normal controls diabetics and evaluate difference in MPV between DM patients with and without macro- and microvascular complications. It was conductedin 145 consecutive T2D patients and 100 non-diabetic control subjects. The result showed that MPV was significantly greater and the mean platelet counts were significantly decreases in DM patients compared to age- and sex-matched non-diabetic healthy controls (P=.001), respectively.T his suggest that platelets have a significant role the diabetic complications.(53)

Another similar case-control study was conducted by AkinsegunA*et al*.in 200 participants It was found that the mean fasting blood glucose for the DM patients was higher as compared to controls. The difference in platelet counts in DM patients and controls statistically significant (p =0.038) while no difference was found in MVP between the two groups (p=0.593).(54)

In a clinical study mean platelet volume (MPV) was compared between T2D and T1D, Also the association between MPV and complications due to diabetes was evaluatetd. This study was conducted in 416 patients which were divided into Group

A and B. It was observed that MPV was significantly greater in group A as compared to group B. In patients with retinopathy MPV was significantly higher than in patients without retinopathy (P = 0.043). Moreover in patients with microalbuminuria, MPV was also significantly higher (P = 0.044) than in patients without microalbuminuria. When MPV was other paramenter like gender, age, duration of diabetes, insulin requirement, BMI, HbA1c, coronary artery disease or dyslipidaemia no association was found in group A. It was concluded that MPV is greater diabetics than in non-diabetic patients. Among T2D individuals too, MPV is increased in those who have microvascular complications such as retinopathy or microalbuminuria.(55)

In a cross-sectional clinical study it was observed that MPV could predict the glycemic control deterioration in patients with T2D.This study included included 106 T2D patients, allocated to into groups as per according to glycated haemoglobin (HbA1c) values: A(n=44, HbA1c ≤7.0%) and B (n=62, HbA1c>7.0%). It was found that the MPV was significantly higher in the group B compared to the group A ($p<0.0005$) with a significant positive correlations of MPV with fasting blood glucose and HbA1c (rho=0.382, $p<0.0005$; rho=0.430, $p<0.0005$, respectively).(56)

The association between platelet indices and blood sugar control may vary diabetic patients. This was investigated in a study in total of 691 T1D and 459 T2D patients and 943 control subjects (blood donors). It was found that the volume-count relationship were significantly related to HbA1c only in T1DM patients. There was no associations seen platelet indices and short-term glycemic control by Long-term glycemic control is affected by platelet mass and the volume-count relationship only in T1DM subjects. These findings suggest different mechanisms are involved in

platelet formation in diabetic patients with long-term glycaemic control being more relevant factor.(57)

A retrospective study was conducted on 595 T2Dindividuals over a period of 1 year out patient to compare the basal and post-treatment MPV and HbA_{1c} levels. It was found that a significant decrease of MPV and HbA_{1c} levels was present($p < 0.001$). There was also a positive correlation between the mean changes of MPV and HbA_{1c} levels after the treatment. The results indicate suggest that tight blood glucose control is associated with a significant lowering of MPV levels and is not affected by the treatment with either insulin or oral antidiabetics.(58)

7. Association within MPV and HbA1c and microvascular complications

Diabetes mellitus is considered as a "prothrombotic state" owing to sustained hyperglycemia, dyslipidemia, and insulin resistance causing endothelial and pericyte injury. Altered platelet morphology and function has been observed in diabetes in the form of enhanced platelet activity which may contribute to this "prothrombotic state".(59) Larger platelets which consist of denser granules are more dynamic than tiny ones in terms of metabolic and enzymatic activity and have higher thrombotic potential. Hence, increased mean platelet volume (MPV) and platelet distribution width (PDW), that might be linked with increased thrombotic potential.

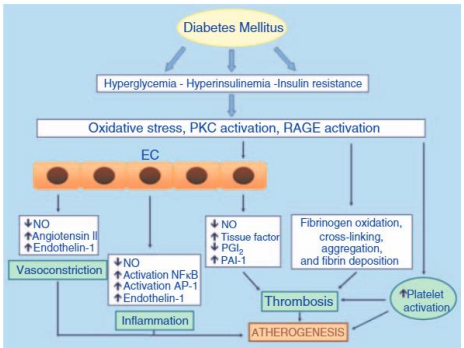

Figure. 1. Increased oxidative stress, protein kinase C (PKC) and receptor for advanced glycation end products (RAGE) causing Metabolic disturbance in diabetes (59)

The association of increased MPV, PDW, P-LCR, and platelet count with diseases related to endothelial dysfunction such as metabolic syndrome, diabetes, coronary artery disease (CAD), and malignancy has been shown in many studies.(60) The newer hematological analyzers are giving variety of platelet parameters which help in easy detection of change in platelet structure. It may help in early detection of prothrombotic state of the platelets. These can act as an alarm for diagnosing initiation/progression of diabetic complications. Hence, in view of this, we aim to study platelet parameters in type 2 diabetes and its predictive role in diabetic angiopathies.

In recent years, there has been renewed interest in hematological parameters such as white blood count (WBC), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), platelet count, platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR). All of the mare designated as predictors of endothelial dysfunction and inflammation. MPV and PDW are predictive biomarkers of diabetic vascular complications. They are more significant in microvascular complications than macrovascular complications.(61)

Elevated white blood cell count (WBC) is a conventional inflammatory indicator and is associated with several cardiovascular disease risk factors and DM. The relationship of raised MPV, PDW, PCT and platelet count with diseases are related to endothelial dysfunction and inflammation such as metabolic syndrome, diabetes, coronary artery disease and malignancy.(62), (63)Activation and size of platelets have significant role in the pathogenesis DM complications.

The mean platelet volume (MPV) and the platelet distribution width (PDW) are indictors of subclinical platelet activation and are involved in the pathogenesis of thrombotic events, such as myocardial infarction, cerebral ischemia, coronary and PAD.

Buch A. et al evaluated platelet volume indices (PVI) in T2D with and without complications in comparison to controls. MPV and PDW can be used to predict micro and macrovascular complications in diabetes especially microvascular complications.(61)

Microvascular complications

Nephropathy

One of the useful predictor of diabetic nephropathy T2D patients is microalbuminuria. It also helps in identifying diabetic microangiopathy. The enhanced activation of platelets plays a significant role in pathological change of vascular complications. To confirm these findings a was conducted in which the association of microalbuminuria with MPV were investigated in 354 T2D. A statistically significant difference was found in the median MPV value of microalbuminuria-positive patients and MPV of patients without microalbuminuria ($p=0.004$). There was positive correlation between MPV and 24-hour urine microalbuminuria ($r=0.14$, $p=0.009$). There were no significant differences between patients with HbA1clevels below and above 7% in terms of MPV ($p>0.05$). No correlation was seen between MPV and HbA1c levels ($r= -0.36$, $p=0.64$).(64)

In another clinical study in T2Dthe association of MVP with microalbuminuria was evaluated. The MPV had a positive correlation with microalbuminuria.MPV values of diabetic patients were higher than those of nondiabetics, the highest levels being in diabetics with microalbuminuria.(65)

In a retrospective study the relationship between MPV and HbA1cwere assessed in 60 T2D patients and 50 age- and sex-matched non-diabetic controls. It was revealed that MPV in the diabetic patients was higher than in the controls ($p = 0.001$). MVP was positively correlated with FBG and HbA1c levels and negatively related to platelet count ($p < 0.001$).(66)

Retinopathy

Retinopathy is one of the common microvascular complications of diabetes whose pathophysiological cause is still undetermined.

AyhanTuzcu E et al retrospectively evaluated the impact of MPV on diabetic retinal complications in 192 T2D patients.A statistically significant difference was observed in MPV values between groups 2 and 4 ($P = 0.001$), between groups 3 and 4 ($P = 0.001$), and between groups 1 and 4 ($P = 0.004$). Logistic regression analysis found a 1.40-fold increase in the risk of retinopathy development (OR: 1.404; $P = 0.002$) and a 1.46-fold increase in the risk of proliferative diabetic retinopathy (OR: 1.466; $P = 0.002$) as the MPV value increased.In diabetic patients, the risk of retinopathy development increased with higher MPV (67)

GüngörAA*et al* investigated the correlation between MPV and diabetic retinal complication in T2D. Mean platelet volume levels were investigated in type 2 diabetic patients with and without retinopathy, and in healthy participants. The mean platelet volume was correlated with hemoglobin A1c and body mass index. It was revealed that the MPV levels were greater in patients with T2D and they were greater in diabetics with retinal complications. No significant difference was found in MPV in diabetic patients with and without hypertension or hyperlipidemia. Moreover, No correlation was found between the MPV levels and hemoglobin A1c or body mass index.(68)

In a retrospective studyTetikoğlu M*et al*evaluated the effect of the platelet indices on the retinal complications of diabetes [diabetic retinopathy (DR) and diabetic macular edema (DME)].

It was revealed that the difference in MPV and PCT values between patients with diabetes and healthy participants was statically significant. The MPV values of patients with DME were significantly higher than those of diabetic patients without DME. This MPV values could be a critical risk factor in the development of PDR and DME in patients with diabetic retinopathy.(69)

In a retrospective study Şahin M1*et al* investigated the MPV in 46 diabetic patientsand 90 control subjects with nonarteritic anterior ischemic optic neuropathy (NAION). The difference between the groups with respect to platelet counts (p = 0.76) was not statistically significant. (70)

Neuropathy

Diabetic neuropathy is a microvascular complications associated with time-dependent uncontrolled blood sugar levels.

In a cross-sectional studyJian-bin Su*et al* investigated the association of HbA1c variability with DPN in 563 T2Ds. It was found that in the study 18.1% (n = 102) had DPN, and these same patients had a higher CV-HbA1c than the patients without DPN ($p < 0.001$). The percentage of patients with DPN increased significantly from 6.9% in the first to 19.1% in the second and 28.5% in the third tertile of CV-HbA1c (p for trend < 0.001). The study findings showed that high HbA1c variability is closely associated with DPN in patient with T2D and useful indicator for early identification of DPN.(71)

Xiao W*et al* evaluated the relationship of platelet volume indices including mean platelet volume (MPV) and platelet distribution width (PDW) with

carotid intima-media thickness (IMT) as well as vibration perception threshold (VPT) in T2D patients. In multivariable analysis, both MPV and PDW were significantly associated with VPT ($P = 0.021$ and $P = 0.007$, respectively). However, only PDW, but not MPV, was significantly associated with carotid IMT ($P = 0.002$). These findings implied the predictive value of platelets volume indices in vascular and peripheral neuropathy complications in T2D.(72)

In another retrospective studyInanc M*et al*assessedthe possible relationship between AAION (arteritic anterior ischemic optic neuropathy) and NAION (non-arteritic anterior ischemic optic neuropathy) with blood platelet parameters and NLR (neutrophil-to-lymphocyte ratio). There was statistical significant higher mean NLR only in AAION group compared to the NAION and control groups. It seems platelet function plays an important role in AIONs and NLR that helps in differentiation AAION from NAION.(73)

MATERIALS AND METHODS

1. SOURCE OF DATA:

The information for the study will be collected from patients admitted to

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———— from October 2017 to June 2019.

