"A STUDY ON NEUTROPHIL-LYMPHOCYTE RATIO AND PLATELETLYMPHOCYTE RATIO AS AN ASSESSMENT TOOL OF GLYCEMIC CONTROL IN TYPE 2 DIABETES MELLITUS PATIENTS"

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Dissertation submitted to BLDE (Deemed to be University), Vijayapura.

In partial fulfilment of the requirements for the award of the degree of

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IN

PATHOLOGY

Under the guidance of

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List of abbreviations used

HbA_{1c}	Glycated Hemoglobin A_{1c}.
NLR	Neutrophil Lymphocyte Ratio
PLR	Platelet Lymphocyte Ratio
WBC	White Blood Cells
RBC	Red Blood Cells
ANC	Absolute Neutrophil Count
ALC	Absolute Lymphocyte Count
Hb	Hemoglobin
HCT/PCV	Hematocrit/ Packed Cell Volume
IDF	International Diabetes Federation
AEC	Absolute Eosinophil Count
AMC	Absolute Monocyte Count
ABC	Absolute Basophil Count
S.TG	Serum Triglycerides
S. Chl	Serum Cholesterol
S.HDL	Serum High density lipoprotein
S.LDL	Serum Low density lipoprotein
S.VLDL	Serum Very low-density lipoprotein
CKD	Chronic Kidney Disease
HSC	Hematopoietic Stem Cells
CHD	Coronary Heart Disease

ABSTRACT

INTRODUCTION

Type 2 diabetes mellitus is one component of metabolic syndrome, which includes impaired glucose tolerance, hypertension, obesity, and dyslipidemia. White blood cell (WBC) count is linked to various components of metabolic syndrome, and subclinical inflammation may be associated with the increased cardiovascular risk in patients with impaired glucose tolerance. Neutrophil lymphocyte ratio (NLR) is an essential marker of systemic inflammation and an indicator of increased risk for cardiovascular events in patients with metabolic syndrome.

OBJECTIVES

1) To understand the role of NLR and PLR in assessment of glucose regulation in type 2 diabetes mellitus.
2) To study the correlation between NLR, PLR & HbA1C in type 2 diabetes mellitus.

METHODS

A total of 300 individuals were divided into three groups of 100 each: Pre-diabetic (HbA1c between 5.7-6.4%), Overt Diabetes Mellitus (HbA1c > 6.4%), and Control (HbA1c <5.7). Total WBC, differential count, and platelet count were determined using an automated blood cell counter. NLRs and PLRS were quantified from the reports as total neutrophil counts divided by lymphocyte counts and total platelet count divided by lymphocyte count respectively using the same blood samples drawn at the time of admission. HbA1c levels were measured using automated ion exchange high performance liquid chromatography (BIO RAD D10).

RESULTS

The absolute neutrophil count showed a statistically significant elevation in the Diabetic group compared to the control and pre-diabetic group (p value 0.000 and 0.003 respectively). The absolute lymphocyte count showed a statistically significant decrease in the Diabetic group in comparison to the control and pre-diabetic group (p value 0.000 and 0.009 respectively). As expected, the NLR had a statistically significant increase in the Diabetic group compared to the control (p value 0.000) and PLR had a statistically significant decrease in the Diabetic group in comparison to the control (p value 0.000)

CONCLUSIONS

NLR and PLR may be used as independent parameters to monitor the progress of Type II Diabetes Mellitus. Owing to their ease of availability and being relatively inexpensive, it may be used in those parts of the country with minimal health infrastructure.

KEYWORDS: Type II Diabetes Mellitus, complications of diabetes mellitus, glycemic control, glycated hemoglobin, NLR, PLR

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A STUDY ON NEUTROPHIL-LYMPHOCYTE RATIO AND PLATELET LYMPHOCYTE AS AN ASSESSMENT TOOL OF GLYCEMIC CONTROL IN TYPE 2 DIABETES MELLITUS PATIENTS"

INTRODUCTION

Diabetes mellitus a chronic preventable metabolic disorder and is considered to be one of the major 'silent killers' across the globe. This is because unless they develop complications, most of the patients remain asymptomatic for years. The burden of diabetes is on the rise in middle- and lower-income countries due to strong influence of urbanization, sedentary lifestyle, nutritional and epidemiological transition. Therefore, drastic steps are required through various health awareness programs in order to control the escalating trends of diabetes in these countries to reduce disease burden. (1)

Type 2 diabetes mellitus is one component of metabolic syndrome, which includes impaired glucose tolerance, hypertension, obesity, and dyslipidemia. White blood cell (WBC) count is linked to various components of metabolic syndrome, and subclinical inflammation may be associated with the increased cardiovascular risk in patients with impaired glucose tolerance. Furthermore, an association has been shown between chronic subclinical inflammation and insulin resistance, metabolic syndrome, and atherosclerosis. Low-grade chronic inflammation is associated with increased cardiometabolic risk. The process of atherosclerosis is known to involve inflammatory mechanisms, and leukocytosis is directly associated with the pathogenesis of both atherosclerosis and metabolic syndrome. The prevalence of macrovascular complications

has been shown to correlate positively with increased WBC count in patients with type 2 diabetes mellitus.(2)

Neutrophil lymphocyte ratio (NLR) is an essential marker of systemic inflammation and an indicator of increased risk for cardiovascular events in patients with metabolic syndrome. In addition, increased NLR may be related to type 2 diabetes mellitus. Thus, it may be proposed that chronic inflammation plays a key role in the pathogenesis of clinical complications including cardiovascular diseases. (2)

The purpose of the present study is to investigate the relationship between NLR and blood glucose regulation. A comparison will be made between the NLR of patients with regulated diabetes mellitus (glycosylated hemoglobin [HbA1c<7%) and patients with unregulated diabetes mellitus (HbA1c>7%). (3)(4)

OBJECTIVES OF THE STUDY:

1) To understand the role of NLR and PLR in assessment of glucose regulation in type 2 diabetes mellitus.
2) To study the correlation between NLR, PLR & HbA1C in type 2 diabetes mellitus.

REVIEW OF LITERATURE

Introduction To Diabetes Mellitus

Diabetes Mellitus (or Diabetes, as it is commonly known) is a term used to describe a disorder of metabolism marked by chronic hyperglycemia and/or disorders of carbohydrate, protein, and fat metabolism. Even though the etiology is heterogeneous, the condition results from either an impaired secretion of insulin or resistance to the action of insulin or both.(5)

Insulin, the hormone that is deficient/non-functional in diabetes is secreted by the beta cells of the pancreas. Insulin helps in the efficient transport of glucose into the cells of the body, particularly muscles and adipose tissue.

Diabetes Mellitus is a serious, long-term (or 'chronic') condition that occurs when there are raised levels of glucose in a person's blood because their body cannot produce enough of the hormone insulin or cannot effectively use the insulin it produces.

Insulin is an essential hormone produced in the pancreas. It allows glucose from the bloodstream to enter the body's cells where that glucose is converted into energy. A lack of insulin, or the inability of cells to respond to it, leads to high levels of blood glucose (hyperglycemia). Insulin deficiency, if left unchecked over the long term, can cause damage to many of the body's organs, leading to disabling and life-threatening health complications such as cardiovascular diseases

(CVD), nerve damage (neuropathy), kidney damage (nephropathy) and eye disease (leading to retinopathy, visual loss and even blindness). On the other hand, early and optimal management of hyperglycemia goes a long way in delaying, and in some cases, preventing the long-term complications(6).

Incidence And Prevalence

It is estimated that Diabetes Mellitus is prevalent in approximately 9.3% of the total population of the world. Worldwide, this translates to around 460 million people between the age group of 20-79 years. By the year 2045, this number is forecasted to reach 578 million by 2030 (10.2%) and to 700 million (10.9%) by 045 (Figure 1).

Individuals diagnosed with Impaired Glucose Tolerance (pre-diabetes) within the age group 20-79 years is approximately 374 million (7.5% of world population in this age group). Just as with the case of overt diabetes, prevalence of impaired glucose tolerance too is expected to climb up, with the most conservative of estimates putting the prevalence by 2030 at 454 million (8%) and 548 million (8.6%) by 2045. Under the age of 20 years, approximately 1.1 million children and adolescents are categorized to have Type I Diabetes Mellitus. An estimate on the number of children and adolescents with Type II Diabetes Mellitus is currently unknown due to the asymptomatic presentation and incomplete diagnosis.

Number of deaths directly attributable to Diabetes Mellitus and its complications is estimated to be around 4.2 million in the year 2019. In the same year, approximately 15.8% of livebirths were affected with transient hyperglycemia, leading to neonatal complications. It is estimated that the world spent an equivalent of 760 billion USD to treat diabetes and diabetes related complications. This figure is said to rise to 825 billion USD by 2030 and 845 billion USD by 2045. The current trends indicate that by the year 2045, not less than 700 million adults worldwide would have diabetes (6).

In India, the incidence and prevalence of Diabetes Mellitus has been on the rise in comparison to the previous years. This trend is noticeable in both the urban and rural areas. As urbanization increases, the middle class grows, and as the proportion of aged individuals increase, it is expected that the prevalence of Diabetes Mellitus too will increase in the future (7). An increase in the disease burden would be a strain on a growing economy like India, which brings to light the need to address this growing problem. The age-adjusted comparative prevalence of diabetes mellitus in India is shown in Figure 2. The age and gender-wise prevalence of diabetes mellitus in India is shown in Figure 3.

According to the World Health Organization (WHO), the death rate from Diabetes Mellitus and its complications would more than double between 2005 and 2030 (Figure 4), with the low-and-middle income group countries contributing to about 80% of the number (8).

