A DISSERTATION ON ASSOCIATION OF CAROTID INTIMAL THICKNESS WITH HEMATOLOGICAL INDICES IN PREDICTING MACRO-VASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS.

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DECLARATION

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on record my sincere thanks to those patients and their relatives who inspite of their sufferings extended their fullest co-operation to this .

LIST OF ABBREVIATIONS

RDW	Red Cell Distribution Width
MetS	Metabolic Syndrome
CIMT	Carotid Intimal Media Thickness
IDF	The International Diabetes Federation
IFG	Impaired Fasting Glucose
FPG	Fasting Plasma Glucose
WHR	Waist Hip Ratio
BMI	Body Mass Index
CVD	Cardiovascular Disease
RBC	Red blood cells
MPV	Mean platelet volume
HDL C	High Density Lipoprotein Cholesterol
ARIC	Atherosclerosis Risk In Communities
CHS	Cardiovascular Health Study.
FFA	Free Fatty Acids
DAG	Diacyl glycerol
FAcoA	Fatty Acyl coA

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ABSTRACT

Background and Objectives

Diabetes mellitus is a global burden in this present era, leading to morbidity physically and economically. The prevalence of dm was 2.8% in 2000 now increased to 4.4% in 2030.Type 2 diabetes mellitus is a chronic inflammatory state with tendency to form thrombosis and leading to vascular diseases, recently inexpensive novel markers easily accessible hematological indices are being evaluated. Measuring the carotid intima media thickness using carotid Doppler has been appeared as a non-invasive and quantitative modality to detect these early macro-vascular changes to assess hematological parameters in type 2 diabetes mellitus, and to correlate with carotid intimal thickness in predicting macro-vascular complications.

Methods

This study was conducted in Rajiv Gandhi Government General Hospital from June2022 to November 2022. A total 100 case were studied. The Hematological indices, HBA1c and CIMT parameter were included and assessed. Data was entered in MS excel sheet and analyzed using SPSS version 21.0. Chi square test and Fisher' s exact test was used for finding association.

Results

Among 100 participants, both male and females are equally represented with 50 each and both are comparable with respect to age. ($p>0.05$). The age group of the study

participants varies from 31 to 70 years. Maximum number of study participants were at 41 to 50 years age group. Mean age of the study participants was 45.38 ± 7.60 years. Among 100 participants, both male and females are equally represented with 50 each and both are comparable with respect to age. ($p>0.05$). HAEMOTOLOGICAL INDICES; 62% had neutrophil lymphocyte ratio of >2. All those with >2 (100%) NLR was found to have increased carotid intimal thickness of >2 compared to 2.6% of those with NLR <2. ($p<0.001$).Among those with RDW >15, 88.7% showed carotid intimal thickness of >0.8 compared to 21.1% in those with RDW 11-15.The Mean Platelet volume was found to be >12 in 58 diabetes mellitus patients in the study. Among them 91.4% had increased carotid intimal thickness of >0.8. However, among those with Mean platelet volume between 7 to 12, the carotid intimal thickness was found to be increased (>0.8) in only 23.8%.

Conclusion

In this study showed among diabetics with poor glycemic control have significant increase in CIMT. and there has been correlation between NLR , RDW , MPV in association with CIMT.

1. INTRODUCTION

ASSOCIATION OF CAROTID INTIMAL THICKNESS WITH HEMATOLOGICAL INDICES IN PREDICTING MACRO-VASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS

Diabetes is a disease of metabolism clinically expressed by chronic hyperglycemia and blood lipid and protein disorders as the result of a defect in insulin secretion, insulin resistance or both.[1] Chronic hyperglycemia of diabetes mellitus is associated with long term damage dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels Insulin resistance is a key component of type2 diabetes mellitus .Obesity and its associated conditions including metabolic syndrome, hypertension and dyslipidemia, is positively associated with concentrations of inflammatory biomarkers, which are predictive of insulin resistance and the incidence of T2D and CVD.

The primary cause of mortality in diabetic patients is cardiovascular diseases (CVD) while major cause of morbidity is microvascular complications. Microvascular complications (retinopathy, nephropathy and neuropathy) and macro vascular complications - (ischemic heart disease, peripheral vascular and cerebrovascular disease) are features of type 2 diabetes mellitus associated with hyperglycemia.

Detection of markers for macro-vascular and microvascular disease could supply new data about pathogenesis of diabetic complications which may help early diagnosis and help to take decisions in terms of prevention and treatment HbA1c is a

clinical standard measure of blood sugar management. HbA1c is the best available biochemical parameter that provides a long-term trend of how high blood glucose levels have been for the period of the last 6-8 weeks (about 2 months). It is closely associated with the risk of developing complications and hence it is used as an evidence- based marker to assess the chances of developing diabetic complications. It is predicted that for every 1% increase in HbA1c is associated with a 30% increase in all-cause mortality and a 0% increase in CVD mortality in T2DM patients. Patients with type 2 diabetes mellitus (T2DM) have an increased risk of coagulation abnormalities and thromboembolic events. Hyperglycemia is thought to trigger vascular damage by creating imbalance between NO bioavailability and accumulation of ROS as well as reactive nitrogen species (RNS), resulting in endothelial dysfunction.

Systematic inflammation, oxidative stress, impaired calcium metabolism, decreased bioavailability of nitric oxide, increased phosphorylation and glycosylation of cellular proteins are responsible for increased platelet activation and increased release of pro thrombotic and pro inflammatory agents in diabetes[5,6]. Platelets have a key role and increased adhesion, activation, and aggregation of platelets due to dysregulation of several signaling pathways and metabolic disturbances including insulin resistance, hyperglycemia, and dyslipidemia have been noted in diabetic patients. Larger platelets which can be demonstrated by increased MPV are more active because of elevated pro thrombotic contents, such as thromboxane A2, thromboxane B2, platelet factor 4, serotonin, and platelet-derived growth factor[2,3,4]. RDW is a quantitative measure of the heterogeneity circulating red blood cell size and is normally

assessed in the differential diagnosis of anemia.

High RDW indicate the presence of anisocytosis which is related to impairment of erythropoiesis and degradation of erythrocytes. Reflecting chronic inflammation and increased level of oxidative stress. WBC, a marker of inflammation, predicts a worsening of insulin action, insulin secretory function, and the development of micro and macro-vascular complications.[7,8] Activation of pro-inflammatory signaling pathways that promote the differentiation and maturation of WBCs are involved in the pathogenesis of insulin resistance, via various cytokines and signaling pathways. Elevated WBC is also associated with atherosclerotic changes which lead to cardiovascular complications.[9,10]

Type 2 diabetes mellitus is a chronic inflammatory state with tendency to form thrombosis and leading to vascular diseases, recently inexpensive novel markers easily accessible hematological indices are being evaluated.[11]

2. RATIONALE OF THE STUDY

Diabetes mellitus is a global burden in this present era, leading to morbidity physically and economically. The prevalence of dm was 2.8% in 2000 now increased to 4.4% in 2030. Diabetes mellitus is a metabolic disorder with chronic inflammatory state and pro thrombotic state as it is global burden, with various vascular complications leading to high mortality, simple and inexpensive tools has been under evaluation to assess vascular complications. The major long-term complications are macro-vascular includes peripheral artery disease, stroke, coronary artery disease and microvascular includes nephropathy, neuropathy, retinopathy. Glycemic control is the common factor that determines the death and complication from diabetes. As the most primary cause of mortality in diabetes mellitus is cardiovascular disease, early markers were identified to prevent mortality.

As diabetes is inflammatory condition with pro thrombotic state several hematological parameters are used to predict early markers of macro-vascular complications. Hematological parameters such as neutrophil -lymphocyte ratio, red cell distribution width is used as inflammatory markers platelet indices are used in order to assess pro thrombotic features in type 2 diabetes mellitus. These parameters are used to assess macro-vascular complication by assessing the Intimal media thickness. As carotid Intimal media thickness is surrogate marker of atherosclerosis asses further vascular complications.

3. REVIEW OF LITERATURE

Diabetes is one of the largest global health emergencies of this century, ranking among the 10 leading causes of mortality. Diabetes caused either by complex interaction of various genetic, environmental factors.

A. CLASSIFICATION

1. Etiologic Classification of Diabetes Mellitus:[12]

- Type 1 Diabetes Mellitus:
    a) Immune-mediated.
    b) Idiopathic
- Type 2 Diabetes Mellitus
- Gestational diabetes mellitus.
- Other specific types of diabetes:

2. Genetic defects of beta cell development or function characterized by mutations of:

- Hepatocyte nuclear transcription factor (HNF) 4α (MODY 1)
- Glucokinase (MODY 2)
- HNF-1α (MODY 3)
- HNF-1β (MODY 5)
- Insulin promoter factor-1 (IPF-1; MODY 4)

- NeuroD1 (MODY 6)
- Subunits of ATP-sensitive potassium channel.
- Mitochondrial DNA
- Other pancreatic islet regulators/ proteins such as KLF11, BLK, PAX4, SLC2A2(GLUT2), GATA4, GATA6, RFX6, GLIS3

3. Genetic defects in insulin action:

- Type A insulin resistance
- Rabson-Mendenhall syndrome
- Leprechaunism
- Syndromes of lipodystrophy.

4. Diseases of exocrine pancreas —pancreatitis, cystic fibrosis, hemochromatosis, fibro calculous, pancreatectomy, pancreatopathy, neoplasia, Mutations in carboxyl ester lipase.

5. Endocrinopathies -

- Acromegaly,
- Cushing's syndrome
- Glucagonoma,
- Hyperthyroidism,

- Pheochromocytoma,
- Somatostatinoma,

6. Drug or chemical-induced—glucocorticoids, nicotinic acid, vacor(a rodenticide), diazoxide β- adrenergic agonists, calcineurin and mTOR inhibitors, pentamidine, thiazides, protease inhibitors, epinephrine, hydantoins, asparaginase, α-interferon.
7. Infections - coxsackievirus, cytomegalovirus, congenital rubella.
8. Uncommon forms of immune-mediated diabetes—stiff-person syndrome, anti-insulin receptor antibodies
9. Other genetic syndromes sometimes associated with diabetes—

- Friedreich's ataxia,
- Huntington's chorea,
- Klinefelter's syndrome,
- Myotonic dystrophy
- Down's syndrome,
- Turner's syndrome,
- Wolfram's syndrome,
- Prader-Willi syndrome,
- Laurence- MoonBiedl syndrome."

The most common type of Diabetes Mellitus is Type 2 Diabetes Mellitus which accounts for around 90% of cases worldwide. Globally, the prevalence of Type 2 Diabetes Mellitus is increasing across all the regions which is driven by increasing urbanization, aging, sedentary lifestyles, and greater consumption of unhealthy foods linked with Obesity[13]. According to the World Health Organization (WHO), noncommunicable diseases (NCDs) accounted for 74% of deaths globally in 2019, of which, diabetes resulted in 1.6 million deaths, thus becoming the ninth leading cause of death globally[13,14].

The International Diabetes Federation (IDF) estimates that worldwide, 415 million people have diabetes, 91% of whom have type 2 diabetes mellitus. People with diabetes comprise 8.8% of the world's population, and IDF predicts that the number of cases of diabetes will rise to 642 million by 2040.[15] The most frequent type of CVD reported was CAD (21.2%) and lowest was stroke (7.6%). Males had higher rates of prevalent disease than females. Recently well accepted theory, that the accumulation of energy due to excessive calorie intake and the lack of physical activity that leads to fat accumulation in the subcutaneous tissue followed by other tissue compartments such as the liver, pancreas, muscles, perivascular and pericardium. This further increases tissues insulin resistance, thereby decreasing insulin sensitivity at tissue and cellular level.

B. INSULIN

Insulin is a peptide hormone secreted by beta cells of pancreatic islets of Langer Hans and maintains normal blood glucose by facilitating cellular glucose uptake, regulating carbohydrate, lipid, protein metabolism.

i. Insulin Secretion

In normal healthy patients, beta cell secretes insulin in response to glucose, free fatty acids, others which is controlled by intrinsic rhythm of intracellular calcium influx leads to pulsatile release of insulin. This integration is mediated by autonomic nervous system and autocrine action of insulin on its own receptors leads to first insulin response, followed by negative feedback to prevent continuous insulin[22].

There are two major oscillatory secretion patterns[20,21]

1. Ultradian oscillations, which have a period of 1-2 hours due to feedback loop between glucose and insulin secretion.
2. Rapid oscillatory phase which has a period of 10-15 minutes.

So that following glucose ingestion, increase in plasma glucose concentration stimulates insulin secretion from beta cells there by hyperinsulinemia increases glucose uptake in liver, skeletal muscle, adipose tissue, suppress hepatic glucose production and maintains euglycemic state.

ii. Insulin Action

Following glucose ingestion, the increase in plasma glucose concentration stimulates insulin secretion from the beta cell and the resultant hyperinsulinemia

suppresses lipolysis, leading to decline in plasma FFA concentration and subsequent decrease in the rate of lipid oxidation. Insulin stimulates glucose uptake in skeletal muscle, and the increased glucose flux into skeletal muscle, together with the activation of key enzymes in glucose metabolism by insulin, leads a marked increase in muscle glucose oxidation.

Insulin receptor is a glycoprotein compromised of two alpha and beta subunits linked by disulphide bonds. The two alpha subunits are on extracellular surface of plasma membrane and has insulin binding domain and transmembrane domain beta subunit which has insulin receptor tyrosine kinase activity . On activation of insulin receptor , tyrosine undergoes phosphorylation , in skeletal muscle IRS-1 (Insulin receptor substrate) serves as major protein and undergoes phosphorylation, which leads to activation PI3K (phosphatidyl inositol 3 -kinase) leads to activation of protein kinase , which is a central intermediate of many metabolic and growth actions of insulin.[33,34,35]

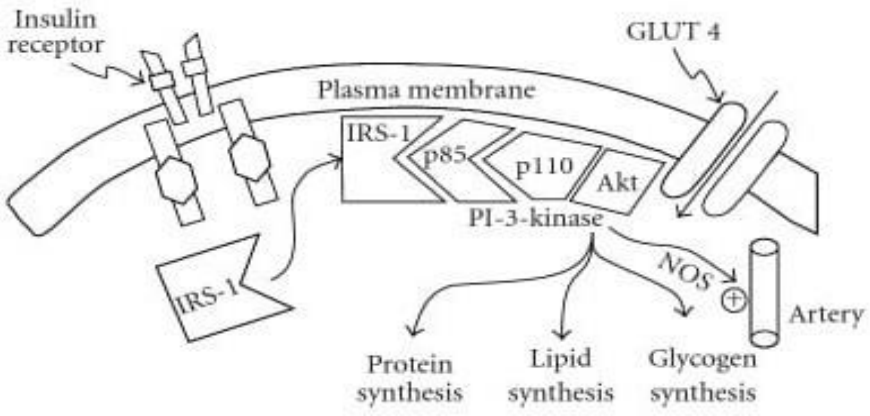


Fig 3.1. Insulin signal transduction system in normal glucose tolerant subjects.

iii. Insulin Resistance

INSULIN RESISTANCE is defined as a decreased tissue effect of insulin per unit delivered.

Following glucose uptake, the balance is disrupted in order to maintain glucose homeostasis following mechanisms operate in a coordinated fashion[16~19]

1. Insulin secretion by the beta cells of Pancreas.
2. Stimulation of glucose uptake by splanchnic (liver and gut) and peripheral (primarily muscle) tissues in response to hyperinsulinemia plus hyperglycemia
3. Suppression of hepatic glucose production.

Glucose homeostasis is altered in type 2 diabetes mellitus as the main pathogenesis is defect in insulin secretion and insulin resistance contributing to glucose intolerance and overt hyperglycemia.

Insulin secretion impaired due to beta cell dysfunction or decreased cell function, so that pulsatile secretion of insulin is lost, leads to hyperglycemia. Insulin resistance is the main component in development of type 2 diabetes mellitus. In order to maintain euglycemic state, demand on beta cells to secrete insulin increases, so that majority of people euglycemia is maintained by a compensatory hyperinsulinemia. However, in individuals who are susceptible to developing type 2 diabetes mellitus beta cells eventually fail to compensate resulting in glucose intolerance and gradually to overt diabetes. Liver and muscles have long been recognized as major contributors of

systemic insulin resistance.[26]

Hepatic glucose production is responsible for endogenous glucose production and necessary for glucose homeostasis, mainly fasting hyperglycemias. In type 2 diabetes mellitus, the rate of hepatic glucose production is increased under basal physiological conditions, and insulin dependent suppression of hepatic glucose production is impaired physiological as well as at supra-physiological plasma levels of insulin and leads to fasting hyperglycemia.[23-27] Skeletal muscle utilizes both glucose and free fatty acid (FFA) as a sources for energy production. During the post absorptive state, the plasma insulin concentration is low. Since the plasma insulin concentration is the principal factor that restrains lipolysis in adipocytes and stimulates glucose uptake in skeletal muscle, during the fasting state, muscle glucose uptake is low and the plasma FFA concentration is elevated. Thus, under fasting conditions, FFA serves as the principal fuel source for energy production in skeletal muscle, while the brain exclusively utilizes glucose.

As insulin resistance glucose uptake is impaired in skeletal muscle leading to postprandial Hyperglycemia.[28,29] A defect in the insulin signaling cascade at the level of IRS-1 is l primary defect that leads to insulin resistance in skeletal muscle. Other defects in the insulin signaling pathway, for example, diminished insulin binding, when present, are modest and secondary to down regulation of the insulin receptor by chronic hyperinsulinemia.[35]

C. METABOLIC SYNDROME

Nutritional status influences the incidence of Type 2 Diabetes mellitus. High BMI (Body Mass Index) has a 2 times greater risk of developing T2DM compared to low BMI. The results showed that general obesity had a risk of 2.24 times while abdominal obesity had a risk of 2.44 times for the occurrence of Diabetes mellitus. The metabolic derangements seen in patients with type2 diabetes mellitus are associated with high risk of cardiovascular diseases. These metabolic derangements are designated as Metabolic syndrome(Mets) or Syndrome X.