2. METHOD OF COLLECTION OF DATA:

Information will be collected through prepared proforma from each patient. Qualifying patients will be undergoing detailed history, clinical examination and laboratory investigations.

- **Inclusion Criteria:**
    1. Patientsalreadydiagnosed to have Type 2 diabetes mellitus with more than five years duration of diabetes.
    2. Controls will be non diabetic.
- **Exclusion Criteria:**
    1. Hb<13gm% - males

       Hb<12gm% - females
    2. Non-diabetic subjects with coronary artery disease(ECG changes).
    3. Patients on antiplatelet drugs such as aspirin and clopidogrel.
    4. Diabetic subject with less than five year duration of diabetes.

3. TYPE OF STUDY:

Comparative study

STUDY DESIGN

- The study will be carried out in 98 patients who are already diagnosed to have Type 2 DM with more than five years duration and 98 non-diabetic subjects without known microvascular complication

- All the diabetic and non-diabetic subjects will undergo complete clinical evaluation with specific reference to any micro-vascular complications as well as any drugs taken. Height and weight of all the subjects will be recorded.
- We will measure MPV in the above target groups who had a complete blood count done using an automatic blood counter.
- Venous blood samples will be collected in di-potassium EDTA and tested within 1 hour of collection to minimize variations due to sample aging. Samples will be maintained at room temperature.
- Samples for plasma glucose estimation and HbA1c will be collected in sodium fluoride and di-potassium EDTA, respectively.

4. SAMPLE SIZE:

With the anticipated mean ± SD of MPV of diabetic and non diabetic patient (i.e 7.5 ± 1.1 and 7.1 ± 1.0) respective group based on previous study at 95% confidence level and 80% power, the sample size calculated is 98 per group.

$$n = \frac{(Z_\alpha + Z_\beta)^2 \times SD^2}{d^2}$$

n = sample size.

Z_α and Z_β = level of confidence and power considered in the study.

SD = common standard deviation.

d = difference between two parameters.

Total sample size is equal to 98 + 98 = 196

Statistical Analysis

Data will be expressed as mean ± SD, percentages and diagrams. Co-variables will be compared using independent ‘t’ test or Mann whitney ‘U’ test. Association between variables will be formed using correlation coefficient.

OBSERVATION AND RESULTS

The study enrolled 100 diabetic patients and demographically matched 100 controls with normal blood sugar and no history of diabetes. The mean age of study group was 62.37 and that of control group was 61.26.

The mean RBS of study and control group was 242.03 242.03 and 118.70 respectively. The fasting and post-prandial blood sugar as well as HbA1C of control group is within normal range. The mean FBS, PPBS and HbA1C values of diabetic group in study are 182.70, 211.83 and 9.069 respectively.

Comparison of Baseline characteristics between two groups

Variables	Compared groups	Mean	Std. Deviation	t test/Mann Whitney U test	P value	Remark
Age	Study group	62.37	10.996	t=0.78	P=0.408	NS
	Control group	61.26	11.576			
RBS	Study group	242.03	108.321	U=1169.500	P=0.0001	HS
	Control group	118.70	27.950			
FBS	Study group	182.70	87.653	U=2486.50	P=0.0001	HS
	Control group	112.00	20.252			
PPBS	Study group	211.83	83.051	U=1934.500	P=0.0001	HS
	Control group	133.04	20.383			
HbA1C	Study group	9.069	2.65	U=727.000	P=0.0001	HS
	Control group	5.723	0.880			
MPV	Study group	12.017	1.227	U=1204.500	P=0.0001	HS
	Control group	9.737	1.365			
Platelet	Study group	2.541	0.837	U=4938.000	P=0.201	NS
	Control group	2.433	0.916			

serum	Study group	1.707	1.827	U=4356.500	P=0.009	HS
creatinine	Control group	0.892	0.272			
Systolic	Study group	136.70	17.330	U=3375.500	P=0.0001	HS
B.P	Control group	124.25	7.335			
Diastolic	Study group	81.74	11.636	U=4203.000	P=0.0001	HS
B.P	Control group	76.65	4.952			

NS: Not significant, HS: Highly significant

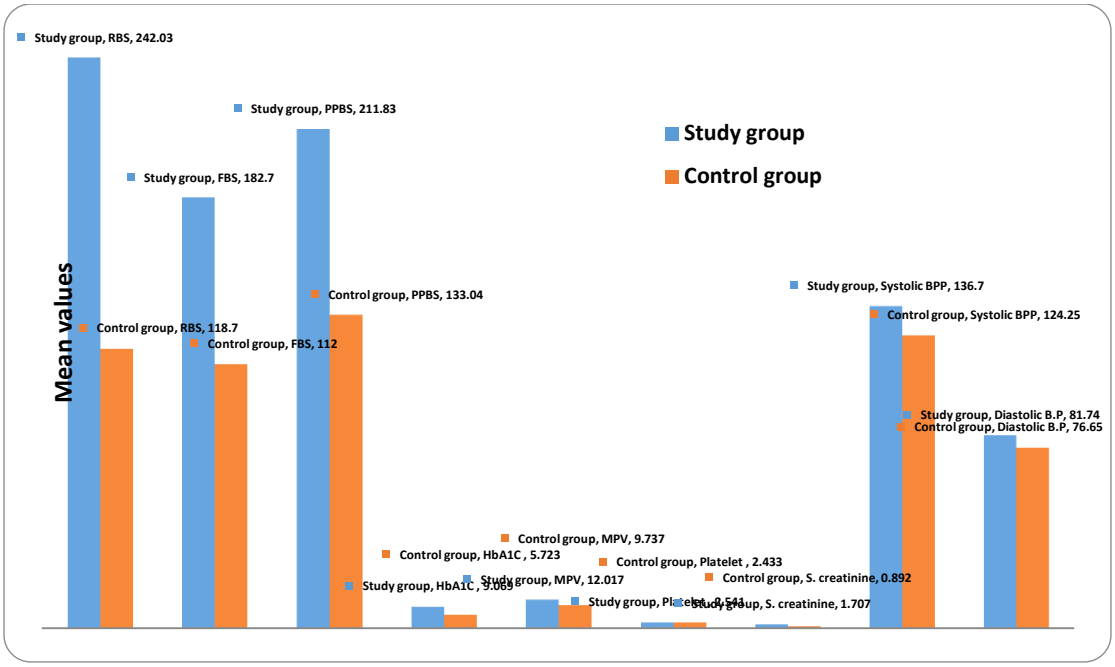
The mean MPV of study group is 12.017 and that of control group is 9.737.

The platelet count of study and control group are 2.541 and 2.433 respectively. This value is not significantly different for study and control group.

The serum creatinine of study group is 1.707 and that of control group is 0.892.

The study groupmean systolic and diastolic blood pressure is 136.70 and 81.74 mm of Hg. Whereas, the control group systolic and diastolic blood pressure is 124.25 and 76.65 mm of Hg.

Other than age and platelet count which are non-significant for study group vs. control group. The values of RBS, FBS, PPBS, HbA1C,MPV, serum creatinine, systolic and diastolic blood pressure were highly significant as compared for study and control group.

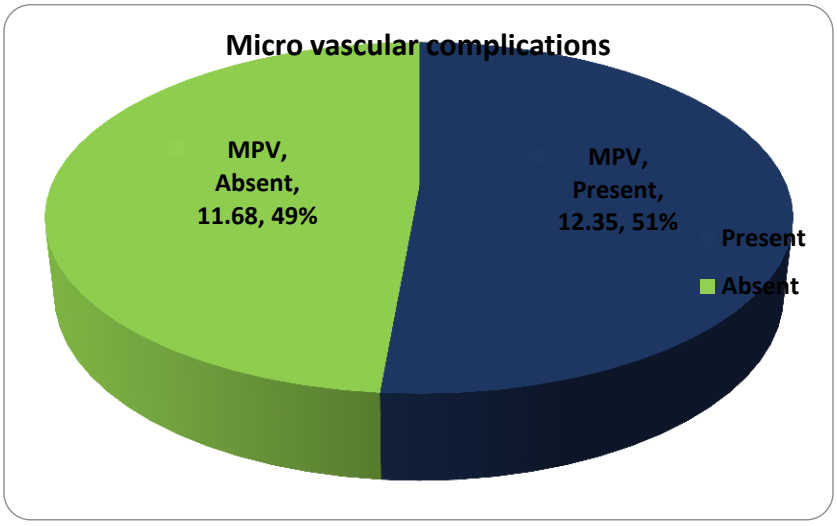


The comparison of MPV with and without micro vascular complications in study group shows that mean(median) MPV in presence of Micro vascular complications was12.35(12.7). It is highly significant with P=0.0139.

Comparison of MPV with and without micro vascular complications in study group

Micro vascular complications	N	Platelet Indices- MPV Mean(Median)	Std. Deviation	Mann Whitney U test	P value	Remark
Present	50	12.35(12.7)	0.89	U=922.00	P=0.0139	HS
Absent	50	11.68(12.5)	1.42			

HS: Highly significant



The correlation between HbA1C and MPV shows that at HbA1C <6.5, correlation coefficient® with MPV was r=0.2645 with P=0.3050. Thus, there is moderate positive correlation which is statistically not significant. At HbA1C ≥ 6.5, correlation coefficient® with MPV was r=**0.428** with P=0.0426. Thus, there is moderate positive correlation which is statistically significant.

Table Correlation between HbA1C and MPV

Correlation between HbA1C and MPV

Correlation between	Correlation coefficient®	P value	Remark
HbA1C(<6.5) and MPV	r=0.2645	P=0.3050 NS	Moderate positive correlation. statistically not significant
HbA1C(≥6.5) and MPV	**r=-0.428**	P=0.0426 Sign	Moderate positive correlation. statistically significant.

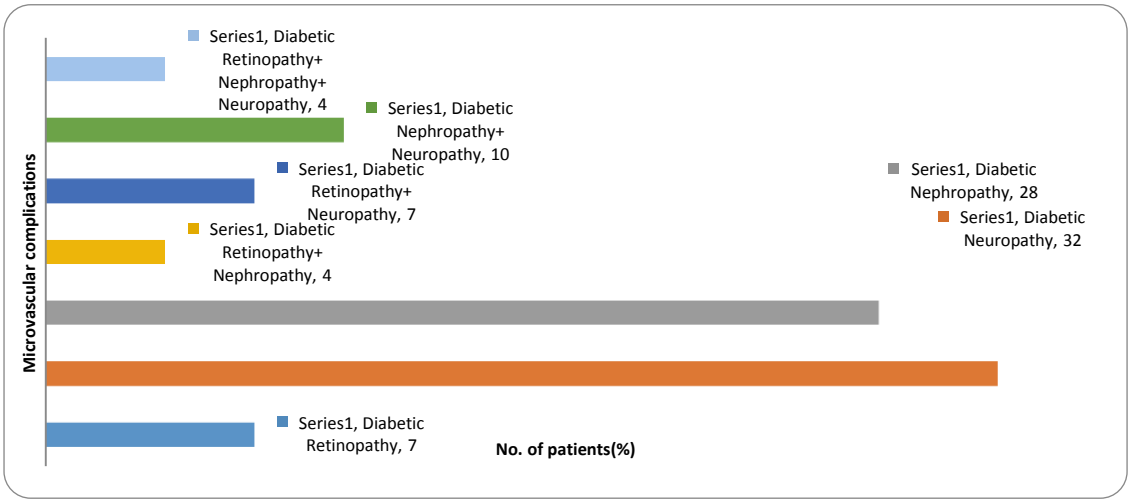
NS: Not significant Sign :Significant

Of the 100 diabetic patients in study group 92 individuals had microvascular complications. They were noted as diabetic retinopathy, diabetic neuropathy and diabetic nephropathy. The diabetic neuropathy was most common as a single microvascular complication. Few patients had any two of the three common microvascular complications.