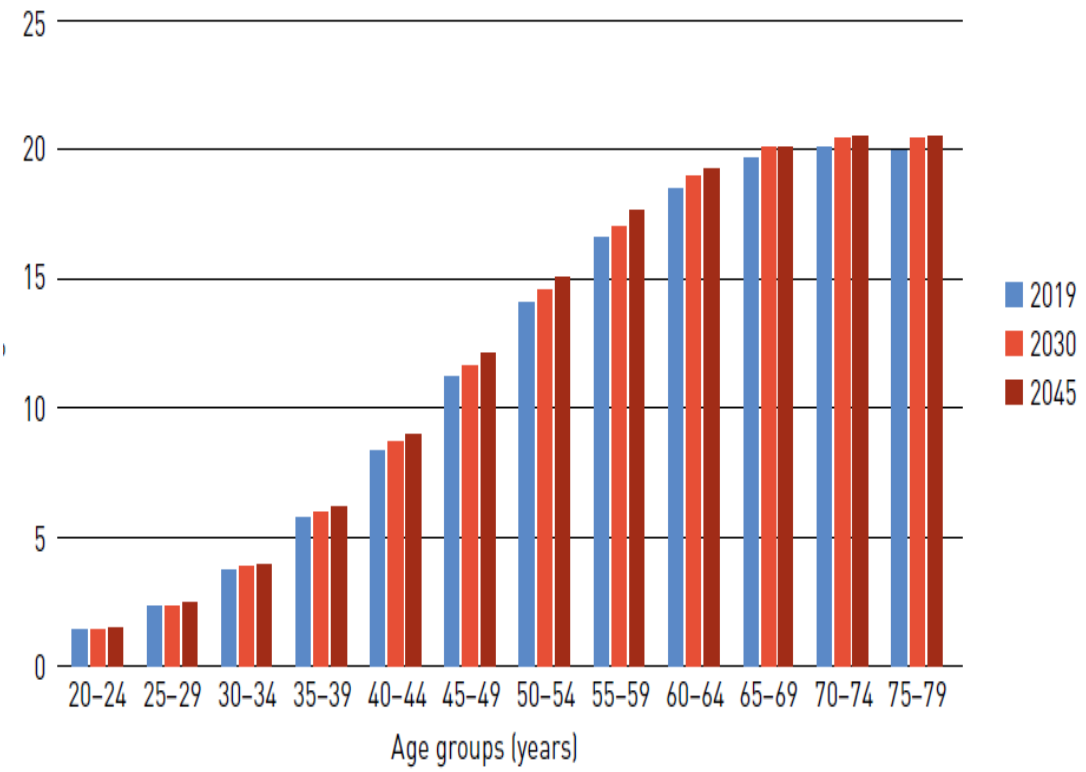

Figure 1: Prevalence of diabetes by age group in adults (20–79 years) in 2019, 2030 and 2045

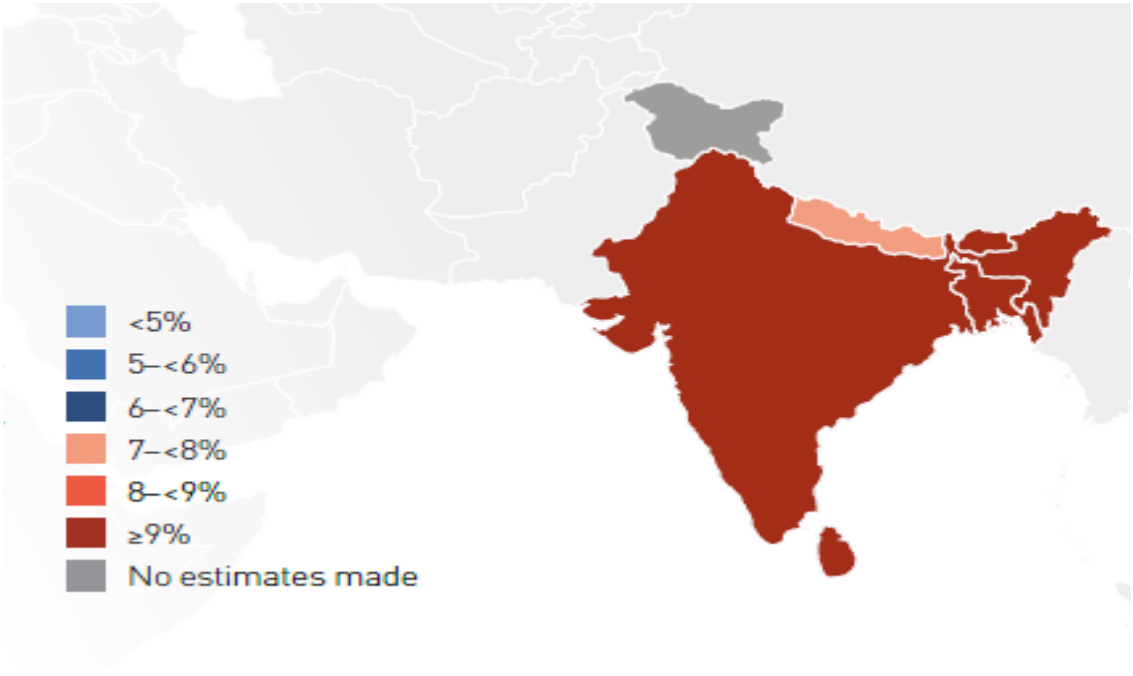

Figure 2: Age-adjusted comparative prevalence (%) of diabetes (20–79 years) in IDF South-East Asia Region

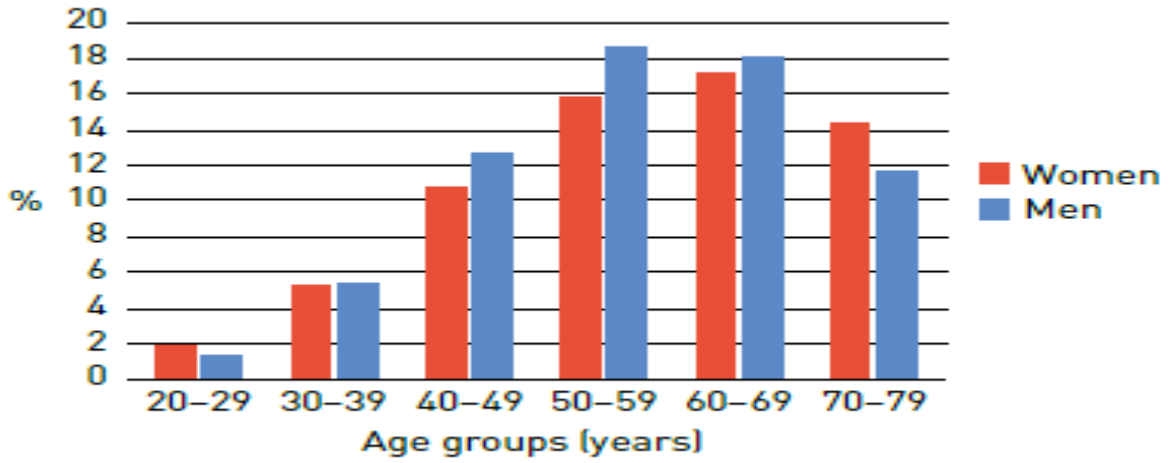

Figure 3: Prevalence (%) estimates of diabetes by age and sex

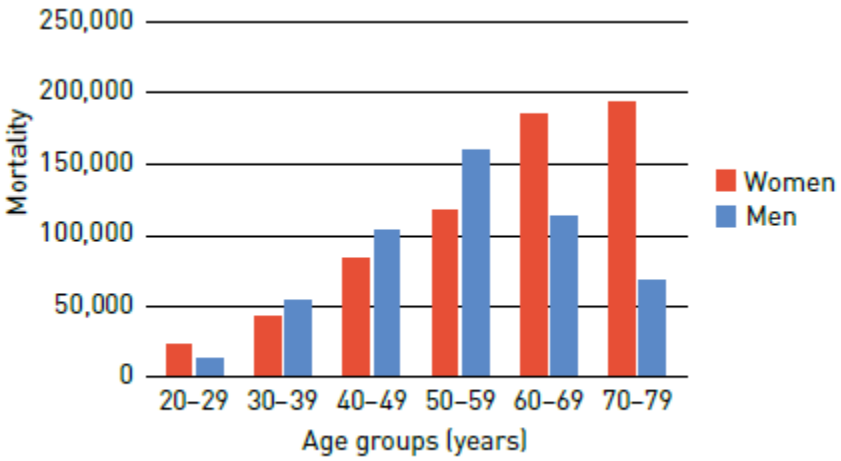

Figure 4: Mortality due to diabetes by age and sex, IDF South-East Asia Region, 2019(6)

Diabetes Mellitus: Etiopathogenesis

The main etiological classification of disorders involving glucose homeostasis is shown in Table 1(3), (9), (10).

Type	**Pathophysiology**	**Etiology**
Type 1	β-cell destruction	Autoimmune Idiopathic Genetic
Type 2	Insulin underproduction/peripheral insulin resistance	Idiopathic Genetic
Genetic defects of β-cell function	TCF7L	Genetic
Genetic defects in insulin action	MTNR1B, FADS1, DGKB, GCK gene defects	Genetic
Diseases of exocrine pancreas	Acute pancreatitis Chronic pancreatitis Neoplasms Neuroendocrine tumors Cystic fibrosis	-
Drug or chemical induced	Thiazide diuretics Beta blockers Quinolones Protease inhibitors Nucleotide reverse transcriptase inhibitors	Iatrogenic
Infections	Chronic bacterial and viral infections	Beta cell destruction secondary to viral infections

		Increased stress response
Gestational Diabetes Mellitus	Pancreatic cell dysfunction Chronic insulin resistance	-
Chromosomal aneuploidy	Prader-Willi Syndrome Down Syndrome Friedreich's ataxia	Genetic

Table 1: Etiological classification of Diabetes Mellitus and glucose homeostasis

Pathophysiology Of Type 1 And Type 2 DM

Disorders of glycaemia: etiological types and clinical stages.(3)

TYPE 1 DIABETES MELLITUS

This is a condition characterized by the pancreas's inability to produce insulin because of the destruction of beta cells of pancreas by the individual's own immune system. The trigger for this attack is not completely elucidated but it is hypothesized that it is a combination of genetic (multiple genes are implicated) and some environmental triggers (predominantly viral infections) that trigger the body's immune system to attack itself. Environmental toxins and diet may also contribute to aggravation of the auto-immune attacks. Although seen predominantly in children and young adults, type 1 diabetes mellitus may be seen in any age group. Type 1 diabetes mellitus is considered one of the most prevalent chronic diseases of childhood although changes in diet, environmental and other epigenetic factors are resulting in an increase in the prevalence of Type 2 diabetes mellitus as well.

TYPE 2 DIABETES MELLITUS

Accounting for about 90% of all cases of diabetes mellitus worldwide, Type 2 diabetes mellitus is by far the most prevalent among all types.

Type 2 Diabetes Mellitus is characterized by two independent, but interrelated conditions:

1. Insulin resistance
2. Inadequate production of insulin

Insulin resistance: This term refers to the body's inability to effectively respond to insulin. Tissues like muscles and adipose, which needs insulin dependent glucose transporters (GLUT-4) for insulin uptake, over time becomes insensitive to the actions of the hormone. This results in increased peripheral glucose levels and a state of hypoglycemia in the tissues.

Inadequate production of insulin: As peripheral insulin resistance increases, the pancreas tries to compensate for that by increasing the production of insulin from β-cells. This results in an undue stress on the cells of the pancreas, and over time, the production becomes ineffective.

The combination of insulin resistance and inadequate insulin production over a sustained period of time results in the signs and symptoms of diabetes mellitus. Historically considered a disease

of middle age, changes in diet and lifestyle changes have resulted in the disease being diagnosed even in young adults and children these days.

The symptoms of Type 2 Diabetes Mellitus may at times mimic those of Type 1 Diabetes Mellitus, but most often is less dramatic and symptomless (10).