Metabolic Syndrome: NCEP-ATPIII Criteria [JAMA 2001; 285: 2486–97.] [Circulation 2004; 109: 433–8. Circulation 2005; 112: 2735–52.]	
At least three of the following five items:	
Glucose or Insulin abnormalities as defined by:	Fasting plasma glucose ≥ 100 mg/dL
Central Obesity as defined by:	Waist circumference ≥ 102 cm (40 in) (men) ≥ 88 cm (35 in) (women)
Dyslipidemia as defined by:	Triglycerides ≥ 150 mg/dL
Dyslipidemia as defined by:	HDL < 40 mg/dL (men), HDL < 50 mg/dL (women)
Elevated Blood Pressure as defined by:	BP ≥ 130/85 mmHg

Table 3.1. Diagnostic criteria of metabolic syndrome

i. Body Mass Index:

Body mass index is a value derived from mass and height of a person. BMI is defined as the body mass divided by square of body height, which is expressed in units of kg/m2.

Quetelet Index = body weight (kilograms) divided by height squared (meters) = BMI.

Table 3.2. BMI Categorization

D. Obesity

Adipose tissue is an essential organ for the regulation of energy homeostasis, as associated with storage of excess energy as triglycerides, adipocytes undergo hyperplasia to increase the number of adipocytes and hypertrophy to increase the size of each adipocyte, allowing adipose tissue to expand in times of nutrient excess so that can be utilized in states of fasting.[30]

Obesity is characterized by dysfunctional adipose tissue, in which adipocytes initially become hypertrophic at the expense of excess calorie intake and secretes adipokines which causes recruitment of pre adipocytes in to mature adipocytes as compensatory protection against metabolic consequences of obesity.[31,32]

i. Waist Circumference

The body fat distribution was referred to as being !android" if it occurred in the upper body and !gynecoid" when it occurred in the lower segment of the body.
This is because men tend to accumulate fat in the abdominal (upper body) area, whereas women tend to accumulate it in the peri pelvic (gluteal) area and the thighs.[35,36,37]

Hence, the anatomic distribution of fat, determines risk for the metabolic syndrome, rather than the overall degree of obesity. The association between increased abdominal (upper body) fat and an increased risk of coronary heart disease is related to visceral fat.

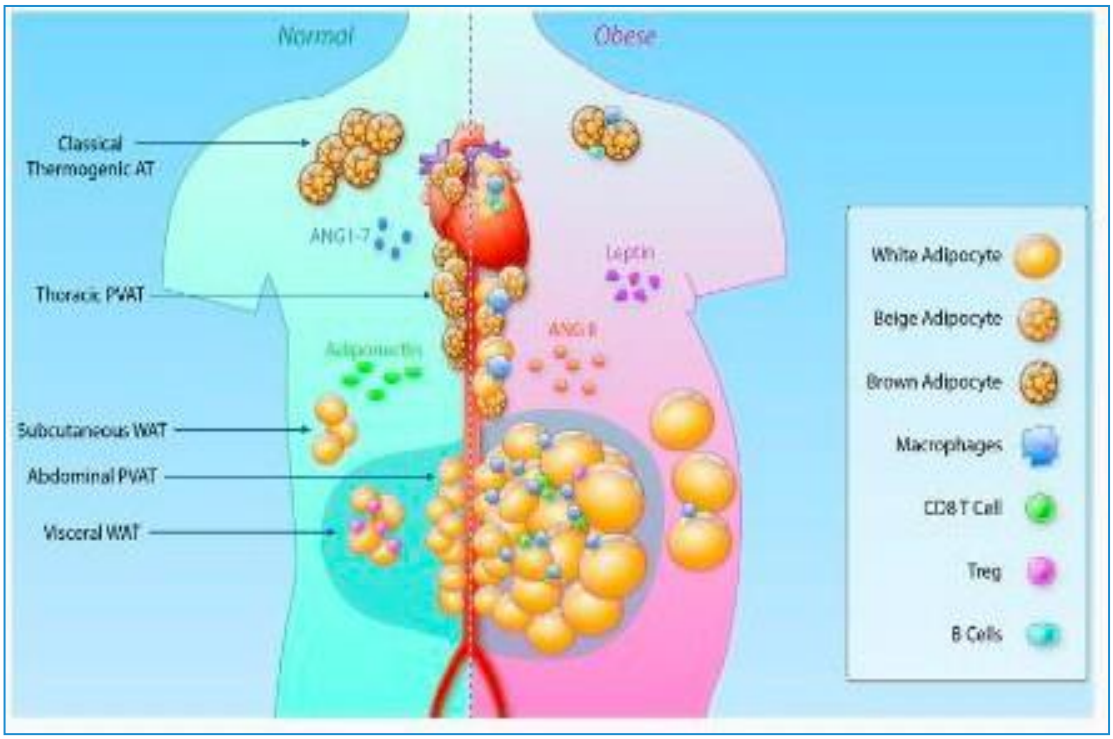


Fig 3.2. Anatomic Distribution of Fat

Increasing waist circumference with subsequent elevation in subcutaneous versus visceral adipose tissue in Asians and Indians may explain the greater prevalence of the syndrome in Asian and Indian populations compared to African- American population for whom there is predominance of subcutaneous fat.

Waist circumference (WC) is a cheap and easy method of measurement. Waist circumference is considered a reasonable indicator of intra-abdominal or visceral fat. This fat is closely associated with increased risk of comorbidity.

How to measure waist circumference

To correctly measure waist circumference:

- Stand and place a tape measure around your middle, just above your hipbones
- Make sure tape is horizontal around the waist

- Keep the tape snug around the waist, but not compressing the skin
- Measure your waist just after you breathe out

At the end of a normal expiration the measurement is taken, while make sure that the participant does not contract the abdominal muscles. (Experimenter can engage in conversation with patient if he is suspected to contract the abdominal muscles)25. The measurement has been made twice and if the difference between the first two measurements is greater than 5% (+1 cm), a third measurement can be taken. The two closest measurements will be averaged.

Detailed instructions

1. Mark with a pencil bony landmarks of the right and left last rib margin.

2. Mark with a pencil bony landmarks of the right and left iliac crest.

3. Mark with a pencil the half way distance between the last rib margin and the top of the iliac crest of the two sides.

4. Measure mid way between the two bony points 1 and 2.

As visceral fat is associated with insulin resistance increases release of free fatty acids which accumulates in various tissues there by decreasing insulin sensitivity in peripheral tissues and mitochondrial fatty acid oxidation leads to overproduction of ROS.

ii. Hepatic Lipid Accumulation:

Visceral derived free fatty acids can directly affect liver via portal vein increasing lipid content in Hepatocytes. Free fatty acids derived triglycerides

accumulate in cytoplasm of hepatocytes as lipid droplets. These lipid droplets show no harm as the intermediate forms such as diacyl glycerol, ceramide shown to increase lipotoxicity and increase insulin resistance. As a result of accumulation of triglycerides in liver leading to hepatic steatosis.[38,39]

In liver Kupffer cells act as resident macrophages, in healthy liver these cells phagocytose pathogens and toxins and, maintain tissue homeostasis. Adipokines imbalance leads from visceral adipose tissue fails to suppress hepatic inflammation and oxidative stress contributing to Kupffer cells activation which secretes pro inflammatory cytokines so that amplifies systemic inflammation[40,41]

iii. Skeletal Muscle

Skeletal muscle is a major site for disposal of ingested glucose, approximately one third of ingested glucose is taken by liver and rest by peripheral tissues, Ie. Skeletal muscle via insulin dependent mechanism through glucose transporter4 (Glut4).

As in obesity accumulation of intramyocellular fat content and fatty acid metabolites, for example fatty acid coenzyme A and diacyl glycerol which impairs insulin activity in skeletal muscle leads to hyperglycemia.[43,44]

As triglycerides accumulated impairs insulin signaling and multiple post receptor intracellular defects thus affects insulin mediated glucose uptake[45].

Increased intramyocellar fat content and fatty acid metabolites, for example , FACoA and DAG, are likely to play a pivotal role in the development of insulin resistance in skeletal muscle. Through activation of serine/threonine kinases and serine

phosphorylate the IRS-1, fatty acid metabolites impair IRS-1 phosphorylation by the insulin receptor and lead to the defect in insulin signaling in insulin resistant individuals.[45,46,47,48]

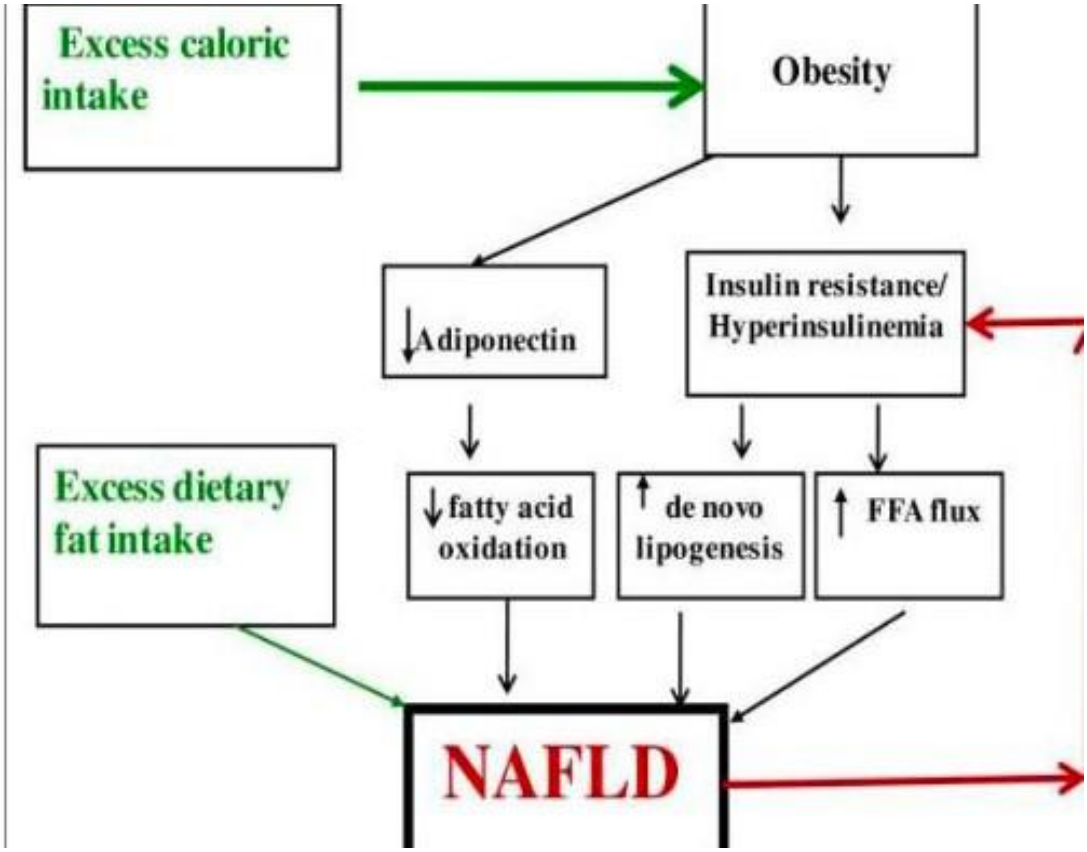


Fig 3.3. Ectopic fat in muscle.

iv. Epicardial fat:

Epicardial adipose tissue (EAT) is the visceral fat unit localized between the myocardium and the inner pericardium, surrounding major coronary vessels Epicardial adipose tissue not only provides fat as a vital energy in periods of elevated energy requirement, but also an active endocrine organ with several vasocrine and paracrine effects. Under physiological circumstances, epicardial fat secretes plenty of beneficial cytokines, such as adiponectin, adrenomedullin and omen tin-1 which present anti-atherogenic, anti-inflammatory and anti-thrombotic properties[49,50,51]

insulin resistance, as excessive epicardial fat provokes imbalance in lipid and glucose metabolism. Redundant epicardial fat secretes a high amount of free fatty acids, which are then accumulated in coronary artery lumen promoting atheromatous plaque development.[53,54]

v. Inflammatory response in obesity

Inflammation is a protective tissue response to injury or destruction of tissues that serves to destroy or dilute both the injurious agent and the injured tissue.

There are two types of inflammation:

1. Acute inflammation that lasts for a short time and is characterized by oedema and migration of leukocytes.
2. Chronic inflammation that lasts for a long time and is characterized by the presence of lymphocytes and macrophages and the proliferation of blood vessels and connective tissue.

Adipose tissue is known to function as an endocrine organ which secretes chemokines, cytokines, adipokines and growth factors. [55] Omental fat produces 2-3-fold higher levels of IL-6 than subcutaneous fat, adipose tissue derived IL-6 promotes hepatic insulin resistance and impaired glucose intolerance. Adipokines includes adiponectin and leptin which are important mediators of various metabolic processes such as fatty acid oxidation, glucose uptake, denovo lipogenesis and gluconeogenesis. Adiponectin is an insulin sensitizing hormone which decreases hepatic gluconeogenesis, increased glucose uptake in skeletal muscle, liver, adipose tissue and fatty acid oxidation. [56,57] .On these organs receptors are highly expressed there by it

promotes glucose homeostasis, in addition it also exhibits anti-inflammatory action. Leptin is another peptide hormone that regulates food intake and energy regulation. Adipose tissue expansion in obesity is accompanied by inflammatory changes within adipose tissue contributes to chronic low-grade inflammation characterized by elevation of IL -6 and TNF-alpha, chemokine MCP-1, responsiblefor recruitment of inflammatory cells such as macrophage and decreased secretion of anti-inflammatory cytokines adiponectin.

AT macrophages (ATMs) represent the largest subpopulation of AT immune cells, encompassing 4% of normal visceral fat with an increase to 12% in excessed adiposity Macrophages are divided into two subpopulation types: a classically activated M1 type and the alternatively activated M2 type. M2 occurs in lean subjects adipose tissue, while M1-type predominates in obese individuals. Activated M1 macrophages are characterized by increased production of pro inflammatory cytokines: interleukin (IL)-6, TNF-α, IL-12, IL-23, and reduced synthesis of anti- inflammatory IL-10.

vi. Oxidative Stress

Oxidative stress is defined by excess endogenous oxidative species, which both damage cells and manipulate signal pathways. Reactive species, especially reactive oxygen species (ROS) like superoxide, hydrogen peroxide, and hydroxyl radical ions are the agents of oxidative stress and are produced at low physiological levels mostly in the mitochondria and peroxisomes. ROS are produced endogenously and have physiological significance at low levels. [60,61,62]

AS there is increased supply of glucose in view of insulin resistance,

mitochondria have more substrate available to make ATP. Thus, the mitochondria are hyperactive and produce more of their natural byproduct, ROS. This increased ROS damages the infrastructure of the cell, ROS directly stimulate stimulating pathways NF-kB (Nuclear factor –kB), JNK and p38 MAPK resulting in mitochondria-induced stress responses., [63,64,65]. Increased ROS levels induce mitochondrial fission resulting in actions on both the insulin receptor pathway and stress proteins. Mitochondrial fission has been directly linked to insulin resistance in skeletal muscle as there is altered insulin signaling pathway, and down regulation of receptors.

In obesity as the level of free fatty acids and glucose increased due to insulin resistance, increases electron transport chain (ETC) activity in mitochondria and active nicotinamide adenine dinucleotide phosphate oxidase (NADPH) causes an increased reactive oxygen species (ROS). BMI is closely related to oxidative stress and ROS content in adipocytes. Oxidative stress leads to an increased infiltration of macrophages into fat tissue.

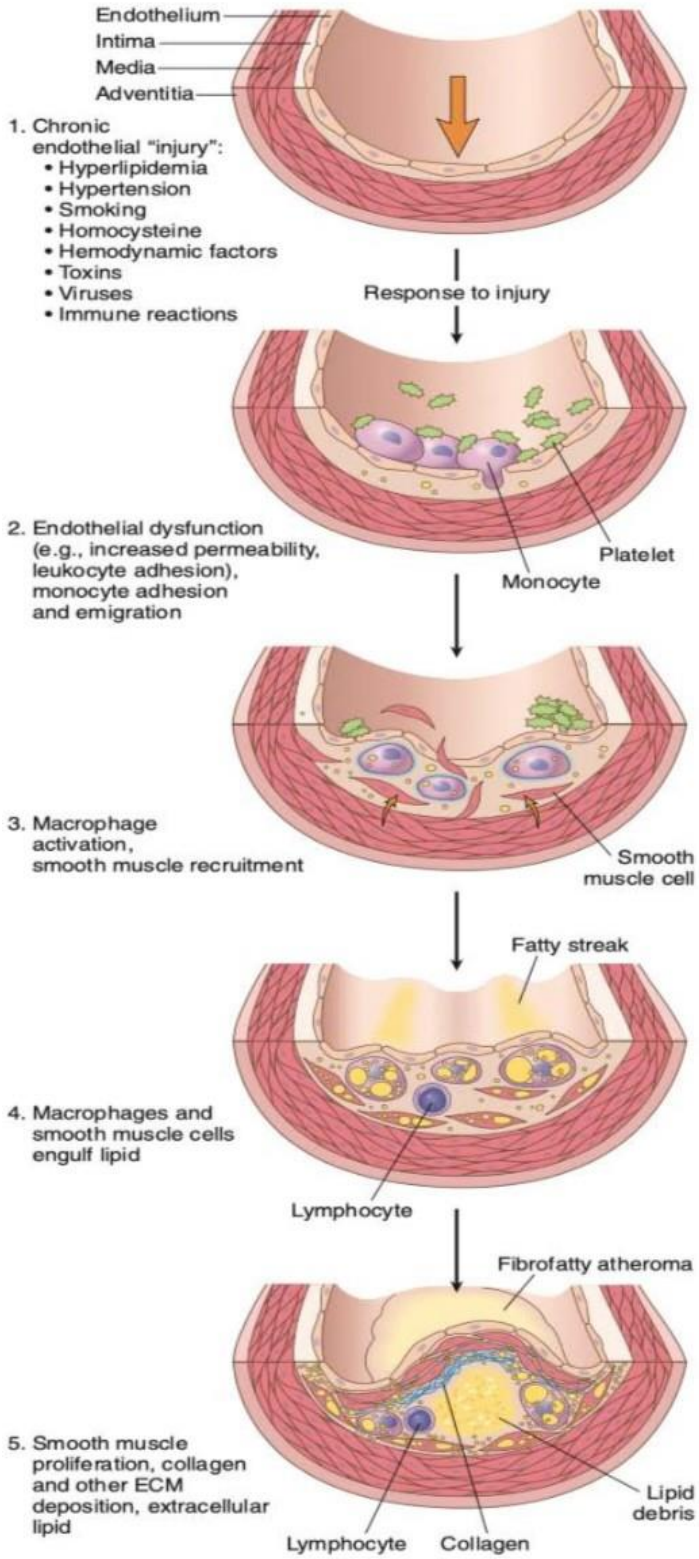


Fig 3.4. PATHOGENESIS of Atherosclerosis

E. Vascular Response

Atherosclerosis is a chronic vascular inflammatory disease associated to oxidative stress and endothelial dysfunction involving large and medium wall arteries such as aorta, carotid, coronary arteries.