Whereas, 4 patients had all three microvascular complications. Among all these presentations, diabetic neuropathy was most common as a single microvascular complication at 28%.

Table:Microvascular complications

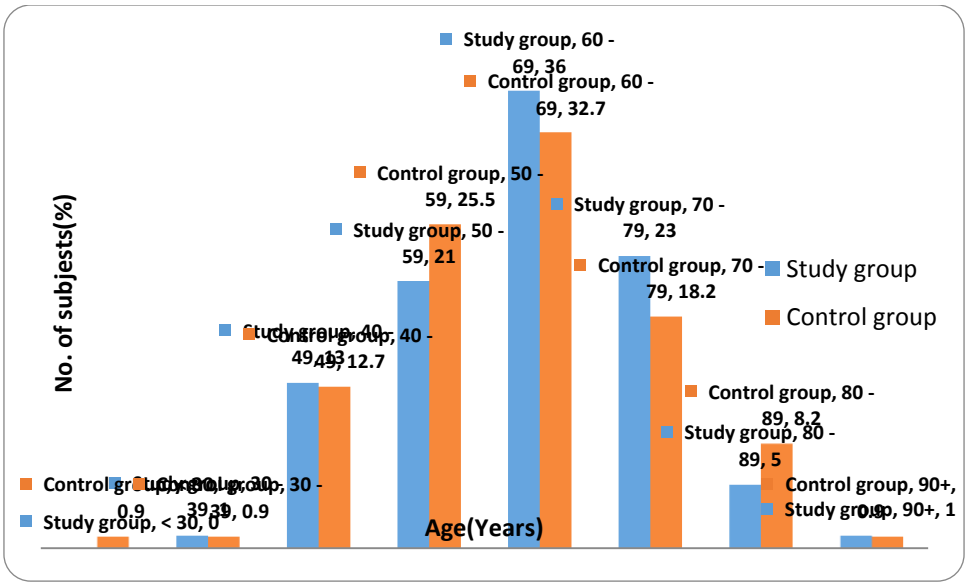
Microvascular complications	No. of subjects	Perecentage
Diabetic Retinopathy	7	7%
Diabetic Neuropathy	32	32%
Diabetic Nephropathy	28	28%
Diabetic Retinopathy+ Diabetic Nephropathy	4	4%
Diabetic Retinopathy+ Diabetic Neuropathy	7	7%
Diabetic Nephropathy+ Diabetic Neuropathy	10	10%
Diabetic Retinopathy+ Diabetic Nephropathy+ Diabetic Neuropathy	4	4%



Amongst the study population, no subject of < 30 years age was enrolled. Maximum number of study subjects belonged to age group 60-69. But as the study population is too small to represent cross section of diabetic patients of the region, the results cannot be extrapolated to general population.

Distribution of respondents according to Age(Years)

Age(Years)	Study group		Control group	
	No. of subjects	Percentage	No. of subjects	Percentage
< 30	0	0	1	0.9
30 - 39	1	1.0	1	0.9
40 - 49	13	13.0	14	12.7
50 - 59	21	21.0	28	25.5
60 - 69	36	36.0	36	32.7
70 - 79	23	23.0	20	18.2
80 - 89	5	5.0	9	8.2
90+	1	1.0	1	0.9
Total	100	100.0	110	100.0

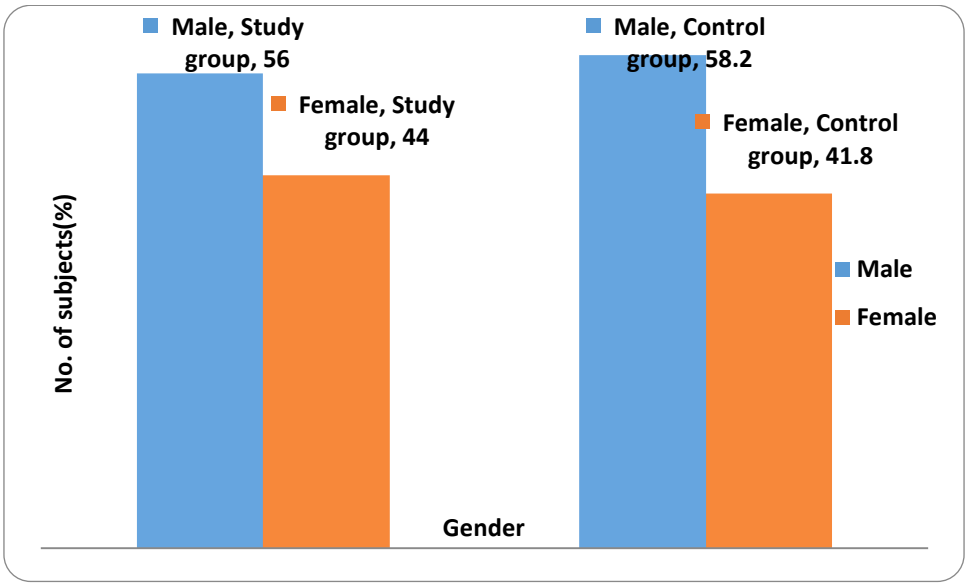


The microvascular complications were totally absent in the control group.

The gender distribution among two groups was 56 male and 44 female in study group and 64 male and 46 female in control group.

Distribution of respondents according to Gender

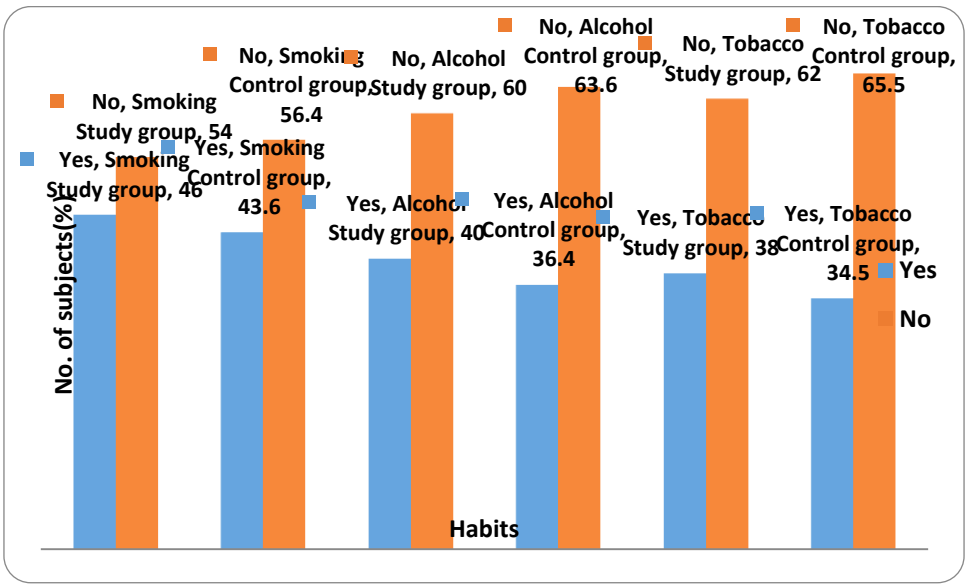
Gender	Study group		Control group	
	No. of subjects	Percentage	No. of subjects	Percentage
Male	56	56.0	64	58.2
Female	44	44.0	46	41.8
Total	100	100.0	110	100.0



The addiction profile of both groups was checked with alcohol, tobacco consumption and smoking. The addiction does not seem to affect MPV profile as both groups have comparable number of members with either of the addictions.

Distribution of respondents according to Habits

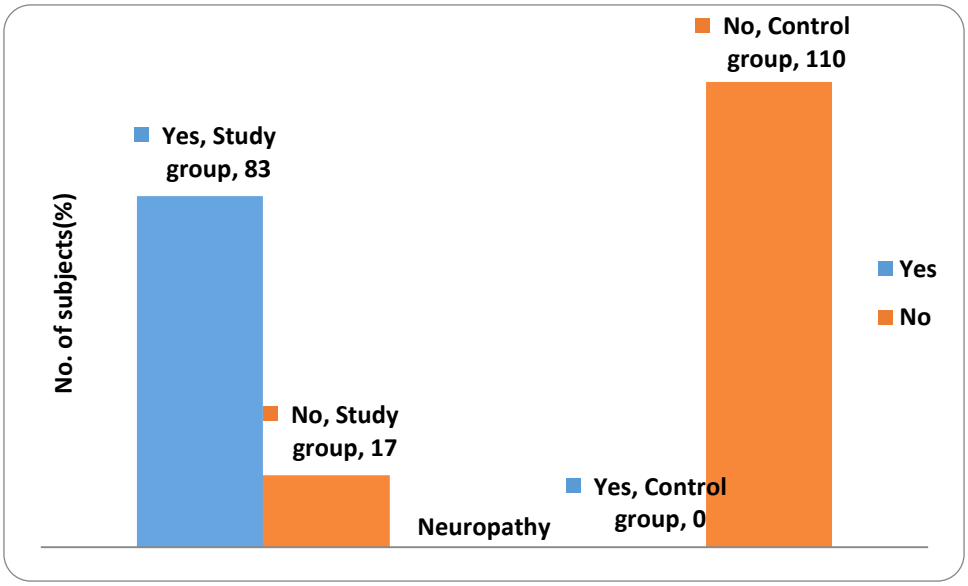
Habits	Study group		Control group	
	No. of subjects	Parecentage	No. of subjects	Parecentage
Smoking				
Yes	46	46.0	48	43.6
No	54	54.0	62	56.4
Alcohol				
Yes	40	40.0	40	36.4
No	60	60.0	70	63.6
Tobacco				
Yes	38	38.0	38	34.5
No	62	62.0	72	65.5
Total	100	100.0	110	100.0



Among the 100 diabetic participants of study group, 83 subjects had neuropathy.