The term prediabetes is a term used interchangeably with impaired glucose tolerance or impaired fasting glucose. This condition is considered the biggest risk factor for developing Type 2 diabetes mellitus and associated complications.

Gestational diabetes, on the other hand, is the development of impaired glucose tolerance for the first-time during pregnancy. Not only does this condition predispose to the development of Type 2 Diabetes Mellitus in the future, but also may result in large for gestational age babies.

Increased glucose levels in blood results in endothelial damage through a variety of mechanisms including elevated oxidative stress, increased induction of atherogenic circulating adhesion molecules, and those cytokines that regulate and induce cell recruitment, migration, growth, and proliferation (11).

Clinical presentation

The clinical symptoms and signs are quite similar for Type 1 and Type 2 Diabetes Mellitus. These symptoms are often vague and hence, results in a delayed diagnosis. The typical symptoms of Diabetes Mellitus are listed in Table 2.

Symptoms
Excessive thirst
Blurred vision
Bedwetting
Frequent urination
Lack of energy/fatigue
Constant hunger
Weight loss

Table 2: Typical symptoms of Diabetes Mellitus

The classical triad of polydipsia, polyuria, and weight loss may not be present in all cases and hence, may result in delayed diagnosis (6)

Acute Complications Of Diabetes Mellitus

Even though acute complications of diabetes mellitus are more common in Type 1 diabetes mellitus, uncontrolled type 2 diabetes mellitus too may result in complications. The most common acute complication of diabetes mellitus is Diabetic Ketoacidosis, a condition resulting in life threatening metabolic acidosis due to the accumulation of lactic acid. Initially presenting with confusion, lethargy, and disorientation, if untreated, the individual quickly will progress to irreversible neurological damage, coma, or even death. The incidence of diabetic ketoacidosis is the hallmark of Type 1 diabetes although uncontrolled type 2 diabetes mellitus too can cause the same.

In individuals with Type 2 diabetes mellitus, hyperglycemic hyperosmolar state is another major complication. Insidious in inset, it progresses rapidly to dehydration, loss of electrolytes, and increased serum osmolality. Triggers for hyperglycemic hyperosmolar state are varied although infections have been implicated as the major precipitating cause. This is more commonly seen in the elderly and chronically ill individuals. The overall mortality for hyperglycemic hyperosmolar state is about 5-20%, about ten times higher than that of diabetic ketoacidosis (6).

Paradoxically, hypoglycemia is another important complication of diabetes mellitus, particularly in those individuals who are on insulin and sulfonylureas for glycemic control.

Long Term Complications Of Diabetes Mellitus

Long term complications of Diabetes Mellitus may be broadly categorized into the following:

1. Macrovascular complications
2. Microvascular complications

The major macrovascular complications are:

- Coronary Artery Disease: Long term diabetics are prone to cardiovascular complications such as angina, myocardial infarction, and/or changes in cardiac imaging.
- Cerebrovascular disease: Incidences of stroke, transient ischemic attacks, hemiplegia, paraplegia etc. are not uncommon in long standing diabetics (12).
- Peripheral vascular disease: History of intermittent claudication, pain in the limbs/extremities, and absence of one or more peripheral pulses may be suggestive of peripheral vascular disease in diabetes mellitus.

The major microvascular complications are:

- Peripheral neuropathy: Involvement of the peripheral nerves is quite common in cases of long-standing diabetes mellitus. The major symptoms are tingling, pins and needles sensation, loss of sense of touch and temperature, and muffled reflexes (13).
- Diabetic nephropathy: Long standing diabetes mellitus may cause diabetic nephropathy, resulting in decreased glomerular filtration rate (14).
- Diabetic retinopathy: A major microvascular complication of diabetic mellitus is diabetic retinopathy, resulting in irreversible damage to sight in uncontrolled cases (15).

Diabetes And Cardiovascular Diseases

Cardiovascular complications contribute to the highest rate of morbidity and mortality among individuals with diabetes mellitus (16). Studies have indicated that the relative risk of cardiovascular disease in individuals with diabetes mellitus is between 1.6 and 2.6, with a higher predisposition seen in young individuals and women (17,18). Currently, there is conclusive evidence that an increase in fasting glucose, glycated hemoglobin, and glucose challenge test is associated with a 6-20% increased risk of cardiovascular events (Table 3).

Outcome	**Impact**	**Data systems/study**	**Reference**
Prevalence of cardiovascular diseases	Any CVD: 32% CHD: 21% MI: 10% Stroke: 7.6%	57 cross-sectional studies	Einarson et.al, 2018
Coronary heart disease	160% increased risk	102 prospective studies	Emerging risk factors collaboration, 2010
Ischemic heart disease	127% increased risk	102 prospective studies	Emerging risk factors collaboration, 2011
Hemorrhagic stroke	56% increased risk	102 prospective studies	Emerging risk factors collaboration, 2011

Cardiovascular diseases/death	132% increased risk	97 prospective studies	
Years of life lost	5.8 years of men aged 50 6.4 years for women aged 50	97 prospective studies	

Table 3: Global estimates of the association and impact of diabetes on cardiovascular diseases

Various mechanisms have been implicated for the increased risk of cardiovascular disease in diabetics:

- Insulin resistance
- Direct damage to endothelium through the effect of free radicals
- Other associated risk factors like hypertension and dyslipidemia and central obesity (Metabolic syndrome) (19)
- Low grade inflammation
- Atherosclerosis
- Concomitant hyperuricemia, albuminuria, and high atherogenic index of plasma (20)

Apart from these, risk is also strongly influenced by lifestyle factors like smoking, lack of exercise, and consumption of junk food. Adopting life style changes, taking measures to lower blood pressure and lipids significantly reduces the risk of cardiovascular diseases (21).

Chronic low-grade inflammation has been shown to derange the metabolic functions by inducing insulin resistance, resulting in cardiovascular complications. Insulin resistance in turn is associated with a variety of inflammatory factors, resulting in a vicious cycle (22).

Other concomitant disorders like albuminuria, hyperuricemia, and high atherogenic index are said to be at an increased risk of developing cardiovascular disorder (23).

Similarly, metabolic syndrome is associated with systemic inflammation and thereby, the risk for cardiovascular accidents too increases (24).

The link between atherosclerosis and insulin resistance too has been recently elucidated and two pro-inflammatory cytokines namely TNF-α and IL-6 (25). Metabolic syndrome is seen more in those individuals who have an increased transcription rates of these cytokines, concomitantly increasing the risk of coronary heart disease (26).

Diabetic Eye Disease

Diabetic eye disease, comprising of diabetic retinopathy, macular edema, cataract, and glaucoma is one of the leading causes of morbidity and lowered quality of life in diabetics. Apart from the above mentioned conditions, diplopia, loss of focusing ability, and repeated infections of the eye too are seen in long standing diabetes (27). Early diagnosis and treatment are imperative as chronic diabetes mellitus may have devastating effects on sight and overall health of the eye. Optimization of plasma glucose and routine screening for diabetic retinopathy can drastically reduce the associated morbidity (6).

Diabetic Kidney Disease

People with diabetes mellitus are predisposed to development of chronic kidney disease, Usually, preexisting conditions such as hypertension and hypertriglyceridemia too are contributing factors to the development of CKD. Just as with diabetic retinopathy, strict glycemic control and regular screening for high blood pressure helps in reducing the morbidity from diabetic kidney disease (28). Another sign of chronic kidney disease can be normocytic normochromic anemia, seen at a higher prevalence in diabetics than those with CKD secondary to non-diabetic causes (29), (30). This has been attributed to the activation of the NLRP3/caspace-1/IL-1β pathway, which happens early in the pathogenesis of diabetic nephropathy (31).

Nerve And/Or Vascular Damage And Diabetic Foot Complications

The most common form of peripheral nephropathy by far is diabetic nephropathy. Affecting the distal nerves of the limbs first, the earliest presentation is usually with an altered conduction of nerve signals resulting in poor perception of pain, temperature, and proprioception. Secondary to this, most people with diabetic neuropathy develops non-healing ulcers resulting from trauma and/or suboptimal distribution of internal bone pressure. The sequalae for this is usually very severe, with cellulitis, amputations, and progression of the ulcer to malignancy (32).

Investigations For Confirmation Of Diabetes Mellitus

Currently, the following tests are used to diagnose diabetes mellitus (see Table 4 for summary) (10):

1. Fasting blood glucose
2. Glucose tolerance test
3. Glycated hemoglobin
4. Fructosamine test (not commonly used)

Fasting blood glucose: At least two different fasting blood glucose readings are necessary to make a diagnosis of diabetes mellitus. A value more than 126 mg/dL is confirmatory for diabetes mellitus.

Glucose tolerance test: Not routinely used, but glucose challenge test currently finds in place in the diagnosis of gestational diabetes mellitus in pregnancy.

Glycated hemoglobin: Considered the gold standard for monitoring glycemic control for the last three months, glycated hemoglobin is another important confirmatory test for diabetes mellitus.

Fructosamine test: Not widely used owing to its difficulty in performance and lack of standardized kits, Fructosamine test nevertheless is a very good indicator for the glycemic control over the previous month. Moreover, Fructosamine levels of well controlled diabetics overlap with normal individuals, further reducing its use as a diagnostic tool.

TEST	BIOLOGICAL REFERENCE INTERVAL
Fasting Blood Glucose	Normal: <100 mg/dL
	Impaired glucose tolerance: 101-125 mg/dL
	Diabetes Mellitus: >160 mg/dL
Glucose tolerance Test	2 hours: <140 mg/dL
Glycated hemoglobin	Normal: <5.6%
	Impaired glucose tolerance test: 5.6-6.4%
	Diabetes Mellitus: >6.4%
Fructosamine test	Normal: 200-285 μmol/L when serum albumin is 5 g/dL

Table 4: Diagnostic tests for diabetes mellitus

Glycated Hemoglobin

Chromatography of hemoglobin of adult human beings reveal two different types (33):

1. Adult type (containing 2α and 2β chains): ~90-92%
2. Fetal hemoglobin (containing 2α and 2γ chains): ~8-10%

Apart from these two, minor percentages of other hemoglobin too may be present in trace quantities.

Adult hemoglobin is further divided into HbA_1, HbA_2, HbA_3, and other forms depending on the changes in the globin chains.

The major globin chain in adults (HbA_1) contains 2α and 2β chains. Glycation, the non-enzymatic addition of glucose to the N-terminal end of β chain is a normal reaction happening in all humans, the rate of which depends on blood glucose. An accurate quantification of this value gives an accurate impression of the individual's glycemic control over the life span of RBCs. These glycated hemoglobin molecules have been named HbA_{1c} or glycated hemoglobin (34).