The World Health Organization redounds an estimated 16.7 million deaths to the atherosclerotic cardiovascular disease Inflammation is a crucial element in progression of atherosclerotic plaque, plaque rupture, and atherothrombosis. Oxidative stress and inflammation are interrelated; they form a vicious feed-forward cycle during atherogenic plaque progress.

i. Layers of vessel:

1. The tunica intima is the innermost and thinnest layer, consisting of a monolayer of endothelial cells on top of a basement membrane with minimal underlying extracellular matrix (ECM).
2. The tunica media is the thickest layer of the arteries, and is composed predominantly of smooth muscle cells and ECM, surrounded by loose connective tissue, nerve fibers, and smaller vessels of the adventitia.
3. The adventitia is the outermost layer of the artery and typically consists of a loose matrix of elastin, smooth muscle cells, fibroblasts, and collagen. Most of the neural input into blood vessels also traverses through the adventitia.

The earliest visible lesion of atherosclerosis is the fatty streak, which is due to an accumulation of lipid-laden foam cells in the intimal layer of the

artery., that leads to thickening of artery. With time, the fatty streak evolves into a fibrous plaque, the hallmark of established atherosclerosis

Atherosclerotic lesions are composed of three major components:

The first is the cellular component comprised predominately of smooth muscle cells and macrophages. The second component is the connective tissue matrix and extracellular lipid. The third component is intracellular lipid that accumulates within macrophages, thereby converting them into foam cells

MECHANISMS:

ENDOTHELIAL DYSFUNCTION:

The initial theories states endothelial dysfunction is the earliest abnormality to occur during the process of atherosclerosis (13,14). Endothelial dysfunction/ injury is said to alter these properties of vessel wall and predispose the arteries wall to atherosclerosis.

Exposure to combination of following stimuli like

• smoking,

• hypertension and

• hyperlipidemia, could lead to increased endothelial permeability, which

enhance leukocyte adhesion and altered gene expression. Inflammatory cytokines (e.g. TNF) plays some role in the expression of pro- atherogenic genes. The following two causes are said to be most important in the process of endothelial dysfunction: **Lipids**:

hyperlipidemia and excess of altered lipoproteins are the cause of endothelial dysfunction which may lead to increased transudation of lipoproteins mainly LDL, chylomicrons into the arterial wall intima.

Hemodynamic disturbances: Atheromatous Plaques have more tendency to occur at the sites of turbulent blood flow patterns like blood-vessel bifurcating points and ostias of the exiting blood vessels. Also, many studies demonstrated that, in regions with laminar blood flow, there is an expression of genes which are atheroprotective .

However, the histopathological examination of vascular endothelium in various studies explains that the earliest atheromatous lesions occur in blood vessels whose endothelium is morphologically intact. Subsequently many studies which have demonstrated that the rate of lipoprotein entry into atherosclerosis- susceptible sites could be either increased or decreased which suggests a nonessential role for alterations in endothelial permeability .

ii. Pathogenesis of atherosclerosis:

Over the past 150 years numerous efforts had been there in order to explain complex events of atherosclerosis. Three different hypothesis had been under investigation currently as follows:

1. The response-injury hypothesis:

In this hypothesis atherosclerosis begins with endothelial injury or dysfunction which is characterized by increased endothelial permeability and low-density lipoprotein (LDL) deposition in the subendothelial space. This is followed by leukocyte

adhesion and transmigration across the endothelium. Later on, atherosclerosis is characterized by foam cell formation that is lips laden macrophages and an inflammatory response including T-cell activation, the adherence and aggregation of platelets, and further entry of leukocytes into the arterial wall. This recruited cell secretes cytokines and growth factors promotes an inflammatory response characterized by migration of smooth muscle into intima. Finally, advanced atherosclerosis (*C*) is characterized by continued macrophage accumulation, fibrous cap formation, and necrosis in the core of the lesion [66,67]

2. The response to retention hypothesis:

This hypothesis submits that the lipoprotein retention is the inciting event for atherosclerosis. Apolipoprotein B-100, the single protein associated with LDL, is retained within the arterial wall in close association with arterial proteoglycans .This interaction is mediated by specific residues that, when mutated, protect experimental animals against the development of atherosclerosis. [68, 69]The accumulation of apolipoprotein B-100-containing lipoproteins within the arterial wall further triggers a proinflammatory cascade.

3. Oxidative modification hypothesis:

LDL becomes entrapped in the subendothelial space where it is subject to oxidative modification by resident vascular cells such as smooth muscle cells, endothelial cells, and macrophages. Oxidized LDL stimulates monocyte chemotaxis,

and supports foam cell formation. Once formed, oxidized LDL also results in endothelial dysfunction and injury, and foam cells become necrotic due to the accumulation of oxidized LDL. CHD is the most common clinical manifestation of atherosclerosis and it is the major cause of death all over the world. As early stage of atherosclerosis due to chronic inflammation leads to increased Intima medial thickness, this can be used as surrogate marker[70,71,72]

MORPHOLOGY:

FATTY STREAKS:

This is the earliest lesions in atherosclerosis. It contains lipid-filled foamy cells (macrophages). Appear as flat yellow spots then coalesce to form elongated streaks. They do not usually cause any alteration in the flow. Not all the fatty steaks develop into advanced lesions. Fatty streaks usually begin to form in the coronary vasculature in adolescence, at the same anatomic sites where it later develop into matured plaques.

FIBRO FATTY PLAQUE.

These plaques have three main components:

1) Cells comprising smooth muscle cells, macrophages, and T cells;

2) Extra Cellular Matrix, such as collagen, elastin, and other proteoglycans;

3) Intracellular and extracellular lipid- which form about 40 % of the plaque.

There is a superficial cap made up of smooth muscle cells and dense collagen fibers . Below and to the side of the cap is a cellular area that contains macrophages. Deep to the cap is a

necrotic core, which contain lipid (cholesterol and cholesterolesters), cellular debris, foam cells and fibrin. There is peripheral neovascularization.

These plaques impinge on the lumen of the artery. The order of involvement of blood vessels in humans are lower abdominal aorta, coronary arteries, popliteal arteries, internal carotid and then circle of Willis.

ADVANCED or COMPLICATED PLAQUE

1. Calcified plaque due to calcium deposition.
2. Rupture, ulceration, or erosion:
3. Bleeding into the plaque.
4. Athero-embolism: due to dislodge of atherosclerotic debris into the blood stream following plaque rupture.
5. Aneurysm formation due to increased pressure

VULNERABLE PLAQUE:

These are ones in which plaques have high probability of undergoing rapid progression & thrombotic complications

These usually favor proximal arteries. They have a lipid- rich necrotic core and a thin fibrous cap which is infiltrated by macrophages.

These plaques impinge on the lumen of the artery. The order of involvement of blood vessels in humans are lower abdominal aorta, coronary arteries, popliteal arteries, internal carotid and then circle of Willis.

iii. Carotid Intima medial thickness:

Intima–media thickness (**IMT**), also called **intimal medial thickness**, is a

measurement of the thickness of tunica intima and tunica media, the innermost two layers of the wall of an artery[73]. IMT can be measured using external ultrasound in large arteries relatively close to the skin (e.g. the carotid, brachial, radial, or femoral arteries). Deeper internal arteries, such as the coronary special intravascular catheters employing ultrasound or optical coherence tomography to measure IMT.

The carotid artery includes 4 segments, beginning with the CCA. This gives rise to the carotid bulb from which arise the external carotid artery and the internal carotid artery (ICA).

In healthy adults, IMT ranges from 0.25 to 1.5 mm and values above 1.0 mm are often regarded as abnormal. IMT has been proposed as a quantitative index of atherosclerosis of value in monitoring disease progression and the effects of treatment and as a surrogate end point in clinical trials.

To quantify the degree of thickening of the carotid artery walls, the many measures of IMT were summarized into two variables: one for the CCA and one for the ICA. The maximum wall thickness of the CCA was defined as the mean of the maximum wall thicknesses for near and far wall on both the left and right sides: (mLNW+mLFW+mRNW+mRFW)/4.maximum wall-thickness variable of the ICA was defined in the same way; the results from the three scans were averaged. The number of measurements available for averaging thus ranged from 1 to 4 for the CCA and 1 to 12 for the ICA.

METHOD :

Patient is placed in supine position with neck minimally extended.

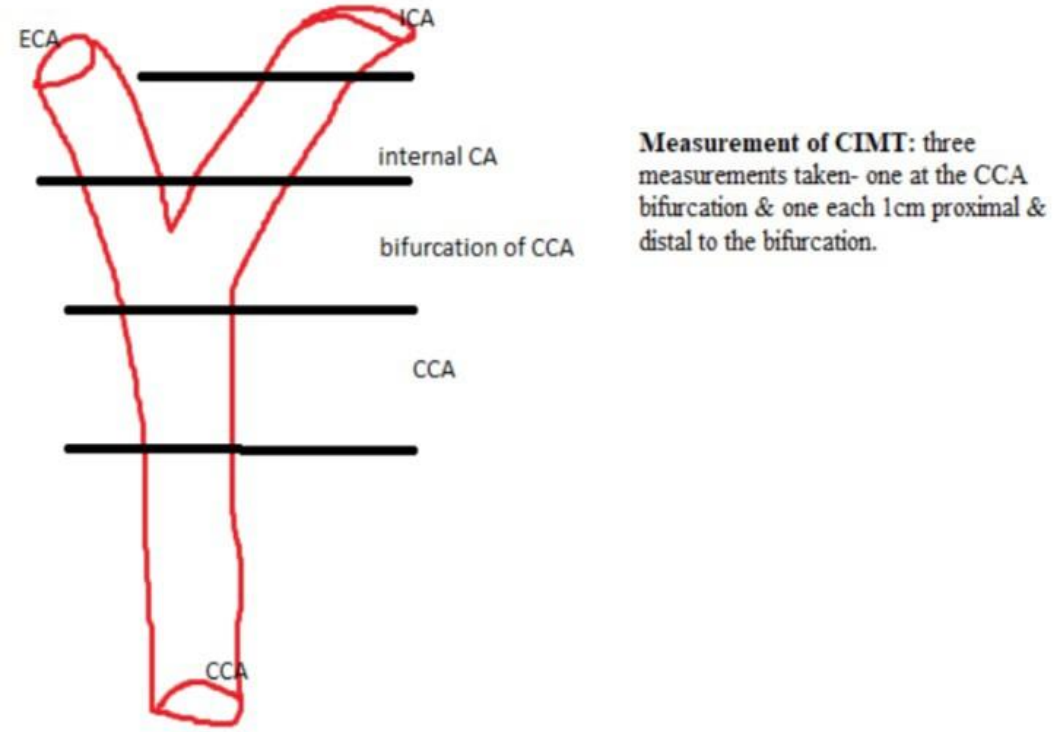


Fig 3.5. Measure of CIMT

Scanning of both side arteries was performed in anteroposterior projections.

Scanning of external and common carotid or internal carotid arteries in neck is performed both sides according to evading edge of second echogenic line. The lumen and intimal interface is represented by the first line and the second line represents the tunica adventitia. At each longitudinal projections, three determinations of IMT are conducted. The first one at the site of greatest thickness and the other two at a point one centimetre downstream and one centimetre upstream from the site of greatest thickness respectively. The three values are then averaged. The normal intimal + medial thickness of common

carotid artery as evaluated by B-mode ultrasound imaging was 0.74 ± 0.14mm (53).

Hematological parameters:

In type 2 Diabetes mellitus, Dyslipidemia, insulin resistance, obesity contributes to low grade inflammation acts as a risk factor of cardiovascular diseases, leading cause of deaths all over the world. the major cause of mortality is cardiovascular diseases, which can be predicted early by hematological parameters.

The chronic inflammatory and hypoxic nature causes a stimulus in the bone marrow and, depending on the intensity of this stimulus, there is a release of immature cells or increase of other cells in the bloodstream. Therefore, their presence in the circulation is an important variable used to diagnose, stratify and predict diseases[74,75,76]

The hematological indices include- Red blood cells, White blood cells which includes granulocytes and agranulocytosis and Platelet, synthesized in the bone marrow, through a process called hematopoiesis. These cells originate from single progenitor cell called the stem cell. These pluripotent cells contribute to all cytological lineages.

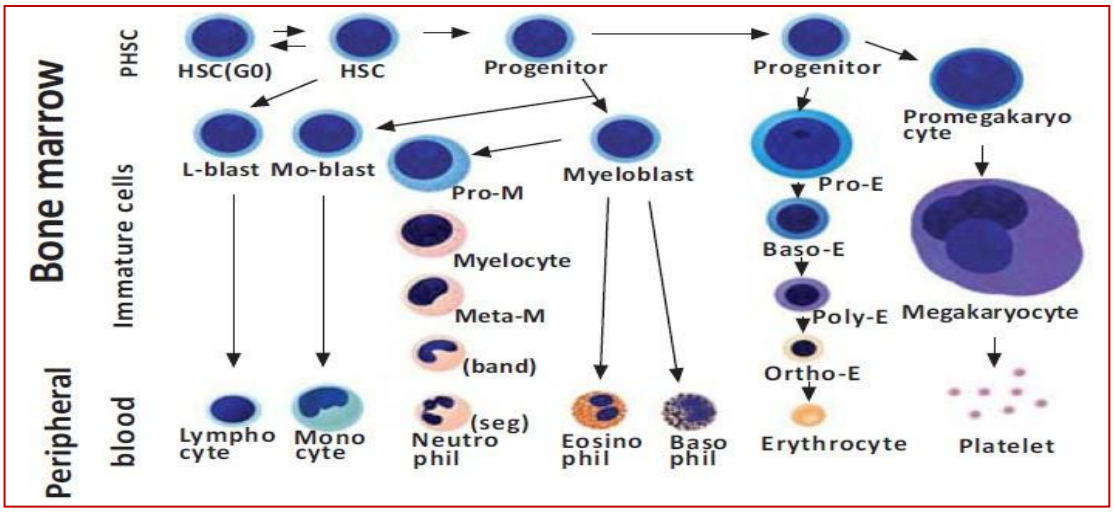


Fig 3.6. Progenitors of blood cells.

iv. Neutrophil - Lymphocyte ratio:

Neutrophils are the most abundant leukocytes in human blood count and they account for 50-70 % of all white load cells in blood stream. Neutrophils are considered as primary effector cells of acute inflammation and first line responders to infection. Neutrophils are phagocytic cells, which engulf, destroys, degranulate and release lytic enzymes, strong oxidants.

The neutrophil to lymphocyte ratio (NLR), a combination of two independent markers of inflammation, is considered a simple and non-specific marker of inflammation. The NLR reflects the relationship between innate (neutrophils) and adaptive (lymphocytes) immune responses in various pathological conditions. Because the NLR correlates with CRP concentrations, it becomes a simple cost- effective biomarker for the detection of subclinical inflammation.

Neutrophils are increased in obesity, and there is a correlation between the level

of neutrophil blood counts and the higher BMI. In addition, neutrophil counts were significantly higher in individuals with metabolic syndrome than in lean individuals. In animal models, neutrophils have also been found to be elevated in blood vessels and infiltrating adipose tissue and the endothelium at atherosclerotic lesions.

In obese individuals activated neutrophils are present which is indicated by elevated plasma concentrations of myeloperoxidase (MPO), neutrophil elastase (NE), as well as an increased expression of CD66b, a marker of neutrophil degranulation .

Activation of neutrophils from obese individuals was also indicated by stimulation of the NF-κB signaling pathway and by a higher ROS generation and enhanced release of pro-inflammatory cytokines. Pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8 increase bone marrow granulopoiesis, releasing neutrophils from the bone marrow to the peripheral circulation. Moreover, these inflammatory mediators induce de-margination of neutrophils from endothelial walls, resulting in neutrophilia.

Neutrophil elastase (NE) and IL-6 contribute to the development of insulin resistance by impairing insulin signaling. IL-1β is an important activator of macrophages in multiple parts of the body. Furthermore, IL-1β, together with TNF-α in pancreatic islets, induces β cell dedifferentiation by down regulating transcription factor Fox01, that regulates β cell proliferation. Together, these events may result in type 2 diabetes mellitus. In addition, TNF-α can alter the adhesion function of endothelial cells by inducing increased low-density lipoprotein (LDL) uptake. These changes have been associated with the progression of atherosclerosis.

The NLR demonstrates role of two inevitable mechanisms necessary to maintain balance of the immune system one of which is neutrophils acting as the nonspecific inflammatory mediator, other one being lymphocytes playing the protective component of inflammation[79,80,81]

In non-diabetic patients lymphocytes contribute to insulin sensitivity whereas neutrophil and lymphocyte contribute to sub clinical inflammation-but also an indicator of poor glycemic control in patients with type 2 diabetes. It can also guide the physician in the resource limited settings like PHC to evaluate a patient with type 2 diabetes for microvascular and macro-vascular complication. So, it can be used as a reliable marker for monitoring the morbidity

NLR is an inexpensive laboratory index calculated by simple division of absolute neutrophil count by absolute lymphocyte count in hemogram. If you only have % count for neutrophils and lymphocytes along with total White blood cell count.

Calculate absolute count-

TOTAL WBC COUNT -*% count for neutrophil or lymphocytes / 100

v. Red cell distribution width:

Red blood cell distribution width (RDW) is a numerical measure of the variability in size of circulating erythrocytes(anisocytosis).