Distribution of respondents according to Neuropathy

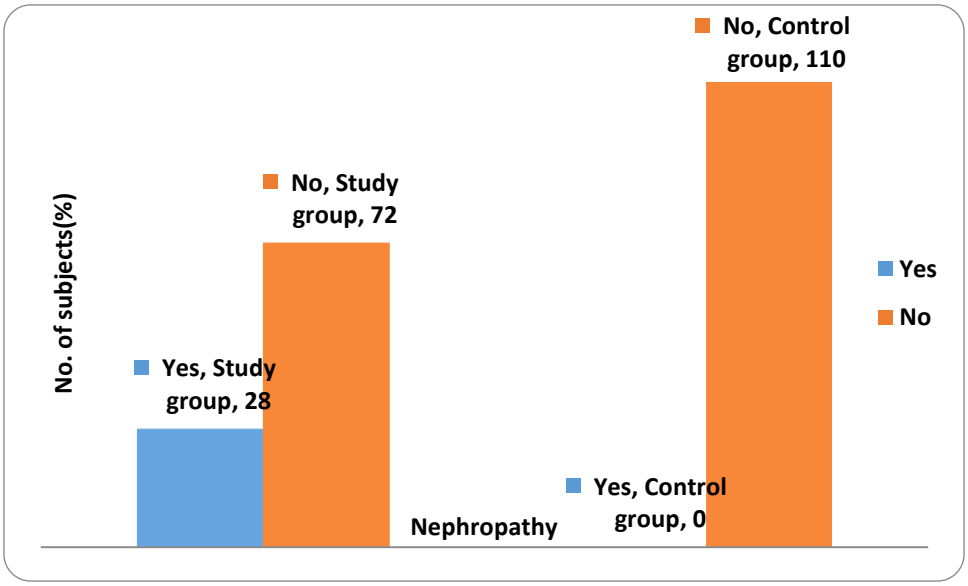
Neuropathy	Study group		Control group	
	No. of subjects	Percentage	No. of subjects	Percentage
Yes	83	83.0	0	0
No	17	17.0	110	110
Total	100	100.0	110	100.0



Among the 100 diabetic participants of study group, 28 subjects had nephropathy.

Distribution of respondents according to Nephropathy

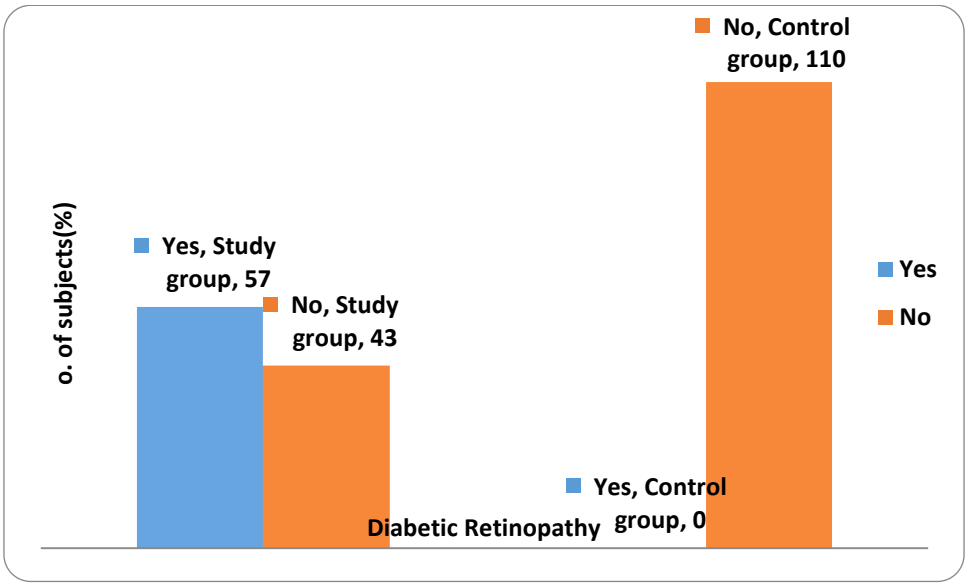
Nephropathy	Study group		Control group	
	No. of subjects	Percentage	No. of subjects	Percentage
Yes	28	28.0	0	0
No	72	72	110	110
Total	100	100.0	110	100.0



Among the 100 diabetic participants of study group, 57 subjects had retinopathy.

Distribution of respondents according to Diabetic Retinopathy

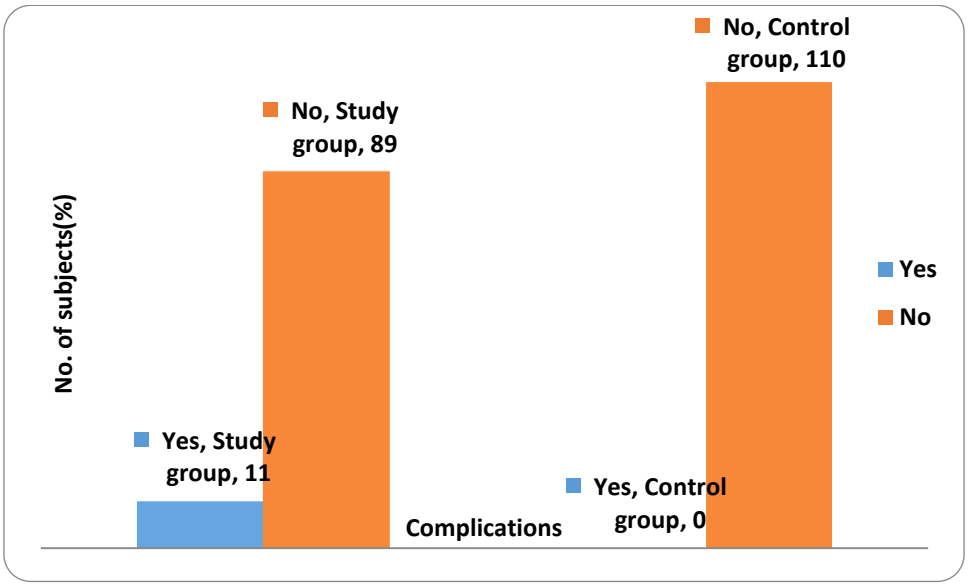
Diabetic	Study group		Control group	
Retinopathy	No. of subjects	Percentage	No. of subjects	Percentage
Yes	57	57.0	0	0
No	43	43.0	110	110
Total	100	100.0	110	100.0



The complications of diabetes were present in 89 subjects of study group.

Distribution of respondents according to Complications

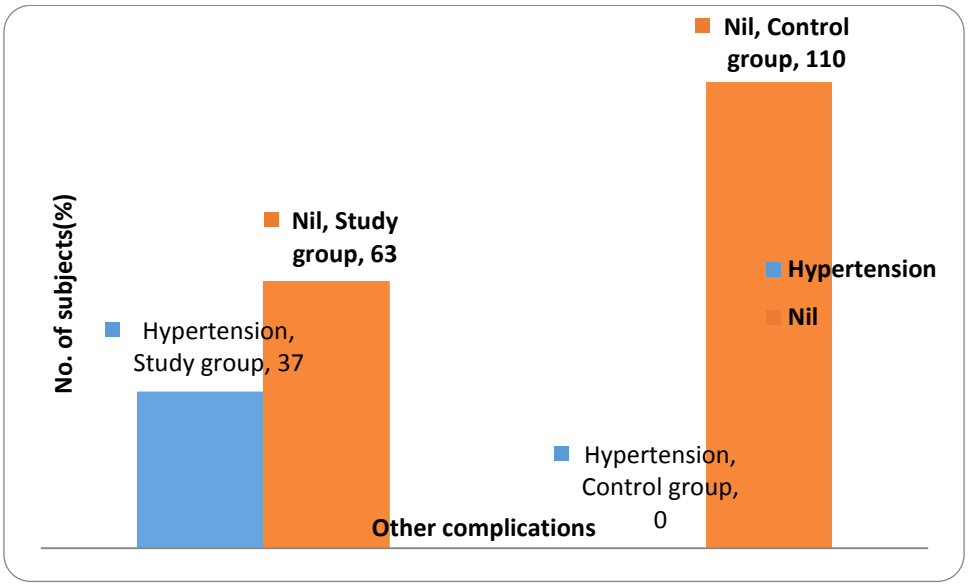
Complications	Study group		Control group	
	No. of subjects	Percentage	No. of subjects	Percentage
Absent	11	11.0	0	0
Present	89	89.0	110	110
Total	100	100.0	110	100.0



The co-morbidity of hypertension was noted in 37 subjects of study group. It was absent in control group.

Distribution of respondents according to Others

Others	Study group		Control group	
	No. of subjects	Percentage	No. of subjects	Percentage
HTN	37	37.0	0	0
Nil	63	63.0	110	110
Total	100	100.0	110	100.0



The minimum and maximum age of study group participants was 35 and 92 years respectively. Their mean systolic blood pressure was 136.70 mm of Hg. The minimum diastolic BP recorded was 70 and maximum was 130 mm of Hg. The maximum recorded RBS among study participants was 625. The maximum FBS and PPBS values were 416 and 429 respectively. The mean HbA1C recorded was 9.069 with SD of 2.654. The mean MPV was 12.017. The platelet count was in range of 0.78 to 5.15. The mean serum creatinine of the study group was 1.707 with SD of 1.827.

Study group	Minimum	Maximum	Mean	Std. Deviation
Age	35	92	62.37	10.996
SYSTILIC B.P	110	190	136.70	17.330
DIOSTOLIC B.P	70	130	81.74	11.636
RBS	82	625	242.03	108.321
FBS	61	416	182.70	87.653
PPBS	69	429	211.83	83.051
HbA1C	5.20	16.90	9.069	2.654
MPV	8.40	13.00	12.017	1.227
Platelet	0.78	5.15	2.542	0.837
serum creatinine	0.4	9.5	1.707	1.827

The minimum and maximum age of control group participants was 22 and 92 years respectively. Their mean systolic blood pressure was 124.25 mm of Hg. The minimum diastolic BP recorded was 70 and maximum was 90 mm of Hg.

The maximum recorded RBS among study participants was 210. The maximum FBS and PPBS values were 162 and 203 respectively. The mean HbA1C recorded was 5.723 with SD of 0.880. The mean MPV was 9.737. The platelet count was in range of 0.70 to 5.61.

Control group	Minimum	Maximum	Mean	Std. Deviation
Age	22	92	61.26	11.576
SYSTILIC B.P	110	136	124.25	7.335
DIOSTOLIC B.P	70	90	76.65	4.952
RBS	53	210	118.70	27.950
FBS	50	162	112.00	20.252
PPBS	75	203	133.04	20.383
HbA1C	3.50	9.70	5.723	0.880
MPV	2.15	12.10	9.737	1.365
Platelet	0.70	5.61	2.434	0.917

DISCUSSION

The diabetes has high global prevalence and its complications are more evident in developing world.(74) The prevalence of microvascular complications of DM is more in patients with poor blood glucose control, chronic DM, uncontrolled blood pressure, tobacco intake and higher BMI.(75), (2)The metabolic syndrome that presents as diabetes is basically uncontrolled and persistent hyperglycemia. It further lead to metabolic dysregulation that present as secondary micro‐ and macrovascular complications.(76)The present study focus on identifying and confirming the usefulness of platelet indices in early identification complications due to DM. Since these indices are easily available and relatively non-invasive and cheap, they would be of immense benefit.