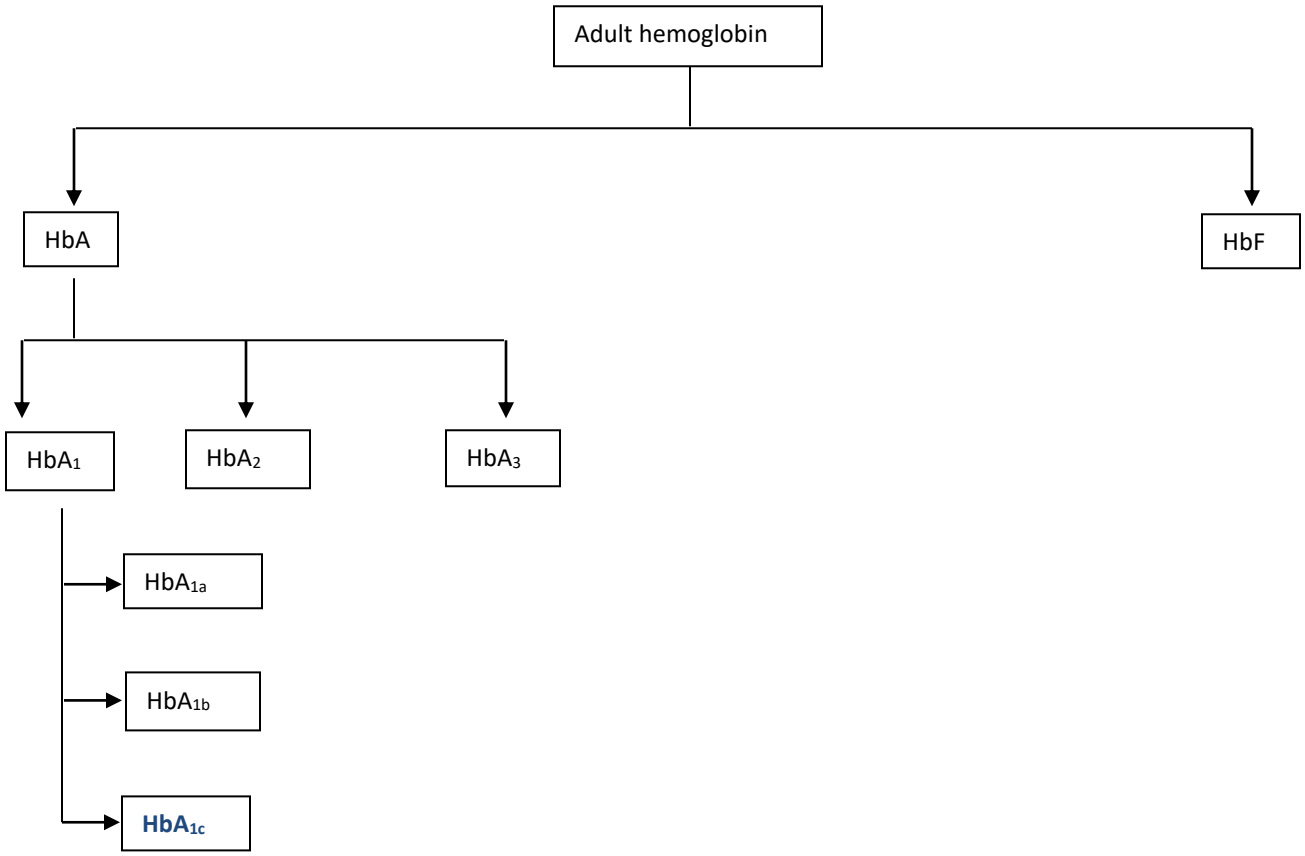

Figure 5: Types of hemoglobin present in adult humans

Methods For Assaying Glycated Hemoglobin

Currently, there are three major methods by which glycated hemoglobin is quantified (35):

1. HPLC assay using ion-selective chromatography
2. Antibody based immunoassay
3. Enzymatic assay

High Performance Liquid Chromatography: The gold standard for measurement of glycated hemoglobin is to make use of high-performance liquid chromatography using ion-exchange chromatography. A negatively charged column is used as the stationary phase to which the positively charged hemoglobin molecules are bound to with varying affinity. A mobile phase, with varying pH is then allowed into the column in a gradient flow, resulting in the elution of various hemoglobin molecules depending on their charge (36).

Antibody based immunoassay: Antibody based immunoassays are based on the latex enhanced immunoassay between the antigen molecules and antibodies specific to HbA_{1c} coated on beads. The cross-linking reaction causes changes in the turbidity of the solution, which is measured and quantified (37)

Enzymatic assays: Recently, a novel direct enzymatic method has been developed which yields a direct measurement of glycated hemoglobin. The advantages of this method include direct glycated hemoglobin and the possibility of not having to calculate the values from the provided measurements (38).

For a comparison of the methods used for the measurement of glycated hemoglobin, please refer to Table 5.

Method with principle	Advantages	Disadvantages
Ion exchange chromatography (HPLC using an anion-exchange column)	• Gold standard • Chromatograms provide information of variants of hemoglobin as well	• Variable interference from hemoglobinopathies
Immunoassay (glucose binding to m-amino phenylboronic acid)	• Easier to implement	• Affected by hemoglobinopathies
Direct enzymatic method	• Minimal interference • No need of calculation	• Measures more than the N-glycosylation in β-chains, but also in the α-chains

Table 5: Comparison of various methods for measurement of glycated hemoglobin

Indications For Testing Glycated Hemoglobin

Since glycated hemoglobin measures the glycation of glucose to beta chain of hemoglobin, the measurement of HbA_{1c} is a fair reflection of the glycemic control over the life span of RBCs, which is approximately 120 days. Hence, after the diagnosis of diabetes mellitus, glycated hemoglobin should be performed every three months in those individuals for assessing their glycemic control until the target level is reached. Once the target glycemic control is attained, the measurements may be necessary only twice a year (39).

Limitations Of Measurement of Glycated Hemoglobin

Since glycated hemoglobin is directly affected by the life span of RBC, any condition that affects the life of RBCs would affect HbA_{1c} measurements as well. Conditions like anemia, hemolysis, blood loss, presence of other hemoglobin variants, and other conditions affecting the life span of RBCs results in potentially wrong measurements. It is also important to keep in mind that a measure of glycated hemoglobin is not a measure of an individual's routine glycemic variability or a measure of blood glucose levels (34)

Factors Affecting Glycated Hemoglobin Levels

Factors affecting the levels of glycated hemoglobin levels are summarized in Table 6 as given below.

FACTORS	INCREASED GLYCATED HEMOGLOBIN	DECREASED GLYCATED HEMOGLOBIN
Erythropoiesis	Iron, Vitamin B12 deficiency, decreased erythropoiesis	Administration of erythropoietin, iron, folic acid, reticulocytes, chronic liver disease
Altered hemoglobin	Genetic or chemical alterations in hemoglobin, hemoglobinopathies, HbF, methemoglobin	Genetic or chemical alterations in hemoglobin, hemoglobinopathies, HbF, methemoglobin
Glycation	Alcoholism, chronic renal failure, decreased intraerythrocytic pH	Aspirin, Vitamin C and E, increased intraerythrocytic pH
Erythrocyte destruction	Increased erythrocyte lifespan, splenectomy	Decreased erythrocyte life span, splenomegaly, rheumatoid arthritis
Assays	Hyperbilirubinemia, carbamylated hemoglobin, alcoholism	Hypertriglyceridemia

Table 6: Factors affecting glycated hemoglobin levels

Hematopoiesis

The process of blood cell production from hematopoietic stem cells is called hematopoiesis. The hematopoietic stem cells have an infinite potential to proliferate and produce more stem cells as well as to differentiate to committed progenitor cells.

If it is committing into progenitor cells, HSCs specialize into either myeloid lineage or lymphoid lineage. Myeloid lineage finally differentiates into monocytes, neutrophils, RBC, platelets, basophils, and eosinophils while lymphoid lineage finally differentiates into T cell, B cell, and NK cells. See Figure 6 for more details.

During its development in the bone marrow, the cells programmed to be leukocytes of the granulocytic series (neutrophils, eosinophils, basophils) synthesize specialized proteins which are then stored as granules in their cytoplasm.

Myeloblast, a primitive, agranular cell, synthesizes proteins and stores them in cytoplasmic granules (primary or azurophilic granules), marking the conversion to promyelocytes. This is followed by the synthesis and storage of secondary granules, which marks the progress of promyelocytes into neutrophilic, basophilic, and eosinophilic myelocytes. What follows next is the conversion of myelocytes into mature amniotic cells capable of chemotaxis and phagocytosis (40).

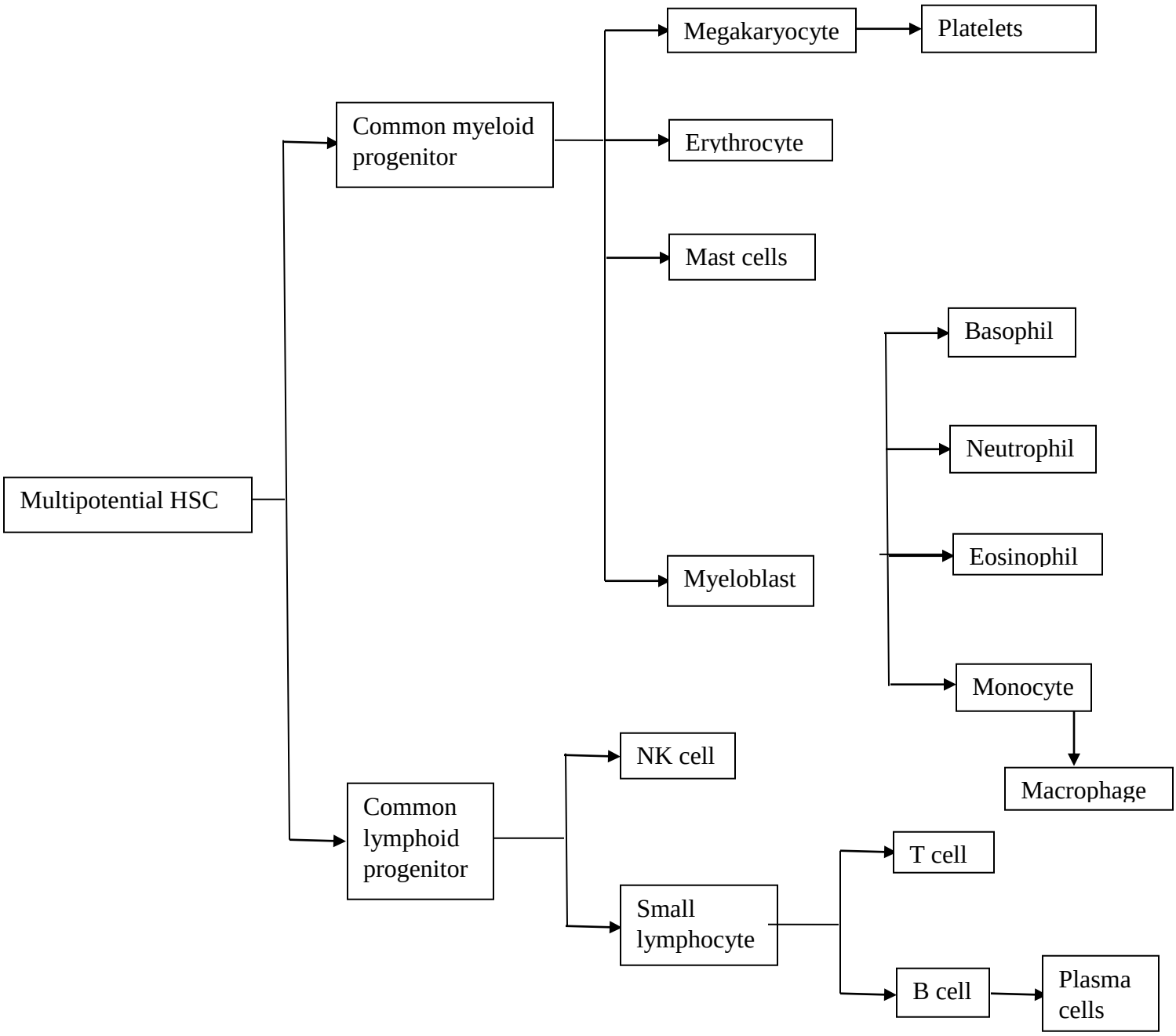

Figure 6: Hematopoiesis

The maturation of neutrophils from myeloblasts is shown in the figure below (Figure 7).