Two methods were used by counters to calculate this value. The first method is referred to as RDW-CV. The RDW- CV is the ratio of the width of the red blood cell distribution curve at 1 SD divided by the MCV (normal RDW-CV = 13 "# 1%). Since it is a ratio, changes in the MCV or the width of the curve would influence the result. Microcytosis would magnifies the change in the RDW-CV simply by reducing the denominator of the ratio. In contrary, macrocytosis will tend to counterbalance the change in the width of the curve and thereby minimizing the change in the RDW-CV. The RDW-SD is independent of the MCV. The RDW-SD ,a second method of measuring the RDW is a direct measurement of the red blood cell distribution width taken at the 20% frequency level (normal RDW- SD = 42 "#4fL).

The normal values for RDW are given below[84,85,86]

• RDW–SD–39-46fL

• RDW – CV – 11.5-14.5% RDW-CV is calculated as follows

• RDW-CV % = 1 standard deviation of RBC volume/MCV x 100%

High RDW indicates the presence of anisocytosis which is related to impaired erythropoiesis and degradation of red blood cells. Chronic inflammation and increased levels of oxidative stress impairs erythropoiesis, resulting in size variation. Chronic

hyperglycaemia causes non enzymatic glycosylation of RBC membrane proteins and leads to accelerated aging of RBC's.Loss of red cell deformability can also lead to impaired microvascular circulation and hypoxia as well as to micro thrombosis.Pro-inflammatory cytokines have been reported to inhibit the maturation of erythrocytes, which is caused by erythropoietin. Thus, inflammation causes immature red blood cells to be released into the peripheral circulation, which may result in anisocytosis [82,83].

Platelets:

Platelets are the anucleate, discoid shaped cells necessary to maintain homeostasis and thrombosis. They are created from megakaryocytes, and persist in blood stream for 5-7 days. Platelets contain at least three major types of granules— α-granules, dense granules, and lysosomes. In physiologic level , platelet adhesion to sub endothelial collagen is mediated by von Willebrand factor and possibly other adhesive proteins, which bind to a glycoprotein receptor (GPIb) on the platelet surface as well as to sub endothelial components.

Platelets adhering to collagen undergo a shape change, secrete their granular contents, and aggregate. In diabetes platelets exhibit enhanced aggregation activity early in the disease course that may precede the development of cardiovascular disease. Among diabetic individuals, following biochemical abnormalities occurs[91]

- Reduces membrane hydration,
- Altered Ca^{2+} and Mg^{2+} homeostasis (increased intracellular Ca^{2+} mobilization and decreased intracellular Mg^{2+}),

- Increased arachidonic acid metabolism- increased thromboxane synthesis, decreased prostacyclin production, decreased NO production, decreased antioxidant levels.
- Increased expression of activation-dependent adhesion molecules (e.g., GpIIb–IIIa, P-select-in).

In healthy individual insulin is natural antagonist for platelet hyperactivity and sensitises prostacyclin and nitric oxide. As there is defect in insulin action disordered platelet activity leads to microvascular and macro vascular complications.

The large platelets having dense granules are more active biochemically, function- ally, and metabolically, have higher thromboxane A2 levels, express more glycoprotein Ib and IIb /IIIa receptors, and could be a risk factor for developing coronary thrombosis.

Mean platelet volume (MPV) is a marker showing platelet function and activation.

Platelets involve in atherosclerosis through secreting proinflammatory cytokines and its bounding to endothelial cells [87,88]. Platelets release the thromboxanes and other mediators, this may cause increased inflammation in patients with higher platelets. Small vascular bleeds due to rupture of a the thrombotic plaques leads to bone marrow stimulation and recruitment of larger hyperactive platelets. These platelets contain denser granules, secrete more serotonin and TXA2, and have a procoagulant effect. Also osmotic swelling of platelets, as a result of hyperglycaemia contributes to platelet size variation and increased MPV in T2DM patients.

HBA1C

HbA1c is a clinical standard measure of blood sugar management. HbA1c is the best available biochemical parameter which provides a long-term trend of how high blood glucose levels have been for the period of the last 6-8 weeks. It is closely associated with the risk of developing complications and hence it is used as an evidence-based marker to assess the chances of developing diabetic complications [89]

HBA1c is also a good predictor of lipid profile;[90] thus, monitoring of glycemic control using HbA1c could have additional benefits of identifying diabetes patients who are at a greater risk of cardiovascular complication. Target levels for HbA1c in type 2 diabetes are individual, but a general aim stated by the National Institute for Health and Excellence is 6.5–7.0% (48–53mmol/ mol).

According to the National Glycohemoglobin Standardization Program (NGSP), which developed the A1C tests, the accuracy has continued to evolve and got more precise over time. The HbA1c is recommended to be performed at least twice a year in diabetes patients with stable blood glucose levels

4. AIM OF THE STUDY

To assess hematological parameters in type 2 diabetes mellitus, and to correlate with carotid intimal thickness in predicting macro-vascular complications.

5. MATERIALS AND METHODOLOGY

STUDY CENTRE	RGGGH
STUDY DURATION	6 MONTHS FROM TIME OF APPROVAL FROM ETHICAl COMMITTEE
STUDY DESIGN	OBSERVATIONAL STUDY

INCLUSION CRITERIA-

1.All patients of age more than 18 Years diagnosed as type 2 diabetes mellitus according to ADA Guidelines at least 2 years on treatment

2. Patients giving consent for the study.

EXCLUSION CRITERIA-

1. Known case of chronic diseases like chronic kidney disease, chronic liver disease, coronary artery disease.
2. Type 1 Diabetes Mellitus

3. Known case of hematological malignancies, abnormalities.
4. Presented with acute infection.
5. Known case of hypertension.

Sample size-

Sample size calculated using the formula

n= Z 2 PQ/ D2

where,

N is the required samples size

Z- confidence interval: is the standard normal deviate corresponding to 95% confidence interval which is 1.96.

P is the prevalence- 29.7%

d-Absolute precision -10%

Q=100-p

Non-responders-10%

Sample size = 1.96*. 1.96/ 29.7*100-29.7/10*10

=88~100 approximately

Sampling method:

The study participants were selected from RGGGH who are diagnosed as diabetes mellitus based on inclusion and exclusion criteria.

All patients of type 2 diabetes mellitus are selected based on above exclusion and inclusion criteria and evaluated for further examination.

Study variables:

COMPLETE BLOOD COUNT, PERIPHERAL SMEAR:

Total leucocyte count		Platelet distribution width	
Absolute neutrophil count		Hemoglobin	
Absolute lymphocyte count		Red cell distribution width	
Platelet Count		Peripheral smear	
Mean platelet volume			

LFT, SERUM ELECTROLYTES, ABG:

Urea		T.BIL	
Creatinine		D.BIL	
Fasting blood sugar		SGOT	
Post prandial blood sugar		SGPT	

Sodium		Protein	
HbA1c		Albumin	
Potassium		ALP	
Fasting lipid profile		ABG	

COAGULATION PROFILE:

PT	
aPTT	
INR	

COMPARISON OF PROFILE

	HAEMATOLOGICAL PARAMETERS	CAROTID INTIMAL THICKNESS> 0.8	CAROTID INTIMAL THICKNESS< 0.8
1.	NEUTROPHIL/ LYMPHOCYTE RATIO		
2.	RED CELL DISTRIBUTION WIDTH		
3.	MEAN PLATELET VOLUME		
4.	HBA1c LEVELS		

6. RESULTS

The results of the study done on 100 participants to assess hematological parameters in type 2 diabetes mellitus, and to compare with carotid intimal thickness in predicting macro-vascular complications are as follows:

Age group of the study participants:

Age group (in years)	**Male**	**Female**	**Total**	**Fischer exact test value**	**P value**
31-40	12 (24%)	17 (34%)	29 (29%)		
41-50	18 (36%)	26 (52%)	44 (44%)		
51-60	20 (40%)	5 (10%)	25 (25%)	8.419	0.213
61-70	0	2 (4%)	2 (2%)		
Total	50 (100%)	50 (100%)	100 (100%)		

Table 6.1. Age group of the study participants

The age group of the study participants varies from 31 to 70 years. Maximum number of study participants were at 41 to 50 years age group. Mean age of the study participants was 45.38 ± 7.60 years. Among 100 participants, both male and females are equally represented with 50 each and both are comparable with respect to age. ($p>0.05$)

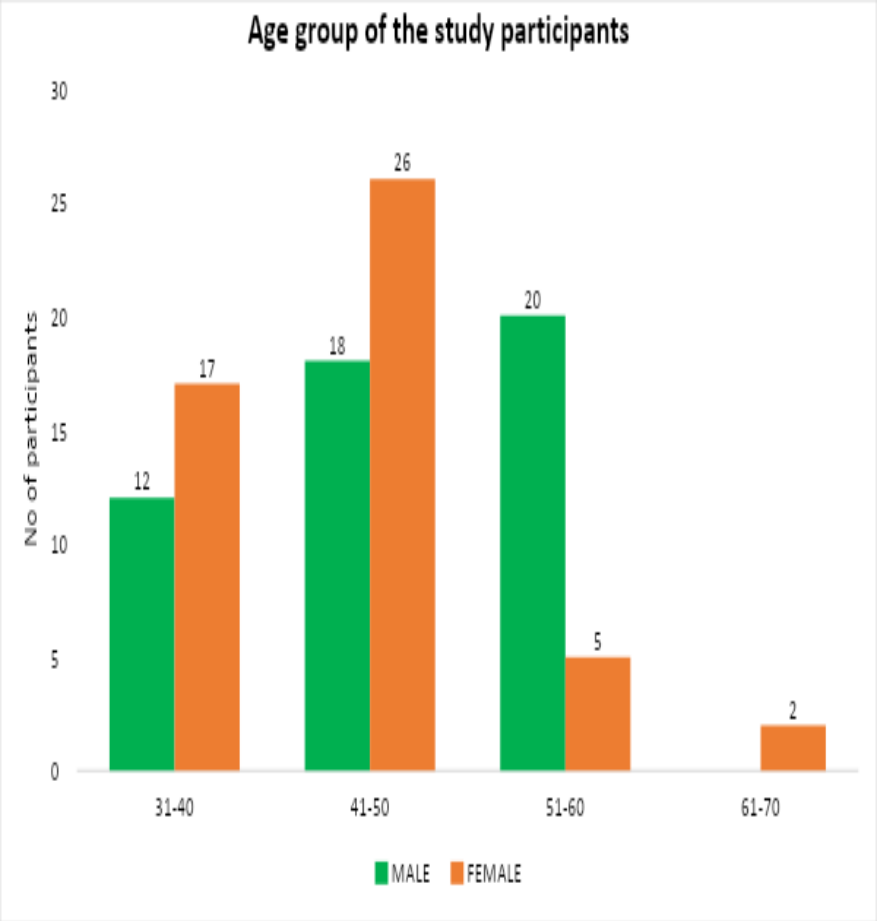


Fig 6.1. Age group of the study participants

HbA1C of the study participants:

HbA1C	Frequency	Percentage (%)
>7.5	59	59
5.5-7.5	41	41
Total	100	100

Table 6.2. HbA1C of the study participants

The Mean HbA1C of the 100 diabetes mellitus patients in the study was found to be 7.27 ± 0.77 with 59% had HBA1C more than 7.5.

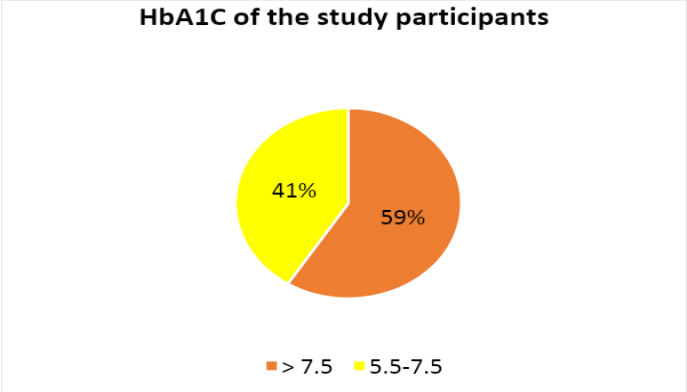


Fig 6.2. HbA1C of the study participants

Neutrophil Lymphocyte ratio of the study participants:

Neutrophil/Lymphocyte ratio	Frequency
>2	62
1-2	38
Total	100

Table 6.3. Neutrophil Lymphocyte ratio of the study participants

Among the 100 study participants 62% had high Neutrophil lymphocyte ratio of more than 2. The overall mean Neutrophil Lymphocyte ratio was found to be 2.23 ± 0.37

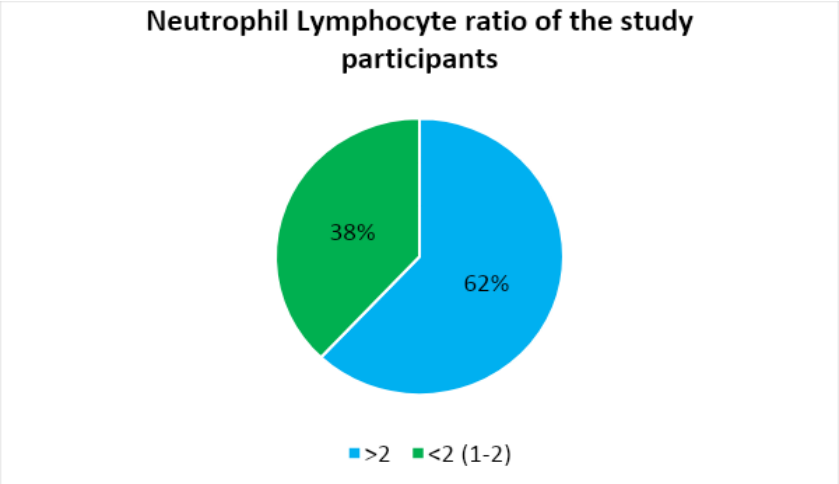


Fig 6.3. Neutrophil Lymphocyte ratio of the study participants

Red cell distribution width of the study participants:

Red cell distribution width	Frequency	Percentage (in %)
>15	62	62
11 to 15	38	38
Total	100	100

Table 6.4. Red cell distribution width of the study participants

The Mean red cell distribution width (RDW) of the study participants was found to be 16.93 ± 3.60 with 38% had RDW more than 15.

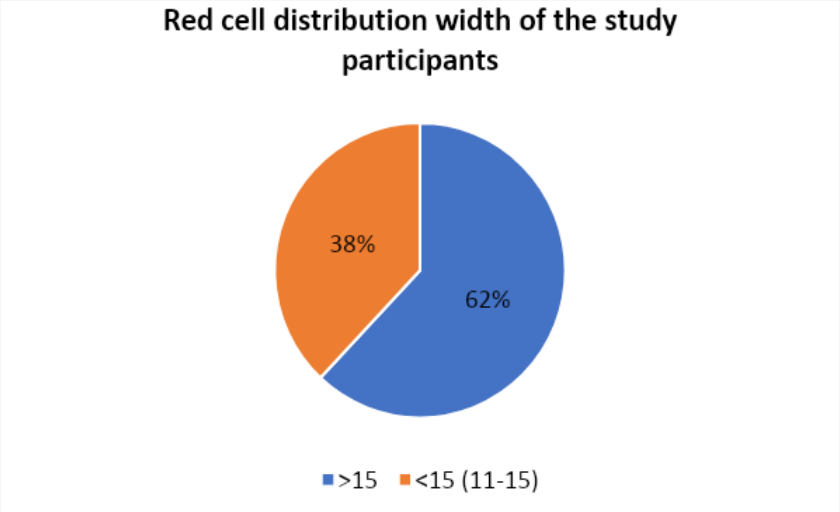


Fig 6.4. Red cell distribution width of the study participants

Mean platelet volume of the study participants:

Mean platelet volume	Frequency	Percentage (%)
>12	58	58
7 to 12	42	42
Total	100	100

Table 6.5. Mean platelet volume of the study participants

The average mean platelet volume of study participants was 12.53 ± 2.46 and 42% showed mean platelet volume of >12.

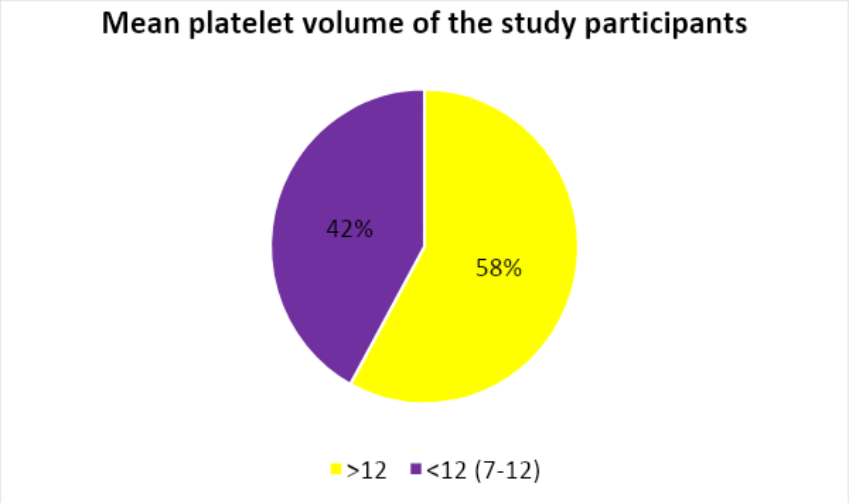


Fig 6.5. Mean platelet volume of the study participants

Comparison of gender and HbA1C among the study participants:

Gender	HbA1C		Total	Chi square value	P value
	>7.5	5.5-7.5			
Male	32 64%	18 36%	50 100%	1.033	0.309
Female	27 54%	23 46%	50 100%		
Total	59 59%	41 41%	100 100%		

Table 6.6. Comparison of gender and HbA1C among the study participants

Among the males 32 males had HbA1c levels more than 7.5 and 18 males had HbA1c levels from 5.5-7.5. Among the females 27 females had HbA1c levels more than 7.5 and 23 females had HbA1c levels from 5.5-7.5. This difference was not statistically significant by Chi square test.