The platelet reactivity is increased in diabetic individuals due to hyperglycemia and insulin resistance. Uncontrolled blood glucose levels are responsible for several interrelated transformations that can lead to dysfunction of endothelial and arterial injuries.(77)

The various mechanisms involved in vascular injuries due to hyperglycemia are the production of advanced glycation end products, stimulation of protein kinase C and disorders in polyol pathways Hyperglycemia reflects as prothrombotic state with increased coagulation, impaired fibrinolysis, and endothelial dysfunction.(78)
Small discoid platelets achieve hemostasis with primary plug formation that seals the vascular defects and its cell wall recruite and activate coagulation factors. Their secretion and aggregation form a thrombus in response to the endothelial stimuli including thrombin, epinephrine, collagen, ADP (dense storage granules), and thromboxane A2 (activated platelets). Platelets change shape, adhere to subendothelial surfaces. Thus, any defect in platelets further advance atherosclerosis

in diabetes.(77), (79), (80)These are an established contributing factors for the pathophysiology of the thrombotic events and its complications.(79)Since anticoagulant drugs too affect platelet indices, the study did not include any subjects taking anticoagulant drugs.(81)

The study enrolled diabetic (n=100) individuals and 100 non-diabetic control whose demographic profile was comparable to avoid bias. The profile took into account their age, gender, habits (alcohol, smoking and tobacco). The study group participants with diabetes belonging to age group 60-69 were highest in number where as no participant below the age of 30 years was included in the study. The age wise distribution of the study population however does not represent the incidence of diabetes among general population. The control group had zero incidence of co-existing morbidity of hypertension. The study has compared baseline characters between the study and control group. Except for the platelet (NS), the RBS, FBS, PPBS, HbA1C, MPV, serum creatinine, systolic and diastolic blood pressure were highly significant in study group. However, when MPV was compared in two subgroups of study group, namely with and without micro vascular complications; the platelet indices measured as MPV was highly significant in 50 participants.When the values of HbA1C and MPV are compared, at HbA1C <6.5, the MPV exhibit moderate positive correlation though, it is statistically not significant. Whereas, at HbA1C(≥6.5) the MPV exhibit moderate positive correlation, that is statistically significant.

More than average size, MPV indicates presence of larger, younger platelets that are more responsive and aggregable. Larger platelets consist of denser granules,produce extra serotonin, thromboxane A2 and β thromboglobulin, than smaller platelets. (53), (80)

The pro-coagulant action of platelet is responsible for vascular complications due to thrombus formation. State of thrombogenesis is the result of MPV and diabetic vascular complications.(2)The rupture of atherothrombotic plaques, hyper reactivity of platelets, and bone marrow stimulation add to complications of diabetes, which itself is a 'prothrombotic state' with higher platelet reactivity. Experimental studies have demonstrated platelet hyperactivity in diabetic animals, both in vivo and in vitro.(82)

Among the 100 study group participants with diabetes, total 92 patients had one or more microvascular complications involving retina, kidney and nervous system. Of the microvascular complications evident in the form of retinopathy, nephropathy and neuropathy, diabetic neuropathy was most common followed by diabetic nephropathy. In patients having more than one form of microvascular complications, diabetic nephropathy along with neuropathy was frequently reported. Few diabetic patients enrolled in the study group had all the three microvascular complications, namely diabetic retinopathy, diabetic nephropathy and diabetic neuropathy.

However, the rate of complications irrespective of optimum diabetic control clearly indicate multifactorial influence.(53), (80), (83) These include biochemical entities such as hyperglycemia and hyperlipidemia, insulin resistance, an inflammatory and oxidant state and nonenzymatic glycation of proteins and growth factors.(84), (85)

The study by Kodiatte et al(1), Demirtunc et al.(76) and Zuberi et al.(2)show significant rise in platelet count in diabetic individuals. But, Hekimsoy Z et al. did not find any correlation between MPV and FSG in patients with type 2 diabetes mellitus.(86)The results of Zaccardi et al (60) show higher mean platelet volume and

platelet distribution width values in diabetics, but nondifferent platelet count as compared with subjects without T2DM. It is in accordance with our findings where platelet count was not significantly different in diabetic and non-diabetic participants. Another study by Zaccardi et al(57)indicate that accounting for confounders and glucose control, the T2DM patients have higher platelet mass and different volume-count kinetics than T1DM. Long-term glucose control seemed to influence platelet mass and the volume-count relationship only in T1DM subjects.

However, in the study by Hekimsoy et al.(86)the diabetic group had lower platelet count among diabetic patients than control group. Thus, the platelet count itself is multifactorial parameter dependent on several variables, that is, mean platelet survival, platelet production rate, and turnover rate in DM. Kakouros et al too suggested that platelet hyperreactivity and increased baseline activation in patients with diabetes is multifactorial and connected to biochemical factors such as hyperglycemia, insulin resistance and hyperlipidemia.(87)Platelets have insulin binding site and it is assumed that insulin reduces platelet responses against aggregant factors including thrombin, ADP and platelet activating factor. Thus it is found that insulin resistance results in platelet dysfunction.(83)

MPV and platelet count exhibit nonlinear, inverse relationship in normal subjects at platelet count within the normal range. The change in MPV is most pronounced at the lower platelet counts. Thus, the definition of ‘normal’ values for MPV requires simultaneous reference to platelet count.(34)

Kaplan ZS and Jackson SP demonstrated that platelets are involved in the initiation and development of atherosclerosis. The increased platelet activity enhances the vascular complications related to T2D.Andersson C. et al. reported an association between high baseline HbA1c in T2D and increased cardiovascular risk.(88)In our

study, the patients in DM group had significantly higher MPV than the non-diabetic individuals. This is in concurrence with the findings seen in studies done by Kodiatte et al,(89)Hekimsoy et al.,(53)Demirtunc et al.,(76)Zuberi et al.,(57)Atea et al., Jindal et al.,(82) and Papanas et al.(55)MPV can also be high because of an atherothrombotic event such as acute coronary syndrome. This is secondary to the faster utilization of smaller platelets in the vascular event and compensatory release of reticulated platelets.(90), (91)

In the present study, increased values of MPV were seenin all patients with diabetes. Among 100 diabetic participants, the percentage microvascular complications observed was retinopathy (57%), nephropathy (28%) and neuropathy (83%). Papanasetal. has also reported similar findings. (55)Thisindicates increased platelet activity is involved the development of vascular complications.

MPV was associated with poor glycometabolic control and it is also reflected in many complications such as retinopathy, nephropathy, CAD, and diabetic foot in study by Buch et al.(61)

A statistically significant correlation of MPV with microvascular complications such as diabetic retinopathy and diabetic nephropathy; (92), (93)and similarly higher values were seen in the studies done by Dindar *et al*., Ates *et al*. (94), (95)MPV was also associated with retinal neovascularization of diabetic retinopathy. Platelets in diabetics are active and have increased aggregation because of dysregulated signaling pathway. This contributes to thrombus formation and microcapillary embolization. The release of constrictive, oxidative, and mitogenic substances such as platelet-derived growth factor and vascular endothelial growth factor accelerates the progression of local vascular lesions such as neovascularization

of lens in diabetic retinopathy. On the other hand, MPV was not significantly different in patients with these complications in studies done by Demirtunc *et al*.(96)

Increased MPV was associated with poor glycometabolic control and it is also reflected in many complications such as retinopathy, nephropathy, CAD, and diabetic foot.(61) The duration of diabetes has a significant relation with increased MPV.(61) But in our study we have not taken duration of diabetes as a parameter. Discordant results were also found in other studies.(1)Platelet count, PDW, P-LCR had no statistically significant correlation with the duration of DM. Platelet number and reactivity along with the cardiovascular comorbidities such as hypertension, albuminuria, obesity, cigarette smoking, and dyslipidemia also contributes to the progression of diabetes and its effect on platelet indices. Thus, it shows that there are many other factors which may account for the thrombotic potential of diabetics with time.(97)

In study by Fahin et al,(98) the MPV values were significantly higher compared to control group which point towards its contribution to the pathogenesis of nonarteritic anterior ischemic optic neuropathy (NAION). As larger platelets are hemostatically more active, they produce more prothrombotic factors.(99) Bath and Butterworth reported that the platelet hyperactivity results in an increase in MPV.(100) Larger platelets aggregate easier than smaller ones. (101)

On the other hand, in the studies done by Hekimsoy et al. and Demirtunc et al. MPV was not significantly different in subjects with diabetic neuropathy/retinopathy from that of diabetics without those complications. Its probable reason is rapid consumption of activated platelets in diabetics with complications.(53), (76)

A study by Güngör AA et al, Ayhan TE et al and Dindar S et al suggest role of raised MPV in diabetic retinopathy. (68),(67),(66)

A study by Tetikoğlu et al suggest that High MPV values may be an important risk factor for the development of proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME) in patients with diabetic retinopathy.(69)

As per the study report by Bayram et al,(65)microalbuminuria might be related with mean platelet volume in diabetic patients. In our study too raised MPV was associated with diabetic nephropathy in 28% enrolled diabetics.

In our study, MPV was significantly higher in diabetics with HbA1c levels ≥ 6.5% than in diabetics with HbA1c levels <6.5%. There was also a significant association between HbA1c and MPV, which was again seen in the study done by Demirtunc et al.(96)It can be concluded that glycemic control decreases the hyper activity of the platelet function and may prevent or delay possible diabetic vascular complications. However, a larger data base is required to draw a guideline. The improper dietary practices and lack of exercise may contribute to poor glucose control and platelet dysfunction.

The results of study by Panova-Noeva et al showed that age, cardiovascular risk factors (CVRFs) such as smoking, hypertension, and high glucose level were linked with higher MPV in males only. In case of females, oral contraceptives and menstruation were strongly associated with higher MPV**.**(102) In our study too fasting and post-prandial blood sugar (FBS, PPBS), HbA1C, serum creatinine, and both systolic and diastolic Blood pressure were significantly related to MPV.

MPV showed statistically significant increase as the blood pressure increases in previous studies.(61) In our study too both systolic and diastolic blood pressure was significantly high in diabetic group with raised MPV. However, blood pressure had no statistically significant co-relation with platelet count, PDW, and P-LCR.(61)MPV was not associated with change in systolic and diastolic BP in a study done by Dindar.

Renin-angiotensin system blockade with medication and a well-controlled BP could be the reason given for this finding.(94)Platelet changes in hypertension may be secondary to changes in MKs, and that anti-hypertensive treatment can alter MKs and the function of platelets they produce. Since antihypertensive therapy reduces the risk of stroke and myocardial infarction, MKs are a novel therapeutic target for the prevention of vascular events.(103)

LIMITATIONS OF THE STUDY

MPV has presently no role in making diagnosis and defining prognosis in any acquired illness. Better knowledge of the role of platelets in the development and progression of diabetic vascular lesions might allow the discovery of novel pharmacological targets. The platelet indices depend on many factors (mean platelet survival, platelet production rate, and turnover rate in DM), thus it is not possible to establish direct cause-effect relation between MPV and other indices to the occurrence and severity of diabetic microvascular complications.