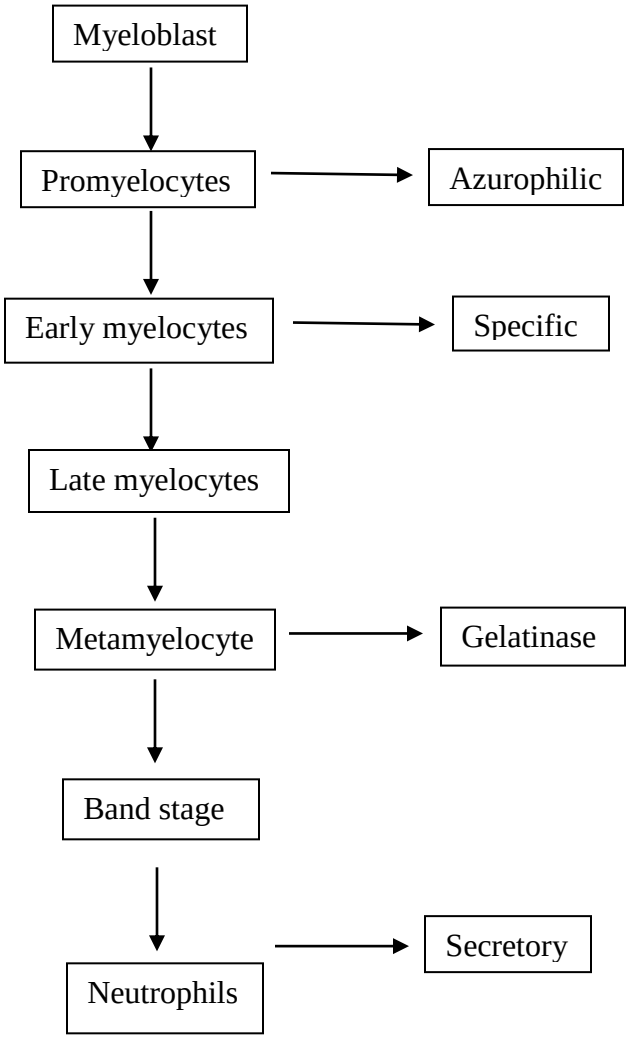

Figure 7: Maturation of myeloblast into neutrophils (with their granules)

A variety of conditions can alter the number of neutrophils, lymphocytes, and platelets. Any increase in the absolute count of neutrophils is depicted by the name neutrophilia while any decrease is depicted by the term neutropenia. Any increase in the absolute count of lymphocytes and platelets are known by the terms lymphocytosis and thrombocytosis respectively while any

decrease is known by the terms lymphocytopenia and thrombocytopenia respectively. The major causes of these are highlighted in Table 7(40).

Neutrophils	**Neutrophilia**	**Neutropenia**
	Physical stimuli: cold, heat, convulsions, pain, labor, anesthesia, surgery	Infections: Hepatitis, Lyme disease
	Hematological disorders: Myeloproliferative disorders, hemorrhage.	Hematological disorders: Aplastic anemia, Fanconi anemia
	Emotional stimuli: Panic, rage, stress, depression	Drugs: Chemotherapy
	Infections: Mycotic, bacterial, and rickettsial	Cancer, bone marrow disorders, hypersplenism
	Inflammation	Nutritional deficiencies like Vitamin B12 and iron deficiency
	Drugs	Autoimmune diseases like Crohn's disease, SLE
	Hormones	
	Toxins	Felty syndrome, Kostman syndrome
Lymphocytes	**Lymphocytosis**	**Lymphopenia**
	Malignancies: ALL, CLL	Inherited: Wiskott-Aldrich syndrome
	Reactive: Dengue, EBV, CMV	Acquired: Aplastic anemia
	Hypersensitivity: Drugs, insect bites	Infectious: Viral (HIV, SARS)
	Persistent: Cancer, leprosy, smoking	Marrow suppression, irradiation
	Serum sickness, post-vaccination	

Thrombocytes	**Thrombocytosis**	**Thrombocytopenia**
	Primary thrombocytosis: ET, CML, MDS, chronic myeloproliferative disorder	Decreased production: Amegakaryocytotic thrombocytopenia, aplastic anemia, MDS
	Reactive: Infection, malignancy, renal disorder, hemolytic anemia, asplenia	Increased destruction: SLE, lymphoproliferative disorders, drugs, viral fevers, DIC
		Abnormal distribution: Dilutional from massive blood transfusion, hypersplenism

Table 7: Major causes of neutrophil, lymphocyte, and thrombocyte alterations

Neutrophil-Lymphocyte Ratio and Platelet-Lymphocyte Ratio

Neutrophil-lymphocyte ratio and Platelet-lymphocyte ratio are important markers of systemic inflammation. Both these ratios are also seen to be elevated in Type 2 diabetes mellitus and has a predictive value in cardiovascular events in diabetics (41). In conditions of systemic inflammation, the WBC constitution changes dramatically, with a predisposition towards lymphopenia and neutrophilia (42). This also results in an altered Platelet-lymphocyte ratio, which may be used as a marker for several conditions like cardiac, rheumatological, and cancerous conditions (43). The comparative stability and minimal cost for calculating NLR and PLR serves as an important advantage when compared to other more expensive markers such as inflammatory cytokines (44).

Elevation in the NLR after an ischemic attack of the heart acts as an independent predictor of mortality in diabetics. The use of this parameter along with other existing risk scoring systems may be beneficial for prognosis and stratification of risk (45). Other biomarkers like TNF-α, interleukin 6, C-reactive protein, VCAM-1, von Willibrand factor, fibrinogen, and adiponectin

are seen to develop with Type 2 Diabetes Mellitus (46). Both NLR and PLR may be used as a marker for end-stage renal disease patients in diabetes mellitus, but PLR is found to be superior in this regard (47). In individuals with long standing diabetes mellitus, an elevation in NLR is reflective of diabetic nephropathy, thereby serving as a reliable early marker for the prediction of the same (48).

NLR and PLR can be used as markers of conditions other than diabetes mellitus as well. For example, NLR may be used as a biomarker for the prediction of IgA vasculitis (49), prognostic marker for the outcome of CABG (50), and as an indicator for the possibility of miscarriage during pregnancy (51), (52).

MATERIALS AND METHODS

STUDY DESIGN: CROSS-SECTIONAL STUDY

SOURCE OF DATA: Data is obtained from patients reporting to Haematology Laboratory, Department of Pathology, Shri B.M. Patik Medical College. Hospital, and Research Centre, B.L.D.E (Deemed to be University), Vijayapura.

STUDY PERIOD: 1st December 2019 to 30th May 2021

INCLUSION AND EXCLUSION CRITERIA:

The Inclusion and exclusion criteria for the study is tabulated in Table 8:

INCLUSION CRITERIA	EXCLUSION CRITERIA
Patients with Type 2 Diabetes Mellitus in Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapura	Suspected medical conditions like sepsis, renal disorders
	On medications (except antidiabetic agents) that may interfere with the results of the study and inflammatory parameters
	Patients with previous/coexisting cardiovascular diseases (as defined by history, medical examination, and cardiac imaging)
	History of smoking

Table 8: Inclusion and Exclusion Criteria for the study

SAMPLE COLLECTION AND ANALYSIS

Informed consent was taken from those patients who fulfil the inclusion criteria for the study. Relevant medical history was taken from them, followed by a routine clinical examination. The archived sample (stored after running the requested tests) were retrieved to confirm the identity of the patient. In the Laboratory Information System, the patient's unique ID was entered, and the relevant values retrieved (HbA1c and Complete Blood Count). Care was taken to run the samples within four hours of collection. Refer the flow chart given below for more the schematic.