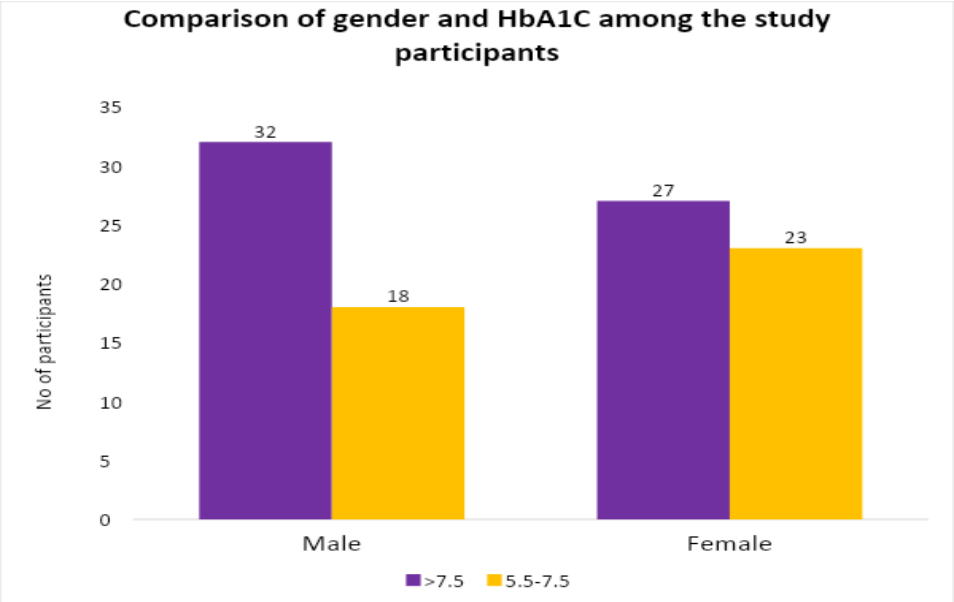


Fig 6.6. Comparison of gender and HbA1C among the study participants

Comparison of gender and Neutrophil/Lymphocyte ratio among the study participants:

Gender	N/L ratio		Total	Chi square value	P value
	>2	1-2			
Male	33 66%	17 34%	50 100%	0.679	0.410
Female	29 58%	21 42%	50 100%		
Total	62 62%	38 38%	100 100%		

Table 6.7. Comparison of gender and N/L ratio among the study participants

Among the males 33 males had N/L ratio more than 2 and 17 males had N/L ratio from 1-2. Among the females 29 females had N/L ratio more than 2 and 21 females had N/L ratio from 1-2. This difference was not statistically significant by Chi square test.

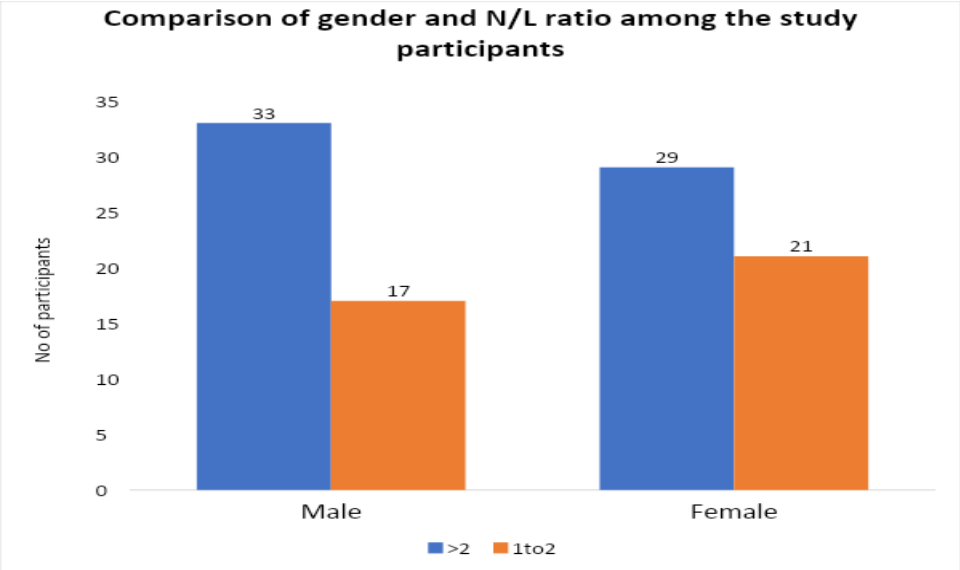


Fig 6.7. Comparison of gender and N/L ratio among the study participants

Comparison of gender and Red cell distribution width among the study participants:

Gender	Red cell distribution width		Total	Chi square value	P value
	>15	11 to			
Male	33 66%	17 34%	50 100%	0.679	0.410
Female	29 58%	21 42%	50 100%		
Total	62 62%	38 38%	100 100%		

Table 6.8. Comparison of gender and Red cell distribution width among the study participants.

Among the males 33 males had Red cell distribution width more than 15 and 17 males had Red cell distribution width from 11 to 15. Among the females29 females had Red cell distribution width more than 15 and 21 females had Red cell distribution width from 11 to 15. This difference was not statistically significant by Chi square test.

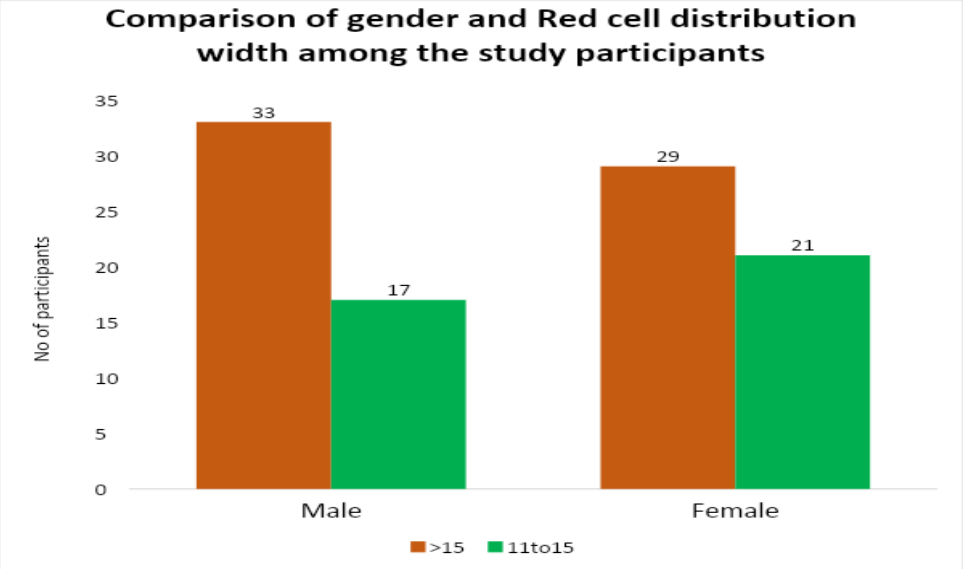


Fig 6.8. Comparison of gender and Red cell distribution width among the study participants

Comparison of gender and Mean platelet volume among the study participants:

Gender	Mean platelet		Total	Chi square value	P value
	>12	7 to 12			
Male	31 62%	19 38%	50 100%	0.657	0.418
Female	27 54%	23 46%	50 100%		
Total	58 58%	42 42%	100 100%		

Table 6.9. Comparison of gender and Mean platelet volume among the study participants

Among the males 31 males had Mean platelet volume more than 12 and 19 males had Mean platelet volume from 7 to 12. Among the females 27 females had Mean platelet volume more than 12 and 23 females had Mean platelet volume from 7 to 12. This difference was not statistically significant by Chi square test.

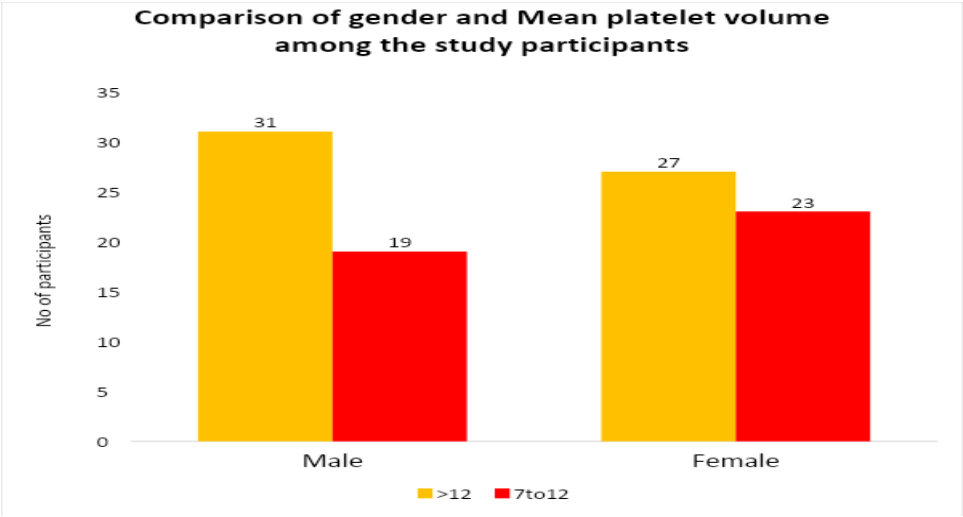


Fig 6.9. Comparison of gender and Mean platelet volume among the study participant

Comparison of HbA1C and carotid intimal thickness among the study participants:

HbA1C	Carotid intimal thickness		Total	Chi square value	P value
	>0.8	<=0.8			
>7.5	58 (98.3%)	1 (1.7%)	59 (100%)	76.949	<0.001*
5.5 to 7.5	5 (12.1%)	36 (87.8%)	41 (100%)		
Total	63 (63%)	37 (37%)	100 (100%)		

Table 6.10. Comparison of HbA1C and carotid intimal thickness among the study participants

*- statistically significant by Chi square test

Among the 100 diabetic mellitus patients in the study, 63 showed carotid intimal thickness of more than 0.8 and 59 patients had HbA1C more than 7.5. Carotid Intima thickness was more among those with HbA1C more than 7.5 (98.3%) compared to 12.1% in those with HbA1C. Hence there is a significant association between increased HbA1C level and Carotid intimal thickness in diabetes mellitus patients.

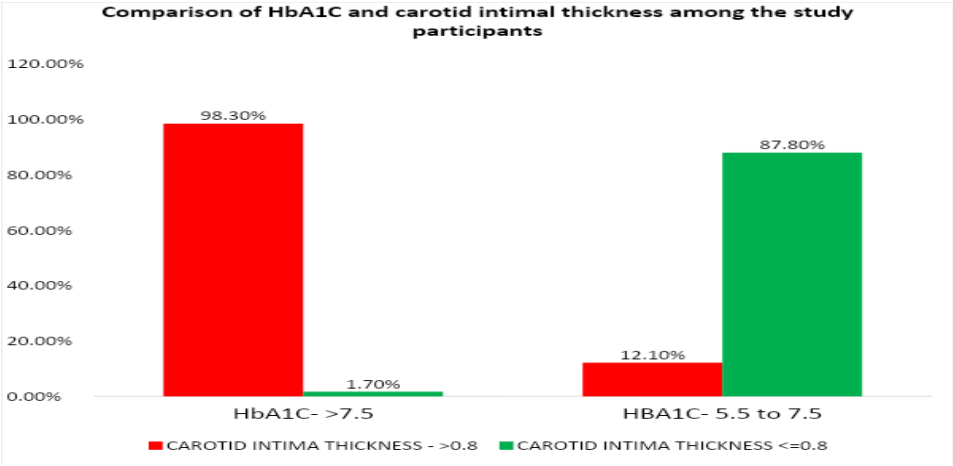


Fig 6.10. Comparison of HbA1C and carotid intimal thickness among the study participants

Comparison of Neutrophil/Lymphocyte ratio and carotid intimal thickness among the study participants:

Neutrophil/ Lymphocyte ratio	Carotid intimal thickness		Total	Chi square value	P value
	>0.8	<=0.8			
>2	62 (100	0	62 (100%	95.823	<0.001 *
1 to 2	1 (2.6%	37 (97.4%)	38 (100%		
Total	63 (63%)	37 (37%)	100 (100%)		

Table 6.11. Comparison of N/L ratio and carotid intimal thickness among the study participants

*- statistically significant by Chi square test

Among the 100 diabetes mellitus patients in this study, 62% had neutrophil lymphocyte ratio of >2. All those with >2 (100%) NLR was found to have increased carotid intimal thickness of >2 compared to 2.6% of those with NLR <2. This difference shows highly significant association of increased Neutrophil Lymphocyte ratio with increased Carotid intima thickness among diabetic patients.

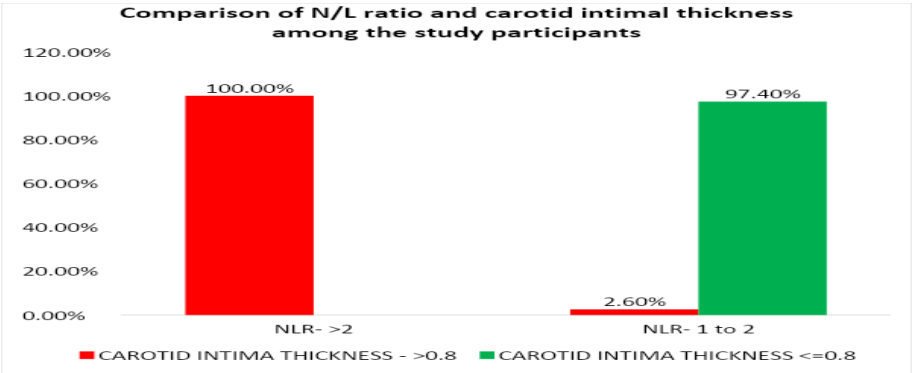


Fig 6.11. Comparison of N/L ratio and carotid intimal thickness among the study participants

Comparison of Red cell distribution width and carotid intimal thickness among the study participants:

Red cell distribution width	Carotid intimal thickness		Total	Chi square value	P value
	>0.8	**<=0.8**			
>15	55 (88.7	7 (11.4	62 (100%)	46.266	**<0.001 ***
11 to 15	8 (21.1	30 (78.9	38 (100%)		
Total	63 (63%)	37 (37%)	100 (100%)		

Table 6.12. Comparison of Red cell distribution width and carotid intimal thickness among the study participants

*- statistically significant by Chi square test

The 62% of the study participants had Red Cell Distribution Width >15. Among those with RDW >15, 88.7% showed carotid intimal thickness of >0.8 compared to 21.1% in those with RDW 11-15. This difference shows significant association of increased red cell distribution width with increased carotid intima thickness among diabetes mellitus patients.

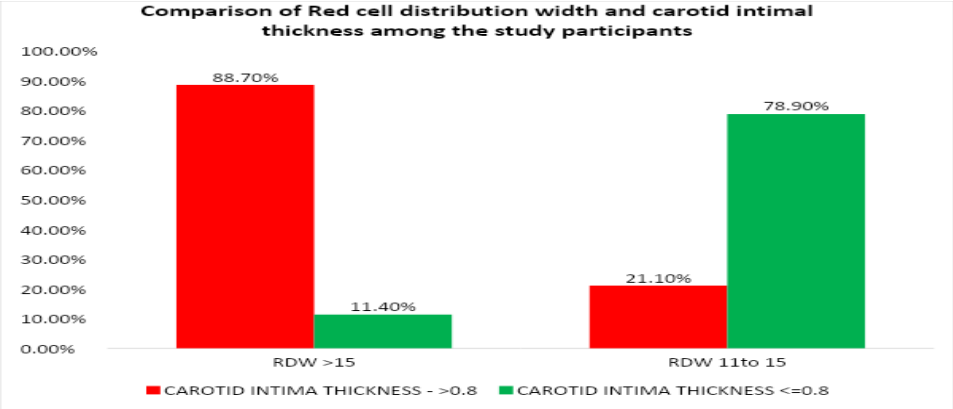


Fig 6.12. Comparison of Red cell distribution width and carotid intimal thickness among the study participants

Comparison of Mean platelet volume and carotid intimal thickness among the study participants:

Mean platelet volume	Carotid intimal thickness		Total	Chi square value	P value
	>0.8	<=0.8			
>12	53 (91.4	5 (8.6%)	58 (100%)	47.713	**<0.001 ***
7 to 12	10 (23.8	32 (76.2%)	42 (100%)		
Total	63 (163%	37 (37%)	100 (100%)		

Table 6.13. Comparison of Mean platelet volume and carotid intimal thickness among the study participants

*- statistically significant by Chi square test

The Mean Platelet volume was found to be >12 in 58 diabetes mellitus patients in the study. Among them 91.4% had increased carotid intimal thickness of >0.8. However among those with Mean platelet volume between 7 to 12, the carotid intimal thickness was found to be increased (>0.8) in only 23.8%. This difference of carotid intimal thickness between those with MPV >12 and 7-12 was found to significant (p<0.05)

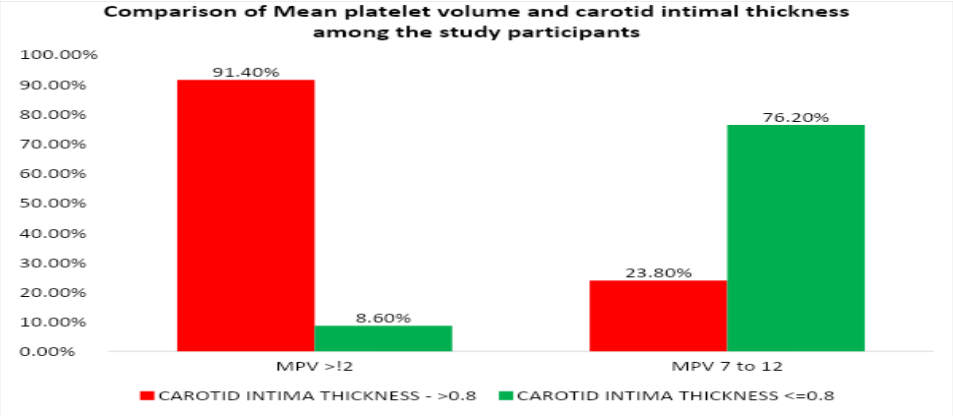


Fig 6.13. Comparison of Mean platelet volume and carotid intimal thickness among the study participants

Correlation between carotid intimal thickness and HbA1C:

Carotid intimal thickness	Pearson correlation coefficient	P value	Interpretation
HbA1C	0.55	<0.001*	Positive correlation

Table 6.14. Correlation between carotid intimal thickness and HbA1C

*- statistically significant correlation

Among the study participants, the increase in Carotid Intima thickness significantly correlates positively with increased HbA1C, showing that among diabetes mellitus patients, increase in HbA1C increases the chances of increased carotid intimal thickness.