The platelet indices are also affected by menstruation, thyroid and rheumatic diseases which were not considered in this study. The study could not assess qualitative platelet disorders. The statins alter platelet indices though, this fact was not taken as study parameter. The follow up of the cases was not done to determine the prognostic significance of our findings. This would have enabled us to compare its association with the progress of the microvascular complications. Moreover, it could have been possible to correlate and check the reversibility of platelet dysfunction with glycaemic control over a period of time.

It is not possible to state a causal relationship between MPV and other findings in diabetics and microvascular complications of diabetes. A prospective study is required to compensate for this weakness. Since our study is on Indian population with type 2 diabetes, and the generalizability of our results have restrictions. The HbA1c variability was not assessed in relation to indices of oxidative stress, inflammation or endothelial dysfunction. The study duration is relatively short. The MPV and severity of complications has not been evaluated.

CONCLUSION

Changes in MPV are seen to be statistically associated with diabetes and its complications. They are easily available, simple, convenient, noninvasive, and easy to interpret method to determine platelet dysfunction and in turn predict the presence of microvascular complications.

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ANNEXURE I

ETHICAL CLEARANCE CERTIFICATE

ANNEXURE –II

INFORMED CONSENT FORM

TITLE OF THE PROJECT - To study the correlation between mean platelet volume and HbA1c in Type 2 diabetes mellitus with special reference to microvascular complications.

PRINCIPAL INVESTIGATOR -

P.G.GUIDE NAME -

All aspects of this consent form are explained to the patient in the language understood by him/her.

I) **INFORMED PART**

1) PURPOSE OF RESEARCH:

I have been informed about this study. I have also been given a free choice of participation in this study.

2) PROCEDURE:

I am aware that in addition to routine care received I will be asked series of questions by the investigator. I have been asked to undergo the necessary investigations and treatment, which will help the investigator in this study.

3) RISK AND DISCOMFORTS:

I understand that I may experience some pain and discomfort during the examination or during my treatment. This is mainly the result of my condition and the procedure of this study is not expected to exaggerate these feelings that are associated with the usual course of treatment.

4) BENEFITS:

I understand that my participation in this study will help to patient's survival and better outcome.

5) CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of Hospital records and will be subject to the confidentiality and privacy regulation. Information of a sensitive personal nature will not be a part of the medical records, but will be stored in the investigator's research file and identified only by code number. The code-key connecting name to numbers will be kept in a separate location.

If the data are used for publication in the medical literature or for teaching purpose, no name will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand that I may see the photographs and videotapes and hear the audiotapes before giving this permission.

6) REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at anytime. _____ is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation.

If during the study, or later, I wish to discuss my participation in or concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital is available to talk with me. A copy of this consent form will be given to me to keep for careful reading.

7) REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that _____ _____may terminate my participation in the study after she has explained the reasons for doing so and has helped arrange for my continued care by my own physician or physical therapist, if this is appropriate

8) INJURY STATEMENT:

I understand that in the unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, the appropriate treatment would be available to me, but no further compensation would be provided. I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

I have explained to _____the purpose of the research, the procedures required and the possible risks and benefits to the best of my ability in patient's own language.

_____ _____

Date (Investigator)

APPENDIX –III

PERFORMA

STUDY OF CORRELATION BETWEEN Hb1Ac AND MEAN PLATELET VOLUME IN TYPE 2 DIABETES MELLITUS.

Name:	CASE NO:
Age:	IP NO:
Sex:	DOA:
Religion:	DOD:
Occupation:	
Residence:	

Presenting complaints with duration:

History of present complaints:

Past History:

Family History:

Personal History:

Diet/appetite

Sleep

Bladder and bowel habits:

Addictions

Drug allergy

Treatment History:

General Physical Examination

Height:

Weight:

Body Mass Index:

Vitals

PR:

BP:

RR:

Temp:

Neck:

Upper Limbs:

Chest:

Abdomen:

Lower Limbs:

Skin:

SYSTEMIC EXAMINATION.

- Respiratory System

- Cardiovascular System

- Central Nervous System

- Per abdomen

INVESTIGATIONS

PATHOLOGY

Complete Blood Count Hemoglobin	Gm/dl
Total Count	Cells/cumm
Differencial counts Neutrophils	%
Lymphocytes	%
Eosinophils	%
Monocytes	%
Platelets	Cells/cumm
ESR	At the end of one hour
Urine routine	
Urine albumin	
Urine sugar	
Urine Microscopy RBC Pus cells Cast Epithelial cell	

BIOCHEMISTRY

Random Blood sugar	
FBS	
PPBS	
Hb1Ac	
Serum Creatinine	

USG ABDOMEN(If done)

ECG

FUNDUS EXAMINATION

FINAL DIAGONOSIS

MASTER CHART

STUDY GROUP (N= 98)																			
					HABITS			CLINICAL PICTURE				LABS							
Sr. No.	I.P No.	Age	Sex	B.P	Smoking	Alcohol	Tobacco	Diabetes	Neuropathy	Nephropathy	Diabetic Retinopathy	RBS	FBS	PPBS	HbA1C	MPV	Platelet	serum creatinine	Others
1	2018/ 7941	45	M	156/100	+	+	-	10yrs	+	-	+	193	294	400	8.3	10	2.26	0.9	HTN
2	2018/ 8313	47	M	110/70	-	+	-	10yrs	+	+	-	420	241	346	6.8	10.8	2.19	2.7	
3	2018/8427	53	F	120/70	-	-	-	10yrs	+	-	+	494	89	402	16	8.4	3.61	0.7	
4	2018/8606	65	F	130/80	-	-	-	10yrs	+	-	-	426	232	302	9.1	9.8	1.5	0.8	
5	2019/21157	48	F	110/70	+	+	+	7yrs	+	-	-	167	209	87	11.3	10.9	2.84	0.6	
6	2018/9351	70	F	150/70	-	-	-	10yrs	+	-	-	267	214	266	5.7	10.1	2.08	0.8	HTN
7	2019/14610	70	M	130/80	-	-	-	12yrs	+	-	-	193	100	240	7.7	10.1	2.18	0.6	
8	2018/10206	62	F	140/70	-	-	-	14yrs	+	-	-	171	123	188	8.6	10.3	2.85	0.8	HTN
9	2018/10671	45	F	110/70	-	-	-	12yrs	+	-	-	500	132	200	9.7	10.8	2.44	0.9	
10	2018/11893	42	M	180/80	+	+	+	7yrs	+	-	+	424	85	212	8.7	9	3.06	0.6	HTN
11	2018/18019	48	F	110/70	-	-	-	15yrs	+	-	+	328	120	246	9	10.1	2.72	1.2	
12	2018/20525	48	F	120/80	-	-	-	10yrs	+	-	-	169	265	314	7.6	9.7	3.51	0.8	
13	2019/23887	75	M	130/80	-	-	-	15yrs	+	-	+	173	147	119	5.8	10.8	2.16	1.4	
14	2018/21960	48	M	140/90	-	-	-	14yrs	-	-	-	130	119	183	8.3	8.4	2.64	1	
15	2018/25095	42	M	160/110	+	+	+	13yrs	-	-	-	356	339	328	5.6	9.1	4.16	1.2	HTN
16	2019/9899	53	M	160/90	+	+	+	12yrs	+	+	+	277	176	202	6.9	11.7	1.91	7.7	HTN
17	2019/7183	92	M	156/90	+	+	+	40yrs	+	+	+	321	214	215	8.1	9.9	2.11	2.7	HTN

18	2019/7504	63	M	130/80	+	+	+	25yrs	+	+	-	237	151	201	8	8.4	1.5	1.9	
19	2019/7892	52	M	130/80	+	+	-	7yrs	-	-	-	220	167	306	13.7	9.2	2.01	0.5	
20	2019/8069	64	M	160/80	+	+	+	15yrs	+	+	+	335	365	372	5.9	10.4	1.84	4	HTN
21	2019/8070	60	M	150/80	+	+	+	12yrs	+	+	+	200	137	203	9.4	10	3.8	6	
22	2019/8305	72	F	130/80	-	-	-	20yrs	+	-	-	143	114	150	7.8	9.5	2.25	1.1	
23	2019/8376	60	M	142/82	+	+	-	8yrs	+	-	-	280	243	97	10.6	10.8	2.51	1.2	
24	2019/8611	60	M	122/80	+	-	-	10yrs	-	-	-	140	110	139	6.2	12.2	1.8	0.7	
25	2019/8988	65	M	170/80	+	+	+	10yrs	-	-	+	163	130	153	7.2	11.2	1.69	1.2	HTN
26	2019/9099	60	M	130/70	+	+	+	9yrs	+	-	+	151	99	221	8.3	10.7	2.6	0.7	
27	2019/9245	55	M	120/70	+	-	-	7yrs	+	-	-	110	104	159	5.8	9.8	2.69	0.8	
28	2019/9284	59	M	162/100	+	+	+	15yrs	+	-	+	138	326	400	10.5	10.7	2.46	0.9	HTN
29	2019/9605	58	F	160/70	-	-	-	10yrs	+	+	+	259	66	147	6.2	11.5	2.13	9.5	HTN
30	2019/9760	55	M	132/80	+	-	-	12yrs	+	+	-	138	113	133	11.1	10.9	1.1	3.4	
31	2019/16632	72	M	130/90	+	-	-	11yrs	+	-	-	140	140	160	5.7	11.5	1.89	1.1	
32	2019/9920	86	M	156/100	+	+	+	25yrs	+	+	+	406	378	376	8.9	10	2.62	2	HTN
33	2019/10210	50	M	124/80	-	+	+	8yrs	+	+	-	90	173	280	8.3	9	1.63	4.4	
34	2019/10944	35	F	120/70	+	-	-	7yrs	-	-	+	412	243	200	13.6	8.8	5.15	0.5	
35	2019/11050	58	M	130/80	+	-	-	11yrs	+	-	-	211	61	69	8.3	9	1.69	0.7	
36	2019/11615	72	F	134/80	-	-	+	12yrs	+	-	+	179	100	145	5.9	8	2.46	1	
37	2019/12183	60	M	140/80	+	+	+	10yrs	+	+	+	211	129	180	9.1	10.1	2.85	3.3	HTN
38	2019/12712	70	M	160/110	+	+	+	12yrs	+	-	+	248	148	184	9.5	9.8	3.68	0.6	HTN
39	2019/12808	63	M	158/90	+	+	-	11yrs	+	+	+	625	416	329	16.9	10.9	3.18	4	HTN
40	2019/13004	76	M	128/80	+	+	+	15yrs	+	+	+	176	122	203	6.1	10.5	1.57	3.9	
41	2019/13212	70	F	110/70	-	-	-	15yrs	+	+	-	257	170	196	7.3	10.9	1.22	3.4	
42	2019/13463	48	F	120/80	-	-	-	11yrs	-	-	-	164	353	158	9.6	10.8	1.98	0.5	
43	2019/13468	75	M	110/70	+	+	+	11yrs	+	+	+	370	312	124	7	9.3	2.2	1.7	
44	2019/13599	75	F	130/80	-	-	-	10yrs	+	+	+	240	198	186	6.3	10	2.75	2.5	
45	2019/14160	60	M	132/80	+	+	+	10yrs	+	-	+	191	231	215	8.3	10	1.52	0.8	
46	2019/14355	75	F	120/80	-	-	-	11yrs	+	-	+	203	116	150	6	11	1.6	0.7	