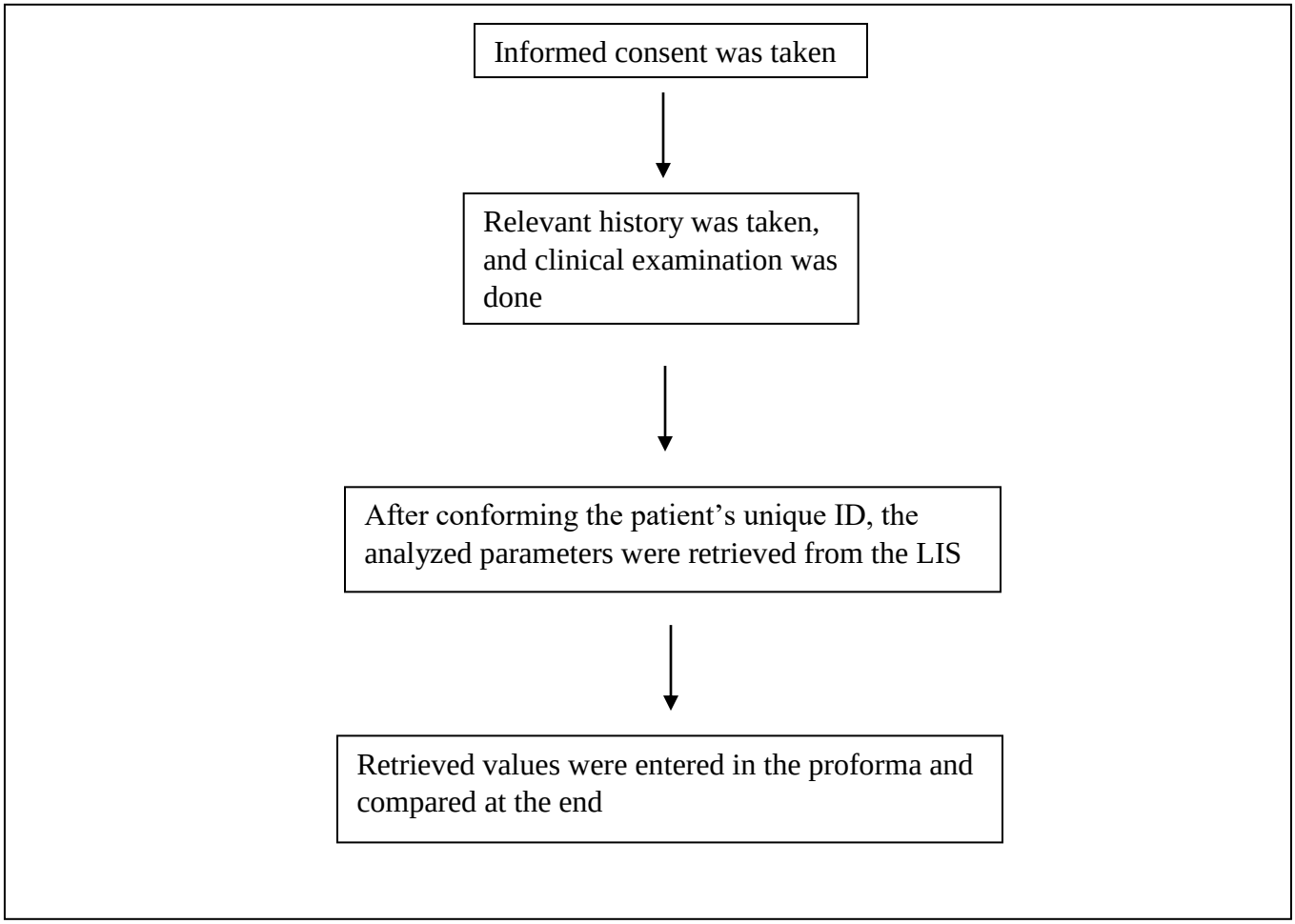

Figure 8: Methods followed for retrieval of data

METHODS OF COLLECTION OF DATA

Data on the analyzed parameters (glycated hemoglobin and complete blood count) from the patients fulfilling the inclusion criteria were evaluated.

Complete blood count was done using an automated blood cell counter (Sysmex XN 1000, Sysmex Corporation, Kobe, Japan, see figure below). Out of the CBC parameters, total WBC count, differential count, and platelet count were of particular importance.

Figure 9: SYSMEX XN 350 and 1000

Neutrophil-lymphocyte ratio was calculated as absolute neutrophil count divided by absolute lymphocyte counts.

$$NLR = \frac{Absolute\ neutrophil\ count}{Absolute\ lymphocyte\ Count}$$

Platelet-lymphocyte ratio was calculated as the total platelet count divided by lymphocyte counts.

$$PLR = \frac{Total\ platelet\ count}{Absolute\ lymphocyte\ count}$$

Glycated hemoglobin was measured using automated ion exchange high performance liquid chromatography (BIORAD D10) according to the manufacturer's instructions.

Figure 10: BIORAD D10

SAMPLE SIZE:

With 95% confidence level and margin of error of ±7.5%, a sample size of 300 subjects will included in the study to determine the association of Neutrophil-Lymphocyte ratio and blood glucose regulation in type 2 Diabetes Mellitus patients with finite population correction (N=5000).

Formula used: $n = z^2 \frac{p(1-p)}{d^2}$

Z= z statistic at 5% level of significance

d is margin of error

p is anticipated prevalence rate (50%)

The sample size of 300 were then divided equally into three groups: Group 1 (prediabetic with HbA_{1c} between 5.7-6.4%), Group 2 (diabetes mellitus with HbA_{1c} more than 6.4%), and Group 3 (control with HbA_{1c} value less than 5.7%).

Statistical analysis:

All statistics were summarized descriptively. For continuous variables, the summary statistics of N, mean, standard deviation (SD) will be used. For categorical data, the number and percentage were used in the data summaries and data were analyzed by comparison of means using t test, ANOVA, post hoc, and diagrammatic presentation.

Biological Reference Interval (40,53)

Parameters	Biological Reference Interval
RBC	3.8-4.8 millions/c mm
Hb	11.6-14.5 g/dl
PCV	36-46 %
Platelet	150-450 $x10^3$µL
WBC	4.8-10.8 $x10^3$µL
Absolute Neutrophil Count	1.4-6.5 $x10^3$µL
Absolute Lymphocyte Count	1.2-3.4 $x10^3$µL
Absolute Eosinophil Count	0-0.5 $x10^3$µL
Absolute Monocyte Count	0.1-0.6 $x10^3$µL
Absolute Basophil Count	0-0.2 $x10^3$µL

Table 9: Reference values

RESULTS

The study was conducted in the Department of Pathology and Department of Biochemistry, Shri B.M. Patel Medical College, Hospital, and Research Centre, B.L.D.E (Deemed to be University), Vijayapura.

The results of study are as follows:

1. Age-distribution (Please refer to Table 10 for more information)
    a. For Group 1 (pre-diabetic group), the mean age is 51.06 with the minimum age being 18 and the maximum age of 84.
    b. For Group 2 (diabetes mellitus), the mean age is 52.65 with the minimum age being 18 and the maximum age being 80.
    c. For Group 3 (control), the mean age is 45.28 with the minimum age of 17 and the maximum age of 92.

Group	Mean	Minimum age	Maximum age
Group 1 (Prediabetic)	51.06	18	84
Group 2 (Diabetic)	52.65	18	80
Group 3 (Control)	45.28	17	92

Table 10: Age-wise distribution of the sample population

2. Distribution of HbA_{1c} levels among the groups (Please refer to the graph for more information)
    a. For Group 1 (pre-diabetic group), the mean HbA1c level is 6.068% with the minimum being 5.7% and the maximum of 6.4%.
    b. For Group 2 (diabetes mellitus), the mean HbA1c level is 9.51% with the minimum being 6.50% and the maximum being 15.3%
    c. For Group 3 (control), the mean HbA1c level is 5.17% with the minimum of 3.90% and the maximum age of 5.60%.

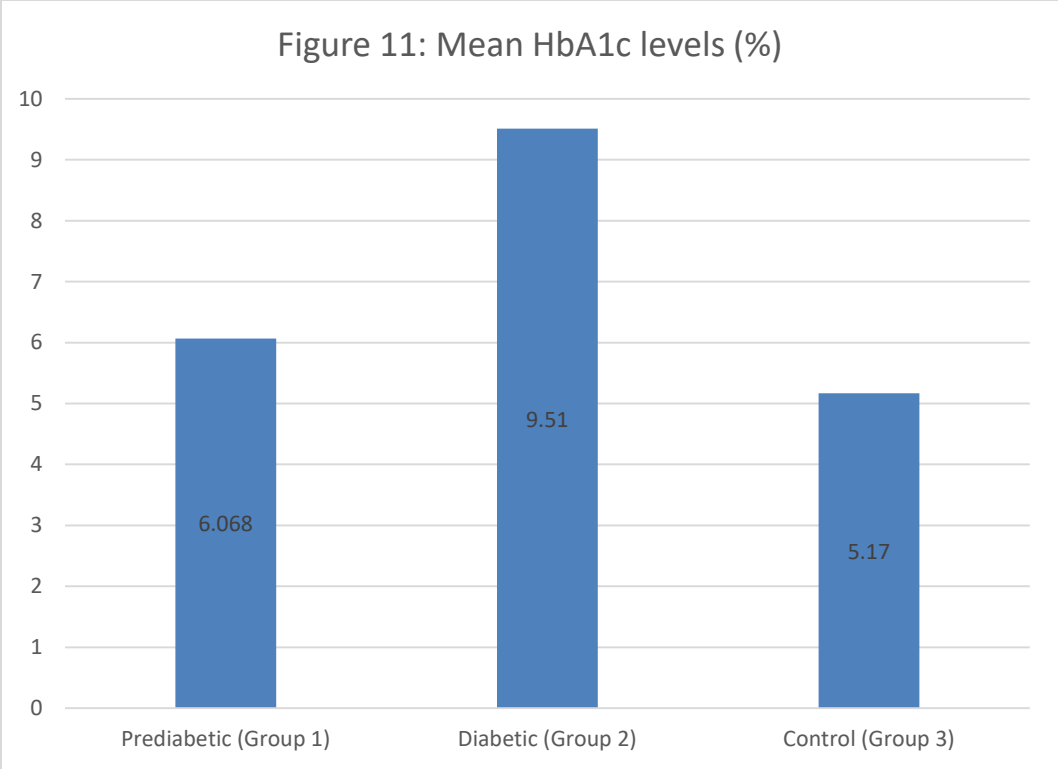

Figure 11: Mean HbA_{1c} levels

3. Gender distribution
    a. The gender distribution for the study is as follows (Figure 12):

	Frequency	**Percentage**
Female	118	39.3%
Male	182	60.7%
Total	300	100%

Table 11: Gender distribution for the study

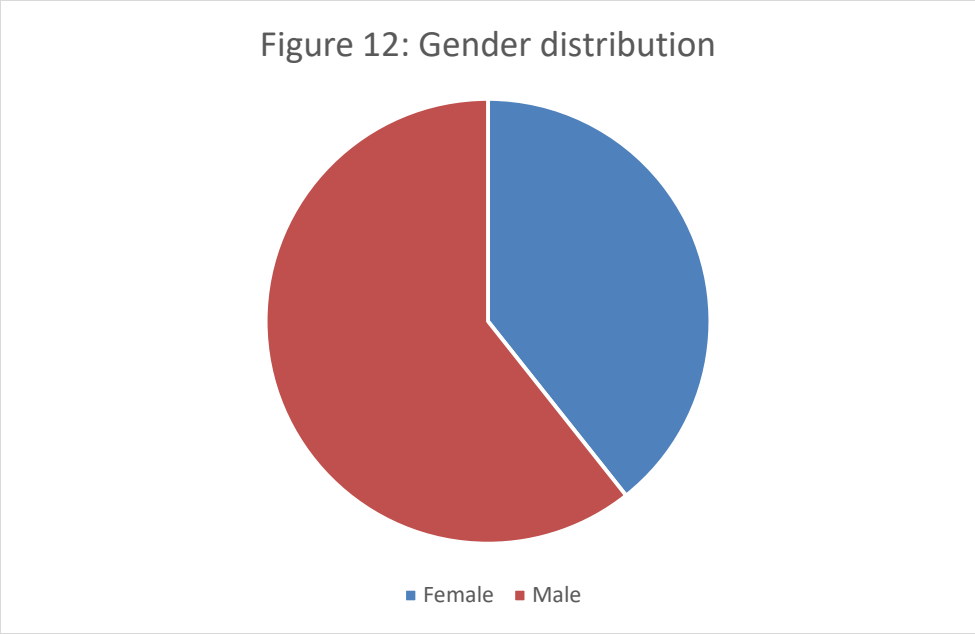

Figure 12: Gender distribution among the participants of the study

b. The group-wise gender distribution is as shown in Figure 13.