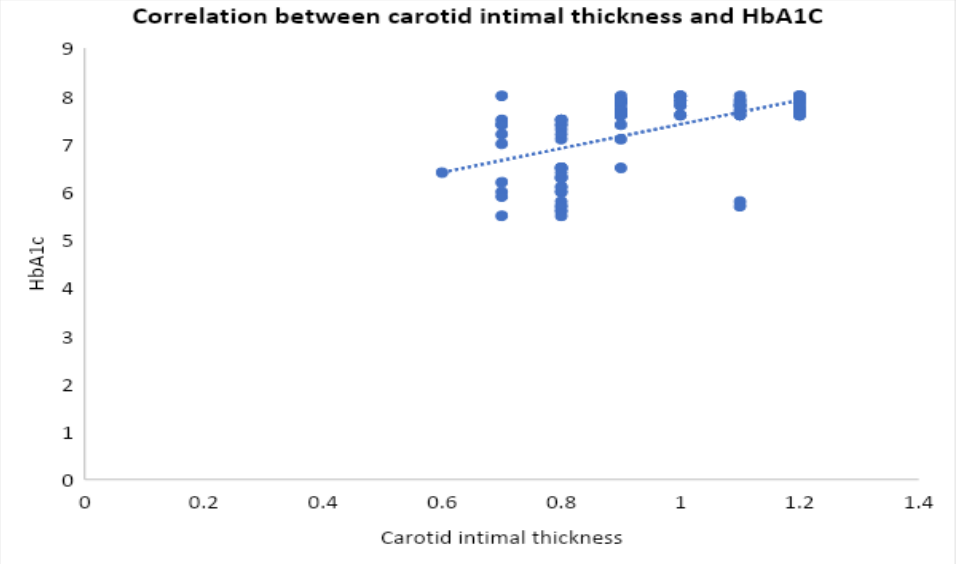


Fig 6.14. Correlation between carotid intimal thickness and HbA1C

Correlation between carotid intimal thickness and Neutrophil/Lymphocyte ratio:

Carotid intimal thickness	Pearson correlation coefficient	P value	Interpretation
Neutrophil/ Lymphocyte ratio	0.628	<0.001*	Positive correlation

Table 6.15. Correlation between carotid intimal thickness and Neutrophil/ Lymphocyte ratio

*- statistically significant correlation

There is a significant positive correlation between increased neutrophil lymphocyte count and carotid intimal thickness. Increase in Neutrophil Lymphocyte ratio >2 in diabetes mellitus patients, increases the chances of increase in carotid intimal thickness.

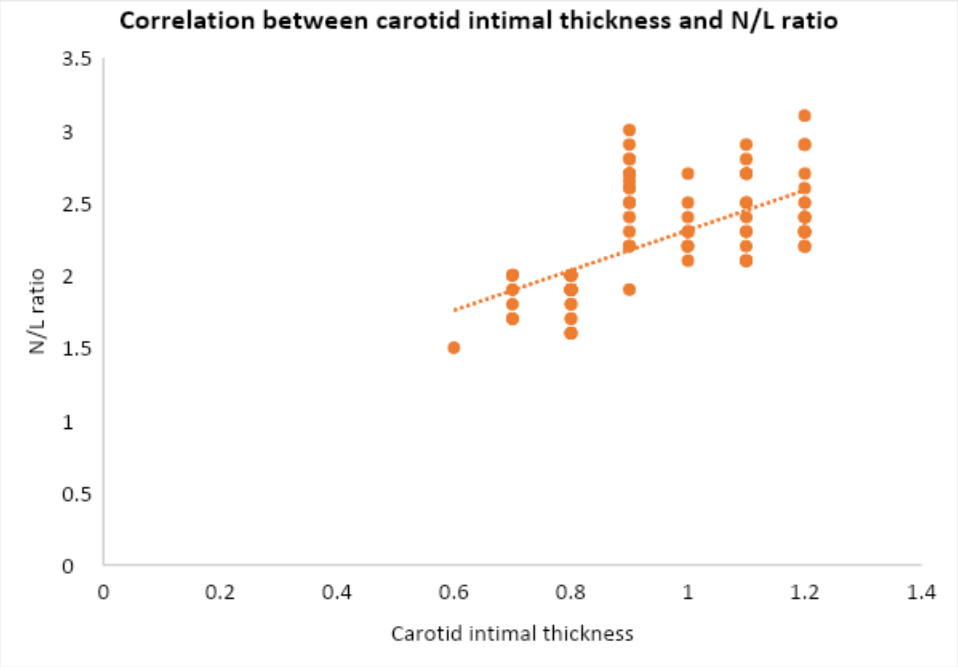


Fig 6.15. Correlation between carotid intimal thickness and Neutrophil/ Lymphocyte ratio

Correlation between carotid intimal thickness and Red cell distribution width:

Carotid intimal thickness	Pearson correlation coefficient	P value	Interpretation
Red cell distribution width	0.494	<0.001*	Positive correlation

Table 6.16. Correlation between carotid intimal thickness and Red cell distribution width

*- statistically significant correlation

Significant positive corelation between Red cell distribution width and carotid intimal thickness in Figure shows that, increase in RDW, increases the risk of increase in Carotid intimal thickness among diabetes mellitus patients.

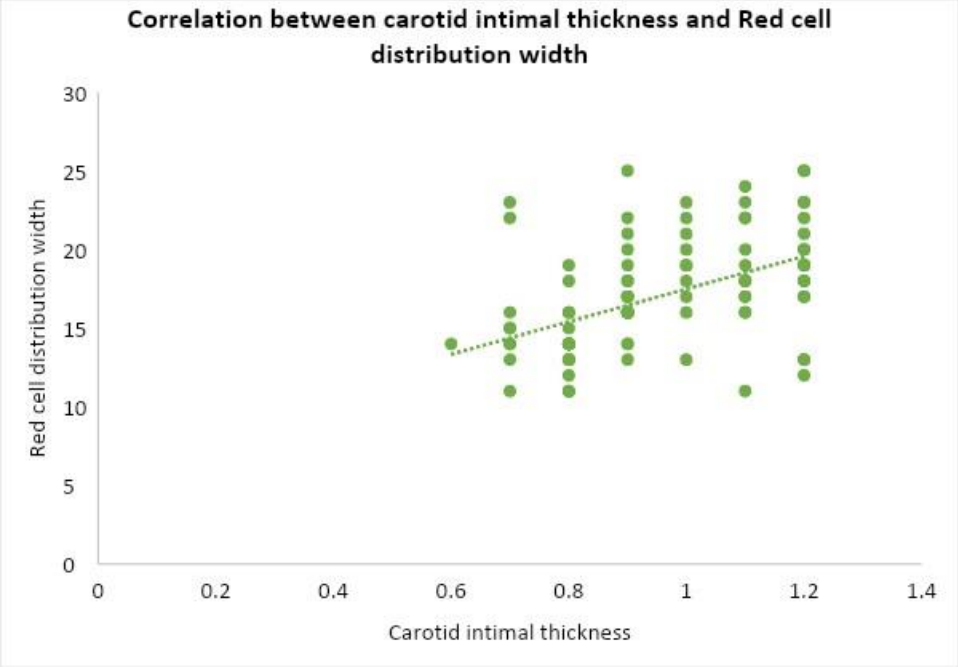


Fig 6.16. Correlation between carotid intimal thickness and Red cell distribution width

Correlation between carotid intimal thickness and Mean platelet volume:

Carotid intimal thickness	Pearson correlation coefficient	P value	Interpretation
Mean platelet volume	0.596	<0.001*	Positive correlation

Table 17. Correlation between carotid intimal thickness and Mean platelet volume

*- statistically significant correlation

There is a significant positive correlation between mean platelet volume and increased carotid intimal thickness ($p<0.05$), which shows that increase in mean platelet volume in diabetes mellitus patients increases the risk of increased carotid intimal thickness.

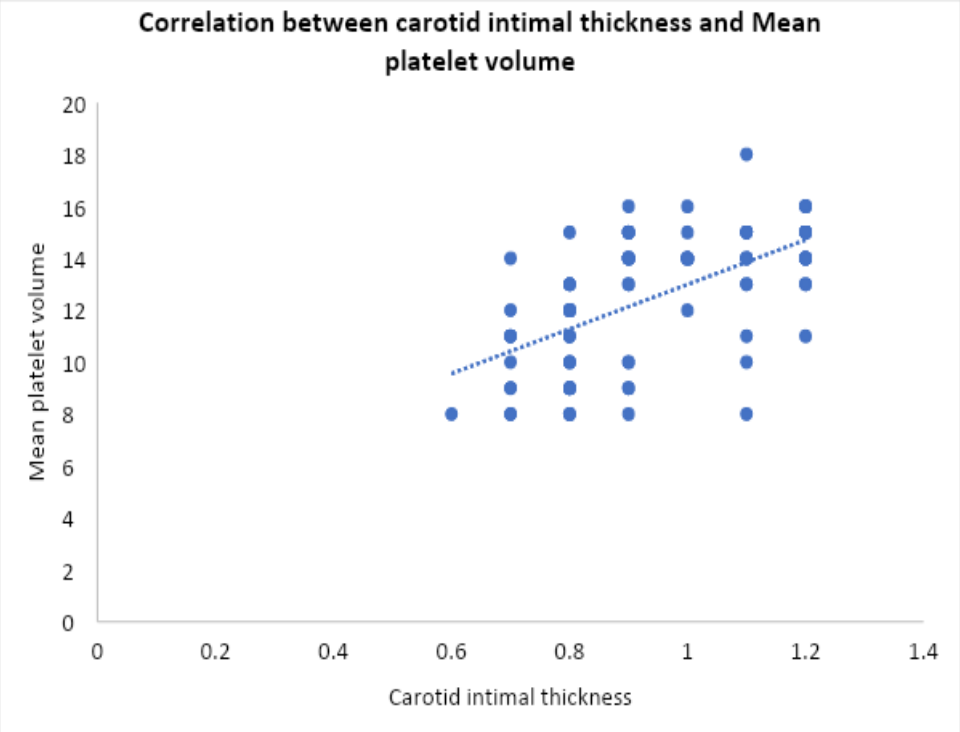


Fig 6.17. Correlation between carotid intimal thickness and Mean platelet volume

7. SUMMARY

This study was conducted in Rajiv Gandhi Government General Hospital from June2022 to November 2022. A total 100 case were studied. The Hemotological indices, HBA1c and CIMT parameter were included and assessed.

AGE OF THE PATIENTS:

Among 100 participants, both male and females are equally represented with 50 each and both are comparable with respect to age. ($p>0.05$). The age group of the study participants varies from 31 to 70 years. Maximum number of study participants were at 41 to 50 years age group. Mean age of the study participants was 45.38 ± 7.60 years. Among 100 participants, both male and females are equally represented with 50 each and both are comparable with respect to age. ($p>0.05$)

Among the 100 diabetic mellitus patients in the study, HBA1c 63 showed carotid intimal thickness of more than 0.8 and 59 patients had HbA1C more than 7.5. Carotid Intimal thickness was more among those with HbA1C more than 7.5 (98.3%) compared to 12.1% in those with HbA1C. HAEMOTOLOGICAL INDICES; 62% had neutrophil lymphocyte ratio of >2. All those with >2 (100%) NLR was found to have increased carotid intimal thickness of >2 compared to 2.6% of those with NLR <2. ($p<0.001$)

Among those with RDW >15, 88.7% showed carotid intimal thickness of >0.8 compared to 21.1% in those with RDW 11-15.

The Mean Platelet volume was found to be >12 in 58 diabetes mellitus patients

in the study. Among them 91.4% had increased carotid intimal thickness of >0.8. However, among those with Mean platelet volume between 7 to 12, the carotid intimal thickness was found to be increased (>0.8) in only 23.8%.

As Type2 Diabetes mellitus is chronic inflammation state with resulting in significant changes of hematological indices which is useful marker to asses complications by associating with CIMT, a surrogate marker of atherosclerosis**.**

8. DISCUSSION

As cardiovascular diseases are the major causes of preventable health in patients of type2 diabetes mellitus , Use of inexpensive tools such as hematological parameters in order to identify the adverse outcomes in association with carotid intimal thickness to asses the macro vascular complications. As IMT considered as surrogate marker of atherosclerosis can be used as cardiovascular risk disease.

Two studies conducted among persons of general population from the CHS and ARIC studies found that IMT markers along with the traditional risk factors such as hs-CRP has shown that B-USG mode of carotid artery as another marker to predict risk CHD. In this study 13,145 ARIC participants followed for ~15 years, using C- IMT and plaque information can improve CHD [92,94]

Cao et al identified an association using carotid IMT measurement, which is a composite measure that merges the maximum internal and common carotid wall thickness of the right and left carotid arteries, and explored the independent effects of carotid IMT and plaque.[93]

Of the 5201 individuals enrolled in the CHS, 2946 (56.8%) were women and 2255 (43.2%) were men. Maximum ICA and CCA wall thicknesses were greater in men than women Wall-thickness measurements of the ICA were consistently greater than those of the CCA. Both measurements were also greater in subjects with CHD when compared with those without.

Red Cell distribution width , an automated measure of RBC size can be used as

marker for CVD. The cardiovascular risk factors of hypertension, hypercholesterolemia, and chronic kidney disease were also associated with higher RDW. Age of the patients, BMI and CRP had positive significant moderate correlation with RDW similar to our study, Vayá et al., mentioned a significant strong correlation between RDW and BMI[95].

Wan et al reported that a higher NLR level was associated with an increased prevalence of cardiovascular and cerebrovascular diseases, and diabetic kidney disease in diabetic adults. It is a cross-sectional survey of 4,813 diabetic adults was conducted in seven communities in China. [96]. Persons underwent several medical examinations, including the measurement of anthropometric factors, blood pressure, routinely analyzed leukocyte characteristics, glucose, lipid profiles, urine albumin/ creatinine ratio, and Fundus photographs. Only the NLR level was positively associated with both CVD and CCA plaque, which suggests that the elevated NLR level may be a more proper predictor of CVD events than leukocyte and neutrophil levels With reference to platelet indices between the control and the diabetic patients, we observed no signicant difference in platelet count, but MPV increased in Diabetics.

Jabeen et al. did a study on 170 diabetic patients to determine the relationship of glycemic control on hematological parameters in diabetes mellitus patients and reported that among hematological parameters MPV were signicantly increased in diabetes patients as compared to non-diabetics

In a study by Swaminathan et al. [97] MPV was found to be higher in subjects with type 2 diabetes and significantly increased in diabet- ics with poor glycemic control and having a longer duration of diabetes. In this study compared between Diabetes and. Non diabetes of 100 individuals each and found increase in MPV in Diabetics.

HbA1c levels and atherosclerotic macrovascular complication. In a study conducted on patients with diabetes mellitus by[99] Brohall G et al. they found significantly increased cIMT values in patients with diabetes mellitus compared to healthy subject . In this study 81 type 2 diabetic patients were enrolled in this study. Patients were divided into two groups according to cIMT values: cIMT < 0,9 mm group and cIMT ≥ 0,9 mm group. Increased cIMT values were accepted as ≥ 0,9 mm. Then compared HbA1c between normal and increased cIMT groups. And found that, HbA1c could not be a marker for subclinical atherosclerosis in diabetic patients.

In the studies conducted on on patients with diabetes mellitus by [100].Mukai N et al Huang Y et al Venkataraman et al and Ma X et al they found significant correlation between high HbA1c and increased cIMT. In a study conducted on patients with diabetes mellitus by Du HW et al. they found no significant relationship between HbA1c and cIMT values. As in this hospital based with prolonged periods in this as HBA1c is king term measure showed no significant changes.

9. CONCLUSION

Poor Glycemic control causes alteration in various biochemical parameters as well as hematological indices. Hyperglycemia induces the production of ROS causing oxidative stress as well as lipid per-oxidation which may accelerate vascular complication in diabetics. The study indicates the clinical usefulness of NLR ,RDW and MPV,HBA1c levels and its association with carotid intimal thickness . As IMT is a surrogate marker of atherosclerosis, to identify complications earlier with inexpensive tools.

10.LIMITATIONS OF STUDY

1. Single institution study where general population cannot be included.

2. Only type2 diabetic individuals on going treatment were included

3. Drugs that can cause hematological parameters changes were not addressed.

11.REFERENCES

1. Kannel WB and McGee DL. Diabetes and cardiovascular disease. The Framingham study. JAMA 1979; 241: 2035-2038. Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. Diabetes 1999; 48: 937-942.

2. El Haouari M and Rosado JA. Platelet signaling abnormalities in patients with type 2 diabetes mellitus: a review. Blood Cells Mol Dis 2008; 41: 119-123.

3. Gasparyan AY, Ayvazyan L, Mikhailidis DP and Kitas GD. Mean platelet volume: a link between thrombosis and inflammation? Curr Pharm Des 2011; 17: 47-58.

4. 4.Zuberi BF, Akhtar N and Afsar S. Comparison of mean platelet volume in patients with diabetes mellitus, impaired fasting glucose and non-diabetic subjects. Singapore Med J 2008; 49: 114-116.

5. Kim JH, Bae HY and Kim SY. Response: clinical marker of platelet hyperreactivity in diabetes mellitus (diabetes metab j 2013;37:423-8). Diabetes Metab J 2014; 38: 160-161.

6. Suslova TE, Sitozhevskii AV, Ogurkova ON, Kravchenko ES, Kologrivova IV, Anfinogenova Y and Karpov RS. Platelet hemostasis in patients with metabolic syndrome and type 2 diabetes mellitus: cGMP-and No-dependent mechanisms in the insulin-mediated platelet aggregation. Front Physiol 2014; 5: 5

7. Twig G, Afek A, Shamiss A, Derazne E, Tzur D, Gordon B and Tirosh A. White blood cells count and incidence of type 2 diabetes in young men. Diabetes Care

2013; 36: 276-282.

8. Jiang H, Yan WH, Li CJ, Wang AP, Dou JT and Mu YM. Elevated white blood cell count is associated with higher risk of glucose metabolism disorders in middle-aged and elderly Chinese people. Int J Environ Res Public Health 2014; 11: 5497-5509.

9. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C and Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002; 51: 455461.