47	2019/14700	76	F	150/90	-	-	-	12yrs	+	-	+	133	130	258	8.2	9.5	2.06	0.8	HTN
48	2019/14793	61	M	130/80	+	+	+	10yrs	+	+	+	147	152	150	6.8	10.3	3.44	2.4	
49	2019/15032	76	F	130/70	-	-	-	10yrs	+	-	+	116	110	130	6.9	9.5	3.42	0.9	
50	2019/15033	66	F	120/70	-	-	-	12yrs	-	-	+	229	193	200	9	11.5	0.78	0.6	
51	2019/15207	54	F	126/80	-	-	-	7yrs	-	-	-	241	139	309	6.7	10.3	1.06	0.5	
52	2019/15672	68	F	156/90	-	-	-	10yrs	+	+	+	132	124	155	7.1	10.5	3.18	3.9	HTN
53	2019/15769	65	F	124/70	-	-	-	10yrs	+	-	+	111	231	137	9.2	5.7	1.4	0.6	
54	2019/15787	55	M	120/70	-	-	-	7yrs	+	-	-	139	151	176	5.8	9.7	1.83	0.8	
55	2019/15908	70	M	130/70	+	+	+	10yrs	+	+	+	170	101	150	11.8	10.5	2.2	1.6	
56	2019/16086	77	F	160/110	-	-	-	12yrs	+	-	+	473	98	133	9.7	10.5	3.1	1.4	HTN
57	2019/16437	55	M	128/80	+	+	+	15yrs	-	+	+	481	352	211	12.5	10.9	2.83	2.2	
58	2019/16488	70	F	130/70	-	-	-	10yrs	+	-	+	242	80	100	7.7	11	1.53	2.4	
59	2019/16626	50	F	120/80	-	-	-	8yrs	+	+	-	345	290	345	8.4	11.8	2.61	2	
60	2019/16632	72	M	130/84	+	+	+	10yrs	+	-	+	152	140	200	5.7	11.5	1.89	1.1	
61	2019/17178	43	M	128/80	-	-	+	8yrs	-	-	-	280	106	122	11.8	7.6	2.92	1.4	
62	2019/17597	49	M	150/90	+	+	+	7yrs	+	-	+	262	185	273	14.5	8.8	2.96	0.8	HTN
63	2019/17714	75	M	140/90	-	+	+	12yrs	+	+	+	108	153	200	6.3	10.8	4.26	6.9	HTN
64	2019/17710	56	F	152/90	-	-	-	9yrs	+	-	+	306	223	153	11	10.4	2.3	0.5	HTN
65	2019/18312	60	F	156/90	-	-	-	15yrs	+	+	-	281	69	117	6	9.3	3.37	6.4	HTN
66	2019/18798	85	M	130/80	+	+	+	15yrs	+	+	+	249	249	110	5.2	10.2	2.34	1.8	
67	2019/18878	65	M	150/80	+	+	+	10yrs	+	-	-	140	69	122	6.5	8.3	3.66	0.8	HTN
68	2019/18929	58	F	128/70	-	-	-	7yrs	-	-	+	98	226	330	10.1	10.2	2.95	0.6	
69	2019/18968	65	F	156/90	-	-	-	7yrs	+	-	-	100	68	95	6.4	9.6	2.43	0.8	HTN
70	2019/18989	75	M	130/70	-	-	-	8yrs	+	-	+	159	265	270	7.8	13.7	3.55	0.5	
71	2019/19324	60	M	120/70	+	-	+	10yrs	+	-	+	300	414	429	13.4	9.9	2.86	1	
72	2019/19657	70	F	150/90	-	-	-	12yrs	+	-	-	135	122	122	7.4	10.7	2.39	0.8	HTN
73	2019/19857	68	M	154/90	+	+	+	10yrs	+	-	+	231	170	180	10.3	11.1	1.87	0.8	HTN
74	2019/19913	57	M	168/70	+	+	+	10yrs	+	-	+	82	105	154	8.1	8.8	3.65	0.6	HTN
75	2019/21741	68	F	154/110	-	-	-	10yrs	+	-	+	383	168	274	12.6	10.1	3.49	0.8	HTN

76	2019/21816	80	M	128/70	+	-	+	12yrs	+		-	142	108	180	14.3	9.9	1.51	1.4	
77	2019/21887	51	M	128/80	-	-	+	7yrs	-	-	-	311	278	300	9.2	12.5	2.33	0.7	
78	2019/22186	80	F	120/90	-	-	-	7yrs	+	-	+	178	124	245	6.3	9.6	3.5	0.8	
79	2019/22485	60	F	160/90	-	-	-	7yrs	-	+	+	209	145	180	9.6	10.6	2.59	6.6	HTN
80	2019/22600	55	M	120/70	+	-	+	8yrs	+	-	+	407	400	353	13	10.7	2.11	0.9	
81	2019/23515	87	M	156/90	-	-	-	15yrs	+	-	-	180	160	200	6.8	9.1	2.43	1.3	HTN
82	2019/23652	70	F	140/80	-	-	-	7yrs	+	-	+	126	132	201	7.9	10	2.19	0.7	HTN
83	2019/21058	70	F	138/70	-	-	-	8yrs	+	-	-	318	386	239	13	10.4	2.58	0.6	
84	2019/21966	50	F	120/80	-	-	-	8yrs	+	-	-	269	156	226	11.9	10.2	2.77	1.3	
85	2019/22527	68	F	126/80	-	-	-	10yrs	+	-	+	415	288	304	7.5	8.6	3.08	0.4	
86	2019/23525	60	M	150/90	+	+	-	7yrs	+	-	+	203	132	188	8.1	9.2	1.59	0.9	HTN
87	2019/24032	50	F	126/70	-	-	-	7yrs	+	-	-	145	166	100	7.3	11	1.55	0.8	
88	2019/24622	50	F	130/90	-	-	-	7yrs	-	-	-	200	263	202	11	10.9	2.72	0.5	
89	2019/24326	65	F	190/100	-	-	-	10yrs	+	-	+	131	137	177	9.6	11.3	2.19	0.8	HTN
90	2019/24618	68	F	130/70	-	-	-	10yrs	+	-	+	316	200	280	16.2	9.9	2.52	0.8	
91	2019/25070	48	F	124/82	-	-	-	7yrs	-	-	-	230	151	132	10.7	10	3.51	0.5	
92	2019/26097	60	M	130/80	+	+	-	10yrs	+	+	+	220	171	226	6.6	10.1	1.07	8.2	
93	2019/26336	68	M	150/80	-	-	+	7yrs	+	+	+	258	181	229	8.5	9.3	4.83	2.8	HTN
94	2019/21157	48	M	120/80	-	+	-	7yrs	-	-	-	167	209	87	11.3	10.9	2.84	0.6	
95	2019/24274	62	M	150/80	+	+	+	7yrs	+	-	-	240	229	201	11.6	10	3.56	0.5	HTN
96	2019/22635	65	M	150/90	+	+	+	8yrs	+	-	-	315	173	209	14.6	10.4	3.17	0.9	HTN
97	2019/21835	70	M	162/110	+	+	-	10yrs	+	-	+	239	92	101	8.7	10.8	3.72	1	HTN
98	2019/21995	65	M	130/80	+	-	+	10yrs	+	-	+	217	90	155	8.3	10.9	1.39	0.9	
99	2019/22683	60	M	110/70	-	-	+	10yrs	+	-	-	266	102	156	10	10	2.93	1.3	
100	2019/23436	66	M	110/70	+	+	-	9yrs	+	+	+	291	95	113	12.4	9.8	2.67	1.7	

CONTROLS GROUP

					HABITS			CLINICAL PICTURE				LABS							
Sr. No.	I.P No.	Age	Sex	B.P	Smoking	Alcohol	Tobacco	Diabetes	Neuropathy	Nephropathy	Diabetic Retinopathy	RBS	FBS	PPBS	HbA1C	MPV	Platelet	serum creatinine	Others
1	2019/7981	45	M	120/80	+	+	+	-	-	-	-	139	107	110	5.6	8.1	3.82	1.5	
2	2019/12452	66	M	126/82	+	+	+	-	-	-	-	141	162	151	6.6	8	2.16	0.8	
3	2019/14610	70	M	130/80	+	-	-	-	-	-	-	150	100	140	7.7	10.1	2.18	0.6	
4	2019/15036	70	M	136/90	-	+	+	-	-	-	-	133	125	140	5.5	11.3	1.52	1	
5	2019/15615	70	F	120/70	-	-	-	-	-	-	-	136	124	140	5.9	11.6	2.44	1.1	
6	2019/15757	56	M	124/70	-	+	+	-	-	-	-	141	88	127	5.1	12.1	1.34	1.1	
7	2019/16084	49	M	130/80	-	-	-	-	-	-	-	210	134	203	5	10.9	4.11	1.2	
8	2019/16048	49	M	130/80	+	+	+	-	-	-	-	140	134	144	5	10.9	4.11	1.2	
9	2019/21475	80	F	120/80	-	-	-	-	-	-	-	109	104	96	5.8	8	1.97	1.1	
10	2019/11831	54	M	110/70	+	-	+	-	-	-	-	153	99	151	4.8	10.5	1.14	0.8	
11	2019/12133	50	M	110/70	+	+	+	-	-	-	-	169	78	130	5.8	10	1.3	1	
12	2019/12116	70	M	130/80	+	-	-	-	-	-	-	102	101	150	6.1	11.2	2.47	1	
13	2019/12941	56	F	132/80	-	-	-	-	-	-	-	139	110	140	3.5	11.8	2.47	0.9	
14	2019/13039	62	F	120/80	-	-	-	-	-	-	-	98	128	130	4.9	12.1	2.38	0.9	
15	2019/13433	63	M	130/80	-	+	+	-	-	-	-	170	104	127	6.2	9.9	3.63	0.7	
16	2019/20587	45	M	120/70	-	-	-	-	-	-	-	101	103	116	5.4	8	2.44	0.7	
17	2019/23597	55	M	124/70	+	+	+	-	-	-	-	143	113	130	5.4	9.4	2.99	0.8	
18	2019/10767	60	F	130/80	-	-	-	-	-	-	-	129	121	142	5.3	9.8	5.61	1	
19	2019/11614	64	M	130/80	+	+	+	-	-	-	-	123	100	105	5.8	9	2.6	0.9	