10. Akbas EM, Demirtas L, Ozcicek A, Timuroglu A, Bakirci EM, Hamur H, Ozcicek F and Turkmen K. Association of epicardial adipose tissue, neutrophil-to-lymphocyte ratio and platelet-tolymphocyte ratio with diabetic nephropathy. Int J Clin Exp Med 2014; 7: 1794-801.

11. Akbas EM, Hamur H, Demirtas L, Bakirci EM, Ozcicek A, Ozcicek F, Kuyrukluyildiz U and Turkmen K. Predictors of epicardial adipose tissue in patients with type 2 diabetes mellitus. Diabetol Metab Syndr 2014; 6: 55

12. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J, Zimmet P: Expert Committee on the Diagnosis and Classification of Diabetes Mellitus2, the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003; 26: 3160– 3167 [PubMed] [Google Scholar]

13. International Diabetes Federation. IDF Diabetes Atlas. 9th ed. Brussels, Belgium: International Diabetes Federation; 2019.

14. World Health Organization. The top 10 causes of death. Available from: http:// www.who.int/en/news-room/fact-sheets/detail/ the-top-10-causes-of-death. [Last accessed on 2021 Jun 04]

15. Pradeepa R, Mohan V. Prevalence of type 2 diabetes and its complications in India and economic costs to the nation. Eur J Clin Nutr 2017;71:816-24.

16. DeFronzo RA (1988) Lilly Lecture. The triumvirate: beta cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 37: 667-687

17. DeFronzo RA, Ferrannini E (1987) Regulation of hepatic glu- cose metabolism in humans. Diab Metab Rev 3:415-459

18. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP (1981) The effect of insulin on the disposal of intravenous 395 glucose: results from indirect calorimetry. Diabetes 30: 1000- 1007

19. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J (1985) Effects of insulin on peripheral and splanchnic glucose metabolism in non-insulin dependent diabetes mellitus. J Clin Invest 76:149-155

20. Ha, J.; Satin, L.S.; Sherman, A.S. A Mathematical Model of the Pathogenesis, Prevention, and Reversal of Type 2 Diabetes. Endocrinology 2016, 157, 624–635.

21. Grapengiesser E, Gylfe E, Hellman B. Three types of cytoplasmic Ca2+ oscillations in stimulated pancreatic beta-cells. Arch Biochem Biophys 1989; 268: 404–407.

22. Gylfe E, Grapengiesser E, Hellman B. Propagation of cytoplasmic Ca2+ oscillations in clusters of pancreatic beta-cells exposed to glucose. Cell Calcium 1991; 12: 229–240.

23. Otani K, Kulkarni RN, Baldwin AC, Krutzfeldt J, Ueki K, Stoffel M et al. Reduced beta-cell mass and altered glucose sensing impair insulin-secretory function in bIRKO mice. Am J Physiol Endocrinol Metab 2004; 286: E41–E49.

24. Firth RG, Bell PM, Rizza RA (1986) Effects of tolazamide and exogenous insulin on insulin action in patients with non-insulin- dependent diabetes mellitus. N Engl J Med 314:1280-1286.

25. Gerich JE (1991) Is muscle the major site of insulin resistance in Type 2 (non-insulin-dependent) diabetes mellitus? Diabetologia 34:607-610

26. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am 2004;88:787–835, ix. https://doi.org/10.1016/ j.mcna.2004.04.013; PMID: 15308380.

27. Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology 2014;59:713–23. https://doi.org/ 10.1002/hep.26672; PMID: 23929732.

28. B. Kiens, “Skeletal muscle lipid metabolism in exercise and insulin resistance,” Physiological Reviews, vol. 86, no. 1, pp. 205–243, 2006.

29. D. Thiebaud, E. Jacot, R. A. DeFronzo, E. Maeder, E. Jequier, and J. P. Felber, “The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and

glucose storage in man," Diabetes, vol. 31, no. 11, pp. 957–963, 1982.

30. Qatanani M, Lazar MA. Mechanisms of obesity-associated insulin resistance: many choices on the menu. Genes Dev. (2007) 21:1443–55. doi: 10.1101/ gad.1550907.

31. Goossens GH, Blaak EE. Adipose tissue dysfunction and impaired metabolic health in human obesity: a matter of oxygen? Front Endocrinol. (2015) 6:55. doi: 10.3389/fendo.2015.00055.

32. Wang QA, Scherer PE. The adipochaser mouse: a model tracking adipogenesis in vivo. Adipocyte. (2014) 3:146–150. doi: 10.4161/adip.27656

33. A. R. Saltiel and C. R. Kahn, "Insulin signaling and the regulation of glucose and lipid metabolism," Nature, vol. 414, no. 6865, pp. 799–806, 2001.

34. J. E. Pessin and A. R. Saltiel, "Signaling pathways in insulin action: molecular targets of insulin resistance," The Journal of Clinical Investigation, vol. 106, no. 2, pp. 165–169, 2000.

35. P. Whitehead, S. F. Clark, B. Ursø, and D. E. James, "Signaling through the insulin receptor," Current Opinion in Cell Biology, vol. 12, no. 2, pp. 222–228, 2000.

36. Kissebah AH, Freedman DS, Peiris AN. Health risks of obesity. Med Clin North Am. 1989; 73(1): 111–138.Cited Here | PubMed | CrossRef

37. Kissebah AH, Vydelingum N, Murray R, et al. Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab. 1982; 54(2): 254–260.

38. Ohlson LO, Larsson B, Svardsudd K, et al. The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 years of follow-up of the participants in

the study of men born in 1913. Diabetes. 1985; 34(10): 1055–1058.

39. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. (2005) 115:1343–51. doi: 10.1172/ JCI23621
40. A, Allen J, Hankey-Giblin PA. Ontogeny and polarization of macrophages in inflammation: blood monocytes versus tissue macrophages. Front Immunol. (2014) 5:683. doi: 10.3389/fimmu.2014.00683 308. Shoelson SE, Lee J, Goldfine AB.
41. Inflammation and insulin resistance. J Clin Invest. (2006) 116:1793–801. doi: 10.1172/JCI29069 309. Jager J, Aparicio-Vergara M, Aouadi M. Liver innate immune cells and insulin resistance: the multiple facets of kupffer cells. J Intern Med. (2016) 280:209–20. doi: 10.1111/joim.12483
42. K. Cusi, K. Maezono, A. Osman, et al., "Insulin resistance differentially affects the PI 3-kinase- and MAP kinasemediated signaling in human muscle," The Journal of Clinical Investigation, vol. 105, no. 3, pp. 311–320, 2000.
43. M.BajajandR.A.DeFronzo,"Metabolicandmolecularbasis of insulin resistance," Journal of Nuclear Cardiology, vol. 10, no. 3, pp. 311–323, 2003.
44. K. Bouzakri, H. A. Koistinen, and J. R. Zierath, "Molecular mechanisms of skeletal muscle insulin resistance in type 2 diabetes," Current Diabetes Reviews, vol. 1, no. 2, pp. 167174, 2005.
45. H. K. R. Karlsson and J. R. Zierath, "Insulin signaling and glucose transport in insulin resistant human skeletal muscle," Cell Biochemistry and Biophysics, vol.

48, no. 2-3, pp. 103113, 2007.

46. T. K. Lam, H. Yoshii, C. A. Haber, et al., “Free fatty acidinduced hepatic insulin resistance: a potential role for protein kinase C-delta,” Am J Physiol Endocrinol Metab, vol. 283, no. 4, pp. E682–91, 2002.

47. G. A. Hitman, K. Hawrami, M. I. McCarthy, et al., “Insulin receptor substrate-1 gene mutations in NIDDM; implications for the study of polygenic disease,” Diabetologia, vol. 38, no. 4, pp. 481–486, 1995.

48. A. Dresner, D. Laurent, M. Marcucci, et al., “Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity,” The Journal of Clinical Investigation, vol. 103, no. 2, pp. 253–259, 1999.

49. W. A. Khan, G. C. Blobe, and Y. A. Hannun, “Arachidonic acid and free fatty acids as second messengers and the role of protein kinase C ,” Cellular Signaling, vol. 7, no. 3, pp. 171184, 1995

50. Nosalski R, Guzik TJ. Perivascular adipose tissue inflammation in vascular disease. Br J Pharmacol. (2017) 174:3496–513. doi: 10.1111/bph.13705 331

51. Fitzgibbons TP, Kogan S, Aouadi M, Hendricks GM, Straubhaar J, Czech MP. Similarity of mouse perivascular and brown adipose tissues and their resistance to diet-induced inflammation. Am J Physiol Heart Circ Physiol. (2011) 301:H1425–37. doi: 10.1152/ajpheart.00376.2011 332.

52. Xia N, Weisenburger S, Koch E, Burkart M, Reifenberg G, Förstermann U, et al.

Restoration of perivascular adipose tissue function in diet-induced obese mice without changing bodyweight. Br J Pharmacol. (2017) 174:344353. doi: 10.1111/ bph.13703 333.

53. Costa RM, Neves KB, Tostes RC, Lobato NS. Perivascular adipose tissue as a relevant fat depot for cardiovascular risk in obesity. Front Physiol. (2018) 9:253. doi: 10.3389/fphys.2018.00253 334.

54. Takaoka M, Nagata D, Kihara S, Shimomura I, Kimura Y, Tabata Y, et al. Periadventitial adipose tissue plays a critical role in vascular remodeling. Circ Res. (2009) 105:906–11. doi: 10.1161/CIRCRESAHA.109.199653 335

55. Gaborit B, Venteclef N, Ancel P, Pelloux V, Gariboldi V, Leprince P, et al. Human epicardial adipose tissue has a specific transcriptomic signature depending on its anatomical peri-atrial, peri-ventricular, or peri-coronary location. Cardiovasc Res. (2015) 108:62–73. doi: 10.1093/cvr/cvv208

56. Ahima RS, Lazar MA. Adipokines and the peripheral and neural control of energy balance. Mol Endocrinol. (2008) 22:1023–31. doi: 10.1210/me.2007-0529

57. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest. (2007) 117:2621–37. doi: 10.1172/JCI31021

58. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. (2003) 423:762–9. doi: 10.1038/nature01705

59. Fang H, Judd RL. Adiponectin regulation and function. Compr Physiol. (2018) 8:1031–63. doi: 10.1002/cphy.c170046

60. Sies H. The concept of oxidative stress after 30 years. In: Gelpi R, Boveris A, Poderoso J, editors. Advances in biochemistry in health and disease. New York: Springer; 2016. p. 3e11.

61. Schrieber M, Chandel N. ROS function in redox signaling and oxidative stress. Curr Biol 2014;24:453e62.

62. Brand M. Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. Free Radic Biol Med 2016;100:14e31

63. Tsai H, Wang W, Lin C, Pai P, Lai T, Tsai C, et al. NADPH oxidase-derived superoxide anion-induced apoptosis is mediated via the JNK dependent activation of NF-kB in cardiomyocytes exposed to high glucose. J Cell Physiol 2012;227:1347e57.

64. Al-Lahham R, Deford J, Papaconstantinou J. Mitochondrial- generated ROS down regulates insulin signaling via activation of p38 MAPK stress response pathway. Mol Cell Endocrinol 2015;419:1e11.

65. Boucher J, Kleinridders A, Kahn C. Insulin receptor signaling in normal and insulin resistant states. Cold Spring Harb Perspect Biol 2014;6:1e23.

66. Rokitansky C. A Manual of Pathological Anatomy. London: Sydenham Society, 1852.

67. Schwenke DC and Carew TE. Initiation of atherosclerotic lesison in cholesterol- fed rabbits. II. Selective retention of LDL vs selective increases in LDL permeability in

susceptible sites of arteries. Arteriosclerosis 9: 908–918, 1989.

68. Nievelstein PFEM, Fogelman AM, Mottino G, and Frank JS. Lipid accumulation in rabbit aortic intima 2 hours after bolus infusion of low density lipoprotein. A deep-etch and immunolocalization study of ultrarapidly frozen tissue. Arterioscl Thromb 11: 1795–1805, 1991.

69. Simionescu M and Simionescu N. Proatherosclerotic events: pathobiochemical changes occurring in the arterial wall before monocyte migration. FASEB J 7: 1359–1366, 1993.

70. Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, and Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. Proc Natl Acad Sci USA 81: 3883–3887, 1984.

71. Heller R, Unbehaun A, Schellenberg B, Mayer B, Werner-Felmayer G, and Werner ER. l-Ascorbic acid potentiates endothelial nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin. J Biol Chem 276: 40–47, 2001.

72. Godin DV, Garnett ME, Cheng KM, and Nichols CR. Sex-related alterations in antioxidant status and susceptibility to atherosclerosis in Japanese quail. Can J Cardiol 11: 945–951, 1995.

73. Kume, Teruyoshi; Akasaka, Takashi; Kawamoto, Takahiro; Watanabe, Nozomi; Toyota, Eiji; Neishi, Yoji; Sukmawan, Renan; Sadahira, Yoshito; Yoshida, Kiyoshi (August 2005). "Assessment of coronary intima--media thickness by optical

coherence tomography: comparison with intravascular ultrasound". Circulation Journal. 69 (8): 903–907. doi:10.1253/circj.69.903. ISSN 1346-9843. PMID 16041157

74. Welty FK, Alfaddagh A, Elajami TK. Targeting inflammation in metabolic syndrome. Transl Res 2016;167:25780.

75. Monteiro Júnior J.G.M., Torres D.O.C., Silva M.C.F.C., et al. Nucleated red blood cells as predictors of all-cause mortality in cardiac intensive care unit patients: A prospective cohort study. PLoS One. 2015;10(12):e0144259.

76. Verbrugge S.E., Huisman A. Verification and standardization of blood cell counters routine clinical laboratory tests. Clin. Lab. Med. 2015;(35):183–196.

77. Fan L., Gui L., Chai E.Q., Wei C.J. Routine hematological parameters are associated with short- and long-term prognosis of patients with ischemic stroke. J. Clin. Lab. Anal. 2018 Epub Ahead of Print.

78. Buyukkaya E, Karakas MF, Karakas E, et al. Correlation of to lymphocyte ratio with the presence and severity of metabolic syndrome. Clin Appl Thromb Hemost 2014;20:15963

79. Bahadir A, Baltaci D, Türker Y, et al. Is the neutrophil-to-lymphocyte ratio indicative of inflammatory state in patients with obesity and metabolic syndrome.Anatol J Cardiol 2015;10:81622

80. Núñez J., Núñez E., Bodí V., et al. Low lymphocyte count in acute phase of ST-segment elevation myocardial infarction predicts long-term recurrent myovardial infarction. Coron. Artery Dis. 2010;21:1–7. [Abstract] [Google Scholar]

81. Basem A., Zaher M., Weiserbs K.F., et al. Usefulness of neutrophil to lymphocyte ratio in predicting short and long-term mortality after non-ST elevation myocardial infarction. Am. J. Cardiol. 2010;106(4):470–476. [Abstract] [Google Scholar]
82. Felker G.M., Allen L.A., Pocock S.J., et al. Red distribution width as a novel prognostic marker in heart failure. J. Am. Coll. Cardiol. 2007;50(1):40–47. [Abstract] [Google Scholar]
83. Tonelli M., Sacks F., Arnold M., Moye L., Davis B., Pfeffer M. Relation between red blood cell distribution width and cardiovascular event rate in people with coronary disease. Circulation. 2008;117(2):163–168. [Abstract] [Google Scholar]
84. EVANS T. and JEHLE D.: The red blood cell distribution width. J. Emmerg. Med., 9 (1): 71-4, 1991.
85. BRIGGS C. AND BAIN B.J.: BASIC HAEMATOLOG- ICAL TECHNIQUES. BAIN B.J., BATES I., LAFFAN M., LEWIS S.M. DACIE and LEWIS: Practical Haema- tology. 11 th ed. Philadelphia, PA: Churchill Livingstone/ Elsevier. Chap. 3, 2012.
86. VAJPAYEE N., GRAHAM S.S., BEM S. BASIC EXAM- INATION OF BLOOD, BONE MARROW. McPHER- SON R.A. and PINCUS M.R.: Henry's Clinical Diagnosis and Management by Laboratory Methods. 22 n d. Elsevier/ Saunders: Philadelphia, PA. 30, 2011.
87. Sakariassen KS, Bolhuis PA, Sixma JJ. Human blood platelet adhesion to artery subendothelium is mediated by factor VIII-Von Willebrand factor bound to the subendothelium. Nature 1979 Jun 14;279(5714):636–638.

88. Holmsen H. Platelet secretion and energy metabolism. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and Thrombosis: Basic Principles and Clinical Practice. 3rd ed. Philadelphia: JB Lippincott; 1994:524–545.

89. Puertas M., Zayas-Castro J.L., Fabri P.J. Statiscal and prognostic analysis of dynamic changes of platelet count in ICU patients. Physiol. Meas. 2015;36:939–953.

90. Zuberi BF, Akhtar N, Afsar S. Comparison of mean platelet volume in patients with diabetes mellitus, impaired fasting glucose and non-diabetic subjects. *Singapore Med J.* 2008;49:114–116. [PubMed] [Google Scholar]

91. El Haouari M, Rosado JA. Platelet signaling abnormalities in patients with type 2 diabetes mellitus: a review. *Blood Cells Mol Dis.* 2008;41:119–123. [PubMed] [Google Scholar]

92. Shariq I. Sherwani, Haseeb A. Khan, Aishah Ekhzaimy, Afshan Masood, Meena K. Sakharkar. significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomark Insights. 2016; 11: 95–104. Published online 2016 Jul 3. doi: 10.4137/BMI.S38440

93. Khan HA, Sobki SH, Khan SA. Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidaemia. Clin Exp Med. 2007; 7:24

94. Ghoshal K, Bhattacharyya M. Overview of platelet physiology: its hemostatic and nonhemostatic role in disease pathogenesis. Sci- entific World Journal. 2014: 781857, doi: 10.1155/2014/781857, indexed in Pubmed: 24729754.

95. Nambi, V.; Chambless, L.; Folsom, A.R.; He, M.; Hu, Y.; Mosley, T.; Volcik, K.; Boerwinkle, E.; Ballantyne, C.M. Carotid intima–media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: The ARIC (Atherosclerosis Risk In Communities) study. *J. Am. Coll. Cardiol.* **2010**, *55*, 1600–1607. [**Google Scholar**] [**CrossRef**] [**PubMed**][**Green Version**]

96. Cao, J.J.; Arnold, A.M.; Manolio, T.A.; Polak, J.F.; Psaty, B.M.; Hirsch, C.H.; Kuller, L.H.; Cushman, M. Association of carotid artery intima–media thickness, plaques, and C–reactive protein with future cardiovascular disease and all–cause mortality: The Cardiovascular Health Study. *Circulation* **2007**, *116*, 32–38. [**Google Scholar**] [**CrossRef**] [**PubMed**][**Green Version**]

97. Howard G, Sharrett AR, Heiss G, Evans GW, Chambless LE, Riley WA, Burke GL, for the ARIC Investigators. Carotid artery intimal-medial thickness: distribution in general populations as evaluated by B-mode ultrasound. Stroke.. 1993;24:1297-1304. [Abstract]

98. Perlstein TS, Weuve J, Pfeffer MA, Beckman JA. Red blood cell distribution width and mortality risk in a community-based prospective cohort. *Arch Intern Med.* 2009 Mar 23;169:588–594. [PMC free article] [PubMed] [Google Scholar].

99. VAYÁ A., SARNAGO A., FUSTER O., ALIS R. and ROMAGNOLI M.: Influence of inflammatory and lipidic parameters on red blood cell distribution width in a healthy population. Clin. Hemorheol. Microcirc., 59: 379-85, 2015.

100. H. Wan, et al.Associations between the neutrophil-to-lymphocyte ratio and diabetic complications in adults with diabetes: a cross-sectional study J. Diabet.

Res. (2020), p. 2020.

101. Swaminathan A, Amitkumar K, Ganapathy S, et al. Evaluation of mean platelet volume and other platelet parameters in subjects with Type-2 diabetes mellitus. Natl J Physiol Pharm Pharmacol. 2017; 7(1): 51–54, doi: 10.5455/njppp.2016.6.0719518072016.

102. Brohall G, Odén A, Fagerberg B. Carotid artery intima-media thickness in patients with Type 2 diabetes mellitus and impaired glucose tolerance: a systematic review. Diabet Med. 2006; 23(6):609-16. PubMed |Google Scholar.

103. Mukai N, Ninomiya T, Hata J, Hirakawa Y, Ikeda F, Fukuhara M et al. Association of hemoglobin A1c and glycated albumin with carotid atherosclerosis in community-dwelling Japanese subjects: the Hisayama Study. Cardiovasc Diabetol. 2015; 14:84. PubMed | Google Scholar.

12.ANNEXURE

INFORMATION SHEET

We are conducting a study titled "**ASSOCIATION OF CAROTID INTIMAL THICKNESS WITH HEMATOLOGICAL INDICES IN PREDICTING MACRO-VASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS"** among patients admitted in Rajiv Gandhi Government General Hospital, Chennai.

The purpose of this study is to examine patients in Type2 Diabetes mellitus to use hematological parameters to asses macro vascular complications.

We are selecting certain cases and if you are found eligible, we may be using your blood samples to do certain tests.

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator

Signature/ left thumb impression of Participant/attender

Date:
Place:

PATIENT CONSENT FORM

"ASSOCIATION OF CAROTID INTIMAL THICKNESS WITH HEMATOLOGICAL INDICES IN PREDICTING MACRO-VASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS"

Participant Name :

Age:

Sex:

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask the questions and all my questions and doubts have been answered to my satisfaction.

I have been explained about the pitfall in the procedure. I have been explained about the safety , advantage and disadvantage of the technique.

I understand that my participation in the study is voluntary and that I'm free to withdraw at anytime without giving any reason.

I understand that investigator, regulatory authorities and the ethics committee will not need my permission to look at my health records both in respect to current study and any further research that may be conducted in relation to it, even if I withdraw from the study.

I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study.I hereby consent to undergo complete physical examination, pathological and radiological investigation pertaining to the study.

Signature/Thumb Impression of Participant

MASTER CHART

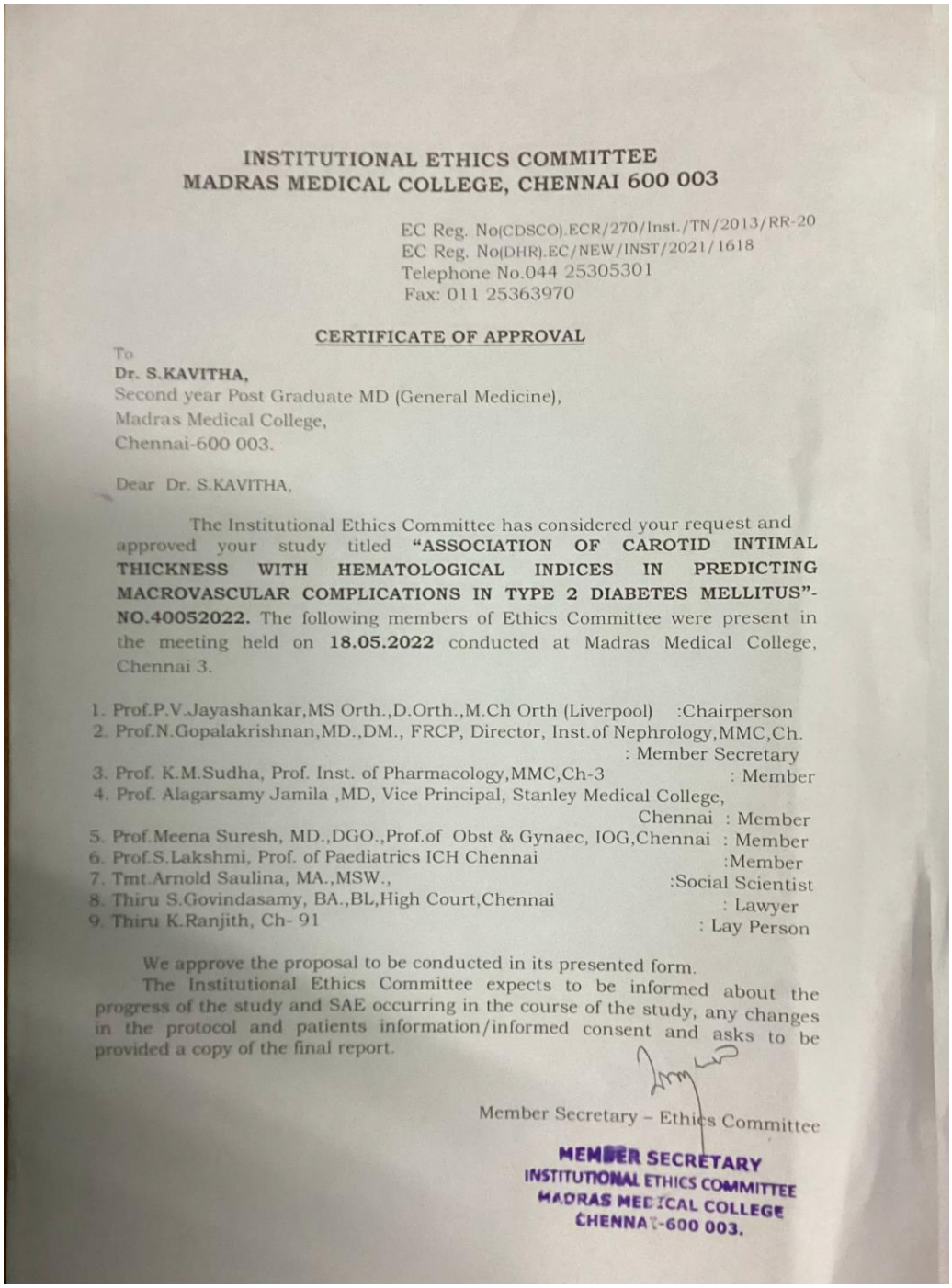
AGE	SEX	NEUTROPHIL COUNT	LYMPHOCYTE COUN	N/L RATIO	RED CELL DISTRIBUTION WID	MEAN PLATLET VALUE	HBA1c LEVEL	CAROTID INTIMAL THICKNES
45	M	91	40	2.3	19	15	7.6	1.2
55	F	99	37	2.7	11	14	8	1.1
38	M	99	40	2.5	16	14	7.7	0.9
40	F	75	40	1.9	11	8	7.5	0.7
50	M	91	40	2.3	17	14	7.7	1.1
39	M	97	34	2.9	22	13	7.7	1.1
45	M	73	31	2.4	18	14	7.6	1.2
46	F	92	39	2	14	12	6.5	0.8
53	M	78	37	2.1	22	13	7.8	1.1
39	F	82	30	2.7	16	14	7.6	1
40	M	94	33	2.8	16	14	7.9	0.9
41	F	73	32	2.3	20	15	8	1
43	F	62	30	2	14	11	6.5	0.8
39	F	92	39	2.4	18	14	7.9	0.9
37	M	85	31	2.7	19	14	7.7	0.9
45	M	95	39	2.4	23	14	8	1
54	M	60	32	1.9	16	12	5.7	0.8
52	F	66	35	1.9	14	13	6	0.8
43	M	84	34	2.5	16	15	7.6	0.9
40	F	86	32	2.7	18	14	7.8	1.1
47	F	64	38	1.7	15	11	6.2	0.7
53	M	85	31	2.7	17	13	7.9	0.9
37	F	92	38	2.4	22	14	7.7	1.2
38	M	64	38	1.7	15	10	7.4	0.7
59	M	94	36	2.6	17	14	7.6	0.9
54	M	70	35	2	13	9	5.6	0.8
47	F	76	35	2.2	20	15	7.7	1.2
39	F	60	37	1.6	11	9	6.3	0.8
32	M	81	38	2.1	19	16	7.6	1

AGE	SEX	NEUTROPHIL COUNT	LYMPHOCYTE COUN	N/L RATIO	RED CELL DISTRIBUTION WID	MEAN PLATLET VALUE	HBA1c LEVELS	CAROTID INTIMAL THICKNES
34	F	75	34	2.2	18	13	8	0.9
37	F	77	35	2.2	17	14	8	1
46	F	64	31	1.7	13	15	7.3	0.8
32	M	66	39	1.7	22	14	6	0.7
39	M	75	39	1.9	19	8	7.1	0.8
39	F	88	33	2.7	16	15	7.6	0.9
45	M	92	38	2.4	18	13	7.8	1.2
53	M	82	38	2.2	17	16	7.6	0.9
49	F	87	31	2.8	16	15	7.9	0.9
59	F	78	38	2	13	12	7.5	0.8
56	M	84	39	2.2	19	14	7.7	1.2
57	M	98	38	2.6	13	14	7.7	1.2
48	F	82	37	2.2	18	14	7.8	1
38	F	79	34	2.3	17	16	7.9	1.2
54	M	64	33	1.9	13	11	6	0.8
56	M	79	34	2.3	19	14	7.9	1
48	M	66	34	1.9	13	9	6.3	0.8
55	M	60	32	1.9	14	10	6.1	0.8
44	M	81	35	2.3	22	14	8	1
54	M	97	40	2.4	16	15	7.6	1.1
56	M	93	37	2.5	23	14	7.9	1.2
45	M	82	37	2.2	24	15	7.9	1.1
46	F	61	40	1.5	14	8	6.4	0.6
45	M	75	32	1.9	12	9	7.5	0.8
35	F	70	33	2.1	18	10	5.8	1.1
47	F	81	36	2.3	21	14	7.7	1.2
32	F	62	39	1.6	14	10	7.4	0.8
54	F	99	36	2.8	18	14	7.6	1.1
43	M	88	39	2.3	19	15	7.9	1.1
34	M	81	31	2.6	20	14	7.7	0.9

AGE	SEX	NEUTROPHIL COUNT	LYMPHOCYTE COUN	N/L RATIO	RED CELL DISTRIBUTION WID	MEAN PLATLET VALUE	HBA1c LEVELS	CAROTID INTIMAL THICKNES
45	F	66	35	1.9	14	9	5.5	0.7
32	F	62	39	1.7	13	11	8	0.7
45	M	75	38	2	14	9	7.2	0.7
56	F	89	38	1.8	11	9	7.4	0.8
43	F	71	31	2.3	20	13	7.6	1.2
42	F	61	32	1.9	18	12	5.8	0.8
42	F	86	34	2.5	21	14	7.8	1
34	M	99	32	3.1	23	15	7.8	1.2
32	F	88	39	2.3	19	16	7.9	1.2
42	F	97	33	2.9	25	14	7.9	1.2
43	M	62	39	1.6	14	12	7.2	0.8
54	M	70	33	2	11	9	6.1	0.8
34	F	61	33	1.8	16	8	5.9	0.7
54	M	61	33	1.8	14	13	5.6	0.8
45	M	89	31	2.9	19	14	7.7	1.2
37	F	68	35	1.9	14	12	6.3	0.8
43	F	70	33	2	14	11	7.5	0.8
65	F	96	39	2.5	25	15	7.6	1.2
45	F	89	35	2.5	16	18	7.8	1.1
43	F	85	37	2.3	21	15	7.9	0.9
47	F	70	33	2	14	11	7	0.7
48	F	92	34	2.7	17	15	8	1.2
46	M	60	31	1.9	14	10	5.5	0.8
47	M	64	40	1.6	15	8	5.7	0.8
65	F	76	38	2	16	8	6.5	0.8
37	M	79	40	2	23	12	7.4	0.7
56	M	89	35	2.5	17	15	7.8	1.1
43	F	70	37	1.9	14	12	6.4	0.8
54	M	93	34	2.7	13	15	7.9	0.9
40	M	98	34	2.9	14	14	7.9	0.9

AGE	SEX	NEUTROPHIL COUNT	LYMPHOCYTE COUN	N/L RATIO	RED CELL DISTRIBUTION WID	MEAN PLATLET VALUE	HBA1c LEVELS	CAROTID INTIMAL THICKNES
53	M	98	36	2.7	20	8	7.6	1.1
45	F	60	35	1.7	14	13	6.5	0.8
46	F	81	37	2.2	13	16	8	1.2
45	M	74	39	1.9	25	10	7.8	0.9
43	F	86	35	2.5	16	8	7.1	0.9
54	M	98	33	3	14	9	7.4	0.9
43	M	71	33	2.2	13	12	7.9	1
54	M	70	33	2.1	23	11	5.7	1.1
46	F	78	32	2.4	12	11	8	1.2
35	F	79	36	2.2	22	9	7.9	0.9
45	F	84	32	2.65	17	10	6.5	0.9

ETHICAL PERMISSION LETTER



INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

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CERTIFICATE OF APPROVAL

To
Dr. S.KAVITHA,
Second year Post Graduate MD (General Medicine),
Madras Medical College,
Chennai-600 003.

Dear Dr. S.KAVITHA,

The Institutional Ethics Committee has considered your request and approved your study titled **"ASSOCIATION OF CAROTID INTIMAL THICKNESS WITH HEMATOLOGICAL INDICES IN PREDICTING MACROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS"-NO.40052022.** The following members of Ethics Committee were present in the meeting held on **18.05.2022** conducted at Madras Medical College, Chennai 3.

1. Prof.P.V.Jayashankar,MS Orth.,D.Orth.,M.Ch Orth (Liverpool) :Chairperson
2. Prof.N.Gopalakrishnan,MD.,DM., FRCP, Director, Inst.of Nephrology,MMC,Ch. : Member Secretary
3. Prof. K.M.Sudha, Prof. Inst. of Pharmacology,MMC,Ch-3 : Member
4. Prof. Alagarsamy Jamila ,MD, Vice Principal, Stanley Medical College, Chennai : Member
5. Prof.Meena Suresh, MD.,DGO.,Prof.of Obst & Gynaec, IOG,Chennai : Member
6. Prof.S.Lakshmi, Prof. of Paediatrics ICH Chennai :Member
7. Tmt.Arnold Saulina, MA.,MSW., :Social Scientist
8. Thiru S.Govindasamy, BA.,BL,High Court,Chennai : Lawyer
9. Thiru K.Ranjith, Ch- 91 : Lay Person

We approve the proposal to be conducted in its presented form.
The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003.

PLAGIARISM CERTIFICATE

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Document Information

Analyzed document 1. ASSOCIATION OF CAROTID INTIMAL THICKNESS WITH HEMATOLOGICAL INDICES IN PREDICTING MACROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS.docx (D152730831)

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Sources included in the report

SA	review 14.9.2018.docx Document review 14.9.2018.docx (D41432215)	1
SA	TO EVALUATE THE ROLE OF RED CELL DISTRIBUTION WIDTH AS A NOVEL PREDICTOR OF POOR GLYCEMIC CONTROL IN PATIENTS WITH TYPE1 AND TYPE 2 DIABETES MELLITUS.pdf Document TO EVALUATE THE ROLE OF RED CELL DISTRIBUTION WIDTH AS A NOVEL PREDICTOR OF POOR GLYCEMIC CONTROL IN PATIENTS WITH TYPE1 AND TYPE 2 DIABETES MELLITUS.pdf (D123169129)	2
SA	KAVIN THESIS.docx Document KAVIN THESIS.docx (D123255691)	1
SA	Bilal Ahmad.docx Document Bilal Ahmad.docx (D137115592)	3
SA	The Tamil Nadu Dr. M.G.R. Medical University / Assessment of platelet activity in type 2 diabetes mellitus patients compared with non diabetics .docx diabetics .docx (D150829821) Submitted by: ba_doc_007@yahoo.co.in Receiver: ba_doc_007.tnmg@analysis.urkund.com	1
SA	A DISSERTATION ON ASSOCIATION OF RED BLOOD CELL DISTRIBUTION WIDTH AND CAROTID INTIMAL MEDIA THICKNESS IN PATIENTS WITH MetS.docx Document A DISSERTATION ON ASSOCIATION OF RED BLOOD CELL DISTRIBUTION WIDTH AND CAROTID INTIMAL MEDIA THICKNESS IN PATIENTS WITH MetS.docx (D56445039)	2
SA	REVIEW OF LITERATURE arun.docx Document REVIEW OF LITERATURE arun.docx (D56394337)	2
SA	Aims.docx Document Aims.docx (D111126073)	1

Document Assessment of platelet activity in type 2 diabetes mellitus patients compared with non

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1. INTRODUCTION

ASSOCIATION OF CAROTID INTIMAL THICKNESS WITH HEMATOLOGICAL INDICES IN PREDICTING MACROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS

Diabetes is a disease of metabolism clinically expressed by chronic hyperglycemia and blood lipid and protein disorders as the result of a defect