20	2019/9845	65	F	130/80	-	-	-	-	-	-	-	111	102	122	6.5	10.3	2.18	1.3	
21	2019/26371	22	M	130/80	+	+	-	-	-	-	-	151	105	136	5.4	8.4	3.16	0.9	
22	2019/23297	56	M	120/80	+	+	+	-	-	-	-	115	122	140	5.6	10.4	1.26	0.7	
23	2019/9935	60	M	110/70	+	+	+	-	-	-	-	75	71	122	6.1	9.6	2.2	1.4	
24	2019/7447	60	M	110/70	-	-	-	-	-	-	-	90	99	117	6.2	9.7	1.52	0.7	
25	2019/22599	65	M	130/80	+	+	-	-	-	-	-	146	122	100	5.7	10.6	2.11	1.4	
26	2019/25450	67	M	132/80	+	-	-	-	-	-	-	146	100	106	6	10.3	2.31	0.8	
27	2019/8692	50	F	120/80	-	-	-	-	-	-	-	99	109	130	6.2	9.4	4.01	0.6	
28	2019/7624	67	M	120/70	+	+	+	-	-	-	-	100	115	130	5.4	10.2	1.58	0.7	
29	2019/17907	48	M	130/80	+	-	-	-	-	-	-	81	110	130	5.7	10.3	1.74	0.9	
30	2019/22151	60	F	110/70	-	-	-	-	-	-	-	115	122	140	5.6	10.4	1.26	0.7	
31	2019/14710	65	M	128/80	-	-	-	-	-	-	-	87	98	120	9.4	2.15	0.7	0.7	
32	2019/18013	70	F	110/70	-	-	-	-	-	-	-	100	88	135	5.4	9.2	3.4	1.4	
33	2019/18841	65	F	120/80	-	-	-	-	-	-	-	87	100	120	5.2	5.7	2.57	0.7	
34	2019/17672	80	F	110/70	-	-	-	-	-	-	-	120	92	113	5.2	10	2.1	0.7	
35	2019/16632	72	M	130/80	-	+	+	-	-	-	-	152	140	160	5.7	11.5	1.89	1.3	
36	2019/14170	54	M	132/80	-	-	-	-	-	-	-	145	82	118	6.2	10.2	1.76	1.2	
37	2019/16937	76	F	120/80	-	-	-	-	-	-	-	103	110	169	5.6	11	1.77	1.3	
38	2019/14861	65	F	130/80	-	-	-	-	-	-	-	98	69	100	5.4	8.9	1.9	0.8	
39	2019/25508	60	M	120/70	+	+	+	-	-	-	-	160	140	150	5.3	10	1.11	0.7	
40	2019/19977	59	M	124/70	+	+	+	-	-	-	-	78	125	144	5.4	10.1	2.4	1.2	
41	2019/19959	45	M	130/80	+	+	-	-	-	-	-	148	122	114	5.6	10.4	2.77	0.8	
42	2019/23652	75	F	130/80	-	-	-	-	-	-	-	126	144	154	5.4	9.8	2.19	0.8	
43	2019/24664	50	F	128/80	-	-	-	-	-	-	-	146	126	150	5.2	10.5	1.09	0.7	
44	2019/19959	45	M	110/70	+	+	+	-	-	-	-	148	122	108	5.6	10	2.77	0.8	
45	2019/19977	59	M	120/80	+	+	+	-	-	-	-	78	88	100	5.4	10.9	1.4	1.2	
46	2019/25045	73	F	110/70	-	-	-	-	-	-	-	155	106	125	5.3	10.4	3.08	1.3	
47	2019/15070	48	F	130/80	-	-	-	-	-	-	-	136	141	152	5.4	10	3.51	0.5	
48	2019/12996	60	F	132/80	-	-	-	-	-	-	-	105	101	126	5.9	9	3.3	0.6	

49	2019/22399	77	M	120/80	+	-	-	-	-	-	-	98	129	160	5.4	10.4	1.5	0.8	
50	2019/3768	70	F	130/80	-	-	-	-	-	-	-	146	126	150	5.2	10.5	1.61	0.7	
51	2019/15787	55	M	130/80	+	+	+	-	-	-	-	142	129	152	5.5	10.6	2.36	0.6	
52	2019/16632	72	M	120/70	-	+	+	-	-	-	-	152	140	160	5.7	10.5	1.89	1.1	
53	2019/18841	65	F	124/70	-	-	-	-	-	-	-	87	92	115	5.2	9.7	2.57	0.7	
54	2019/13212	70	F	130/80	-	-	-	-	-	-	-	100	88	135	5.4	9.2	3.4	1.4	
55	2019/13463	48	F	130/80	-	-	+	-	-	-	-	87	100	120	5.2	5.7	2.57	0.7	
56	2019/21235	38	F	128/80	-	-	-	-	-	-	-	127	75	127	8.1	9.7	1.91	1.1	
57	2019/23151	55	F	110/70	-	-	-	-	-	-	-	135	133	140	6	10.9	3.06	0.9	
58	2019/19857	68	M	120/80	+	+	-	-	-	-	-	126	144	154	5.4	9.8	2.19	0.8	
59	2019/23253	60	F	110/70	-	-	-	-	-	-	-	97	89	91	5.2	11	2.14	0.6	
60	2019/23636	80	F	130/80	-	-	-	-	-	-	-	167	104	166	9.7	10.3	3.22	1.2	
61	2019/18878	65	M	132/80	+	+	+	-	-	-	-	105	101	126	5.9	9	3.3	0.6	
62	2019/18929	58	F	120/80	-	-	-	-	-	-	-	98	129	160	5.4	10.4	1.5	0.8	
63	2019/23697	48	M	120/70	+	-	-	-	-	-	-	172	144	150	8.4	10.5	3.68	0.9	
64	2019/23673	70	F	124/70	-	-	-	-	-	-	-	101	120	130	4.1	9.2	2.91	1	
65	2019/7183	92	F	130/80	-	-	-	-	-	-	-	129	104	144	5.5	9.9	1.74	0.7	
66	2019/7504	63	M	130/80	+	-	-	-	-	-	-	109	110	140	5.7	9.3	3.17	0.7	
67	2019/23887	75	M	110/70	-	-	-	-	-	-	-	173	147	119	5.8	10.8	2.16	1.4	
68	2019/24330	68	M	130/80	-	-	+	-	-	-	-	152	85	140	6.9	9.73	3.34	1.4	
69	2019/8988	65	M	132/80	+	+	+	-	-	-	-	109	130	160	6.3	9.4	1.45	0.6	
60	2019/9099	60	M	120/80	+	+	+	-	-	-	-	140	140	135	5.6	9.1	2.42	0.6	
61	2019/24572	54	M	120/70	+	+	-	-	-	-	-	113	143	144	6.7	9.3	5.53	1	
62	2019/24708	70	F	124/70	-	-	-	-	-	-	-	100	82	120	4.9	9.8	1.69	0.6	
63	2019/16632	72	M	130/80	+	-	-	-	-	-	-	172	144	150	8.4	10.5	3.68	0.9	
64	2019/17178	43	M	130/80	+	+	+	-	-	-	-	101	120	130	4.1	9.2	2.91	1	
65	2019/26334	50	M	130/80	+	+	+	-	-	-	-	53	100	140	5.8	10.5	1.4	0.8	
66	2019/26550	74	F	110/70	-	-	-	-	-	-	-	100	130	107	5.7	5.7	1.79	1.2	
67	2019/18878	65	M	130/80	+	+	+	-	-	-	-	103	110	169	5.6	11	1.77	1.3	

68	2019/18929	58	F	132/80	-	-	-	-	-	-	-	98	69	100	5.4	8.9	1.9	0.8	
69	2019/26342	60	M	120/80	+	-	-	-	-	-	-	129	104	144	5.5	9.9	1.74	0.7	
70	2019/26463	50	F	120/70	-	-	-	-	-	-	-	109	110	140	5.7	9.3	3.17	0.7	
71	2019/22485	60	F	124/70	-	-	-	-	-	-	-	87	108	144	5.8	9.6	3.37	1.3	
72	2019/22600	55	M	130/80	-	+	+	-	-	-	-	108	110	120	6	9.6	1.96	0.6	
73	2019/26320	80	M	130/80	-	-	-	-	-	-	-	126	50	75	5.3	10.6	1.36	0.9	
74	2019/21689	68	M	120/70	+	-	-	-	-	-	-	110	120	140	5.6	10.3	1.79	0.6	
75	2019/23525	60	M	124/70	-	-	+	-	-	-	-	116	111	130	5	9	3.13	0.5	
76	2019/24032	50	F	130/80	-	-	-	-	-	-	-	97	100	120	4.7	10	3.07	0.6	
77	2019/22318	42	M	130/80	+	+	+	-	-	-	-	109	130	160	6.3	9.4	1.45	0.6	
78	2019/22856	83	M	130/80	+	+	-	-	-	-	-	140	140	135	5.6	9.1	2.42	0.6	
79	2019/22186	80	F	110/70	-	-	-	-	-	-	-	148	122	108	5.6	10	2.77	0.8	
80	2019/22485	60	F	130/80	-	-	-	-	-	-	-	78	88	100	5.4	10.9	1.4	1.2	
81	2019/24074	55	M	132/80	+	+	+	-	-	-	-	87	108	144	5.8	9.6	3.37	1.3	
82	2019/24368	86	M	120/80	+	+	+	-	-	-	-	108	110	120	6	9.6	1.96	0.6	
83	2019/24622	50	M	120/70	+	-	-	-	-	-	-	123	100	105	5.8	9	2.6	0.9	
84	2019/24326	65	F	124/70	-	-	-	-	-	-	-	111	102	122	6.5	10.3	2.18	1.3	
85	2019/24695	47	F	130/80	-	-	-	-	-	-	-	116	111	130	5	9	3.13	0.5	
86	2019/29941	42	M	130/80	-	-	+	-	-	-	-	97	100	120	4.7	10	3.07	0.6	
87	2019/16488	70	F	124/70	-	-	-	-	-	-	-	136	141	152	5.4	10	3.51	0.5	
88	2019/16626	50	F	130/80	-	-	-	-	-	-	-	105	101	126	5.9	9	3.3	0.6	
89	2019/26012	65	M	130/80	+	+	-	-	-	-	-	117	112	140	5.5	9.8	1.51	0.8	
90	2019/26169	81	M	130/80	+	+	+	-	-	-	-	97	100	110	5.7	8	4.47	1.3	
91	2019/22186	80	F	110/70	-	-	-	-	-	-	-	97	100	120	4.7	10	3.07	0.6	
92	2019/22485	60	F	130/80	-	-	-	-	-	-	-	109	130	160	6.3	9.4	1.45	0.6	
93	2019/26220	52	M	132/80	+	+	+	-	-	-	-	78	100	126	5.5	10	2.36	0.8	
94	2019/26343	55	M	120/80	+	-	-	-	-	-	-	151	128	146	5.8	10.9	2.79	0.9	
95	2019/19324	60	M	120/70	+	+	+	-	-	-	-	97	100	120	4.7	10	3.07	0.6	
96	2019/19657	70	F	124/70	-	-	-	-	-	-	-	109	130	160	6.3	9.4	1.45	0.6	

97	2019/24074	55	M	130/80	+	-	-	-	-	-	-	87	151	164	5.8	9.6	3.37	1.2	
98	2019/24032	50	F	130/80	-	-	-	-	-	-	-	53	100	140	5.8	10.5	1.4	0.8	
99	2019/24622	50	M	130/80	+	+	+	-	-	-	-	100	130	107	5.7	5.7	1.79	1.2	
100	2019/24326	65	F	110/70	-	-	-	-	-	-	-	103	110	169	5.6	11	1.77	1.3	