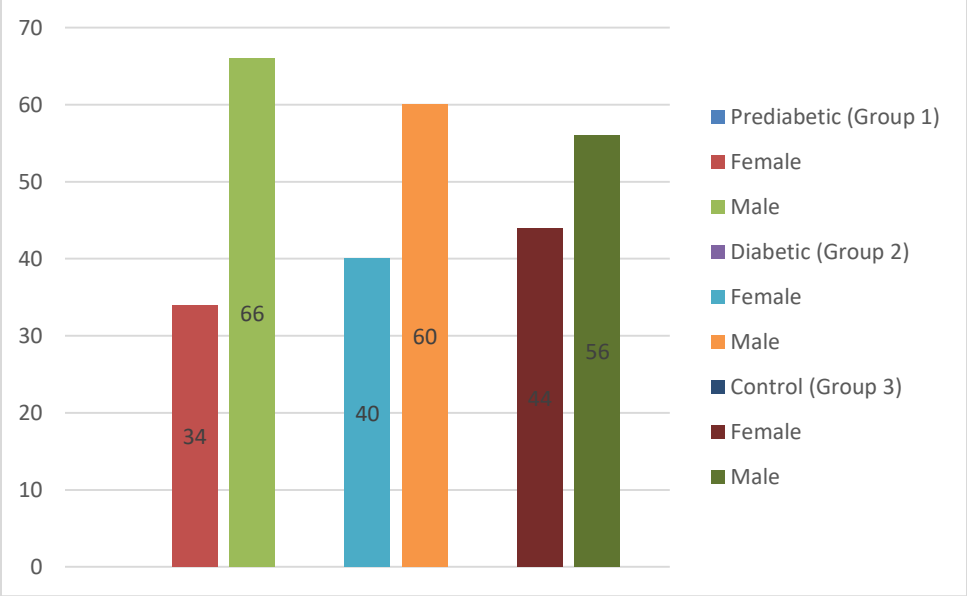

Figure 13: Gender distribution among the groups(%).

4. Descriptive Statistics

The mean, standard deviation, and the range for HbA1c, NLR, and PLR are shown in Table 12 given below:

	HbA_{1c}			**NLR**			**PLR**		
	Group 1	**Group 2**	**Group 3**	**Group 1**	**Group 2**	**Group 3**	**Group 1**	**Group 2**	**Group 3**
Mean	6.068	9.515	5.175	2.088	3.950	1.677	113.963	152.127	94.199
Std. Deviation	0.220	2.399	0.370	0.640	2.008	0.729	54.114	70.633	39.331
Minimum	5.70	6.50	3.90	1.020	1.190	0.709	31.532	31.389	18.446
Maximum	6.4	15.30	5.60	4.324	9.652	4.414	372.54	426.20	222.22

Table 12: Descriptive data among the three different groups

The box plots for HbA1c, NLR, and PLR among the different groups is shown below (Figures 14,15,16)

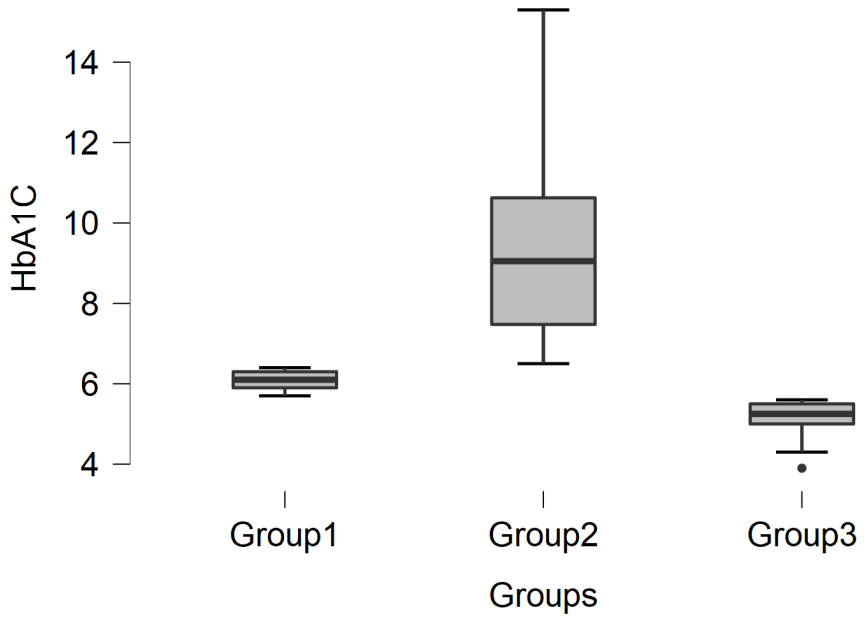

Figure 14

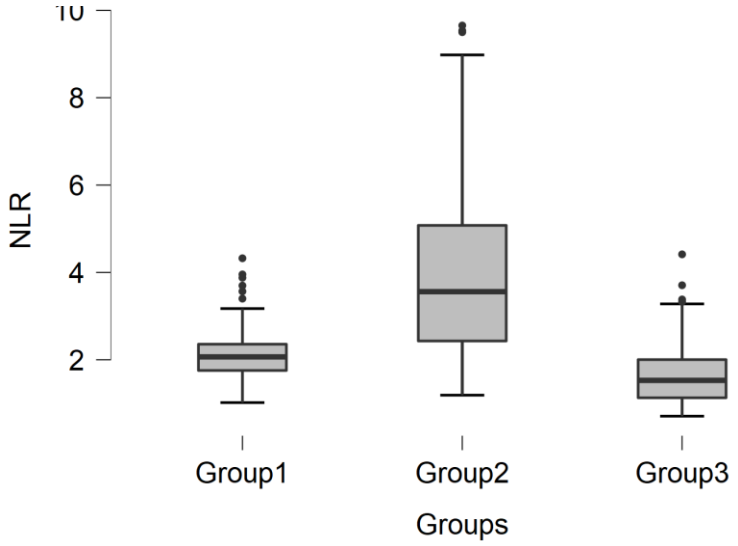

Figure 15

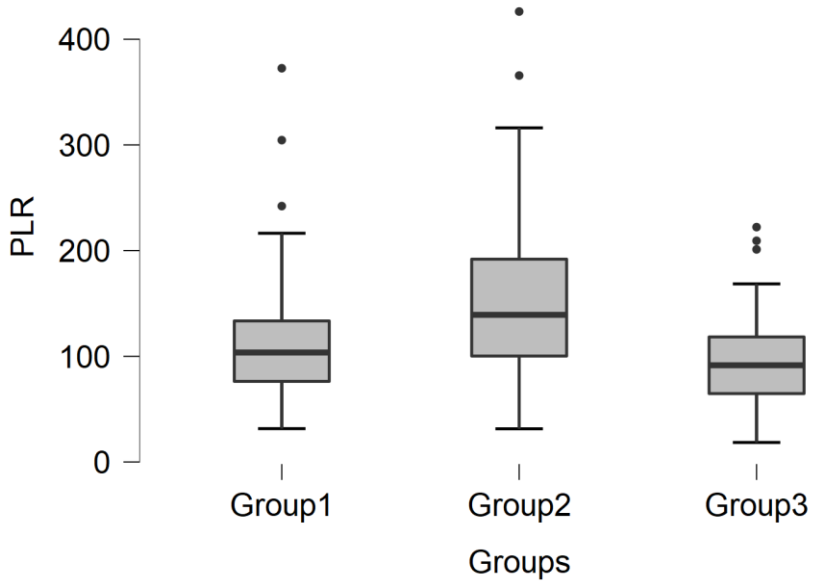

Figure 16

The scatterplot of HbA1c against NLR is shown below (Figure 17):

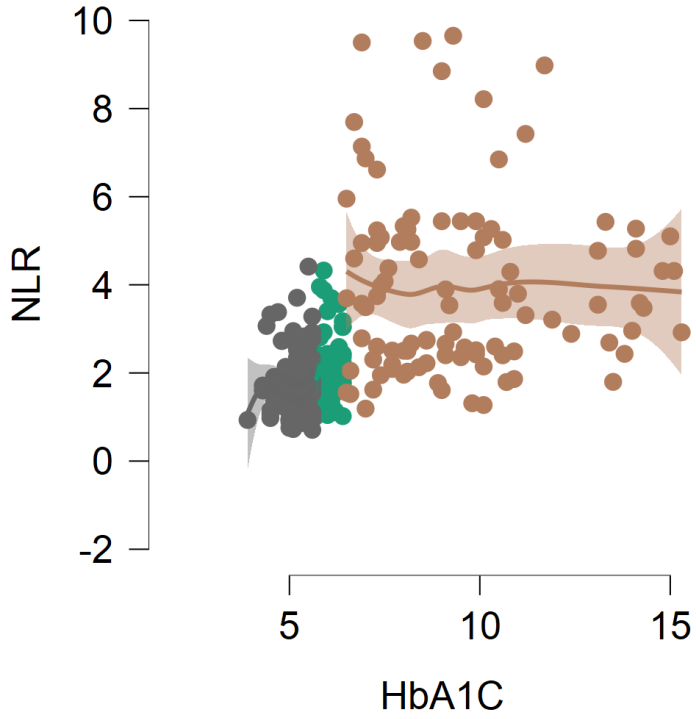

Figure 17:

The scatterplot for HbA1c against PLR is shown below (Figure 18):

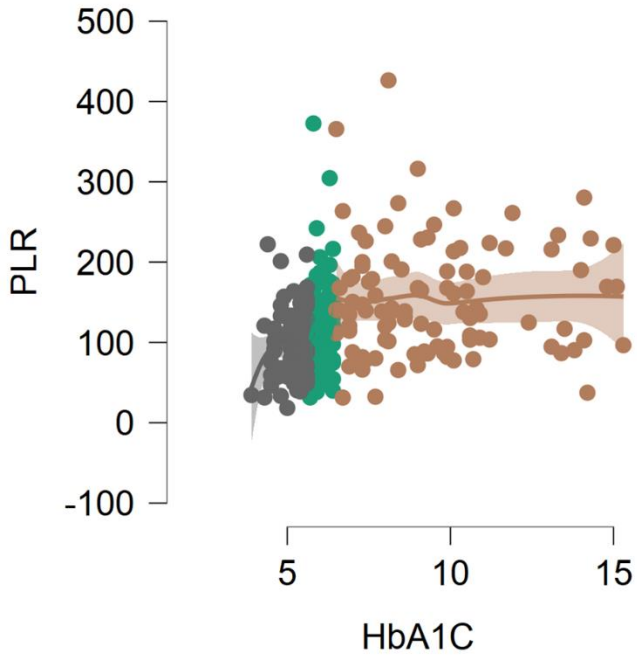

Figure 18:

The scatterplot for NLR vs PLR is shown in the figure below (Figure 19):

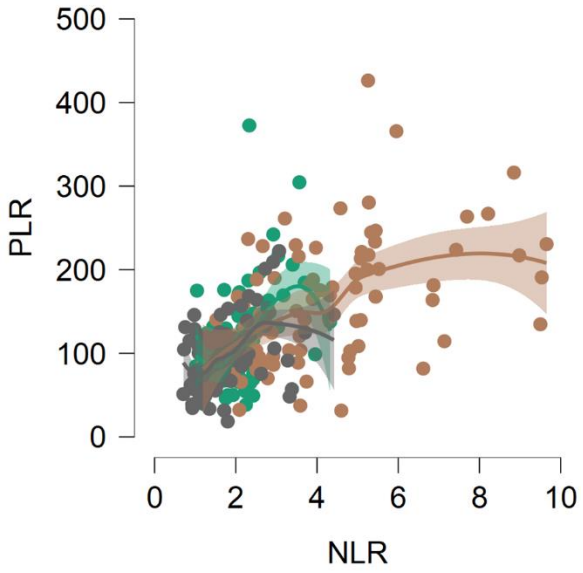

Figure 19:

POST-HOC ANALYSIS

One way ANOVA was used to compare the total WBC count between the different groups and no statistically significant difference was seen between the groups (Table 13).

Group	**Comparison group**	**Mean value**	**p-value**	**Significance**
Group 1	Group 2	9258.2	0.438	Not significant
	Group 3 (Control)	8555.9	0.251	Not significant
Group 2	Group 1	8975.2	0.438	Not significant
	Group 3 (Control)	8555.9	0.055	Not significant
Group 3 (Control)	Group 1	8975.2	0.251	Not significant
	Group 2	9258.2	0.055	Not significant

Table 13: Total WBC count between different groups

One way ANOVA was used to compare the absolute neutrophil count between the different groups and a statistically significant increase was seen in Group 1 (pre-diabetic) and Group 2 (diabetic) compared to Group 3 (Control). Similarly, the absolute neutrophil count was found to be increased in Group 2 (Diabetes) in comparison to Group 1 (pre-diabetic). See the table 14 below for more information.

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	6641.7	0.000*	**Significant**
	Group 3 (Control)	4758.7	0.003*	**Significant**
Group 2	Group 1	5523.01	0.000*	**Significant**
	Group 3 (Control)	4758.7	0.000*	**Significant**
Group 3 (Control)	Group 1	5523.01	0.003*	**Significant**
	Group 2	6641.7	0.000*	**Significant**

Table 14: Absolute neutrophil count between different groups

The mean difference was found to be significant at a p-value of 0.05

One way ANOVA was used to compare the absolute lymphocyte count between the different groups and a statistically significant decrease was seen in Group 1 (pre-diabetic) and Group 2 (diabetic) compared to Group 3 (Control). Similarly, the absolute lymphocyte count was found to be decreased in Group 2 (Diabetes) in comparison to Group 1 (pre-diabetic). See the table 15 below for more information.

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	1983.16	0.000*	**Significant**
	Group 3 (Control)	3163.01	0.009*	**Significant**
Group 2	Group 1	2776.56	0.000*	**Significant**
	Group 3 (Control)	3163.01	0.000*	**Significant**
Group 3 (Control)	Group 1	2776.56	0.009*	**Significant**
	Group 2	1983.16	0.000*	**Significant**

Table 15: Absolute lymphocyte count between different groups

The mean difference was found to be significant at a p-value of 0.05

One way ANOVA was used to compare the absolute eosinophil count between the different groups. It was found that the absolute eosinophile count was found to be to be decreased in the Group 1 (Pre-diabetes) and Group 2 (Diabetes) compared to Group 3 (Control). Comparisons among the other groups yielded no statistically significant results (Table 16).

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	223.3	**.025**	**Significant**
	Group 3 (Control)	326.23	.534	Not significant
Group 2	Group 1	303.91	**.025**	**Significant**
	Group 3 (Control)	326.23	**.004**	**Significant**
Group 3 (Control)	Group 1	303.91	.534	Not significant
	Group 2	223.3	**.004**	**Significant**

Table 16: Absolute eosinophil count between different groups

The mean difference was found to be significant at a p-value of 0.05

One way ANOVA was used to compare the absolute monocyte count between the different groups and no statistically significant difference was seen between the groups (Table 17).

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	379.11	.075	Not significant
	Group 3 (Control)	321.98	.864	Not significant
Group 2	Group 1	317.69	.075	Not significant
	Group 3 (Control)	321.98	.063	Not significant
Group 3 (Control)	Group 1	317.69	.864	Not significant
	Group 2	379.11	.063	Not significant

Table 17: Absolute monocyte count between different groups

One way ANOVA was used to compare the absolute basophil count between the different groups. It was found that the absolute basophil count was found to be decreased in the Group 2 (Diabetes) compared to Group 3 (Control). Similarly, the absolute basophil count was found to be statistically decreased in Group 2 compared to Group 1. Comparisons among the other groups yielded no statistically significant results (Table 18).

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	27.89	**.002**	**Significant**
	Group 3 (Control)	50.36	.987	Not significant
Group 2	Group 1	50.47	**.002**	**Significant**
	Group 3 (Control)	50.36	**.002**	**Significant**
Group 3 (Control)	Group 1	50.47	.987	Not significant
	Group 2	27.89	**.002**	**Significant**

Table 18: Absolute basophil count between different groups

The mean difference was found to be significant at a p-value of 0.05

The Neutrophil-Lymphocyte ratio was compared between different groups using one-way ANOVA. It was found that NLR had a statistically significant increase in Group 2 (Diabetes) compared to both Group 1 (Pre-diabetic) and Group 3 (Control). There was no significant difference between Control and Pre-diabetic group (Table 19).

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	3.94	**.000**	**Significant**
	Group 3 (Control)	1.67	**.08**	Not significant
Group 2	Group 1	2.08	**.000**	**Significant**
	Group 3 (Control)	1.67	**.000**	**Significant**
Group 3 (Control)	Group 1	1.6768	**.08**	Not significant
	Group 2	3.94	**.000**	**Significant**

Table 19: Neutrophil-Lymphocyte Ratio between different groups

The mean difference was found to be significant at a p-value of 0.05

One way ANOVA was used to compare the platelet count between the different groups and no statistically significant difference was seen between the groups (Table 20).

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	265900	.205	Not significant
	Group 3 (Control)	265690	.199	Not significant
Group 2	Group 1	281560	.205	Not significant
	Group 3 (Control)	265690	.986	Not significant
Group 3 (Control)	Group 1	281560	.199	Not significant
	Group 2	265900	.986	Not significant

Table 20: Platelet count between different groups

The Platelet-Lymphocyte ratio was compared between different groups using one-way ANOVA. It was found that PLR had a statistically significant increase in Group 2 (Diabetes) compared to both Group 1 (Pre-diabetic) and Group 3 (Control). The PLR was also found to have a statistically significant increase in Group 2 (Diabetes) in comparison to Group 1 (Pre-diabetic). There was no significant difference between Control and Pre-diabetic group (Table 21).

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	153.12	**.000**	**Significant**
	Group 3 (Control)	94.19	.062	Not significant
Group 2	Group 1	113.96	**.000**	**Significant**
	Group 3 (Control)	94.19	**.000**	**Significant**
Group 3 (Control)	Group 1	113.96	.062	Not significant
	Group 2	153.12	**.000**	**Significant**

Table 21: PLR between different groups

The mean difference was found to be significant at a p-value of 0.05

One way ANOVA was used to compare the RBC count, hemoglobin, and PCV between the different groups and no statistically significant difference was seen between the groups (Table 22, 23, 24).

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	4.47	.980	Not significant
	Group 3 (Control)	4.39	.571	Not significant
Group 2	Group 1	4.46	.980	Not significant
	Group 3 (Control)	4.39	.554	Not significant
Group 3 (Control)	Group 1	4.46	.571	Not significant
	Group 2	4.47	.554	Not significant

Table 22: RBC count between different groups

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	12.42	.762	Not significant
	Group 3 (Control)	12.71	.350	Not significant
Group 2	Group 1	12.35	.762	Not significant
	Group 3 (Control)	12.71	.217	Not significant
Group 3 (Control)	Group 1	12.35	.350	Not significant
	Group 2	12.42	.217	Not significant

Table 23: Hemoglobin between different groups

Group	Comparison group	Mean	p-value	Significance
Group 1	Group 2	36.28	.339	Not significant
	Group 3 (Control)	38.02	.425	Not significant
Group 2	Group 1	37.21	.339	Not significant
	Group 3 (Control)	38.02	.081	Not significant
Group 3 (Control)	Group 1	37.21	.425	Not significant
	Group 2	36.28	.081	Not significant

Table 24: PCV between different groups

The lipid profile parameters were compared between the groups and the results are given below:

One way ANOVA was used to compare the serum triglyceride levels between the different groups and a statistically significant increase was seen in the diabetic group in comparison to prediabetic group (Table 25).

Group	Comparison group	Mean	p-value	Significance
Group 1	Group 2	211.14	.035	**Significant**
	Group 3 (Control)	163.68	.839	Not significant
Group 2	Group 1	158.21	.035	**Significant**
	Group 3 (Control)	163.68	.066	Not significant
Group 3 (Control)	Group 1	158.21	.839	Not significant
	Group 2	211.14	.066	Not significant

Table 25: Serum triglyceride levels between different groups

One way ANOVA was used to compare the serum cholesterol levels between the different groups and no statistically significant difference was seen between the groups (Table 26).

Group	Comparison group	Mean	p-value	Significance
Group 1	Group 2	164.1	.098	Not significant
	Group 3 (Control)	176.87	.474	Not significant
Group 2	Group 1	185.12	.098	Not significant
	Group 3 (Control)	176.87	.367	Not significant
Group 3 (Control)	Group 1	185.12	.474	Not significant
	Group 2	164.1	.367	Not significant

Table 26: Serum cholesterol levels between different groups

One way ANOVA was used to compare the serum HDL between the different groups and no statistically significant difference was seen between the groups (Table 27).

Group	Comparison group	Mean	p-value	Significance
Group 1	Group 2	35.44	.538	Not significant
	Group 3 (Control)	38.44	.658	Not significant
Group 2	Group 1	37.17	.538	Not significant
	Group 3 (Control)	38.44	.305	Not significant
Group 3 (Control)	Group 1	37.17	.658	Not significant
	Group 2	35.44	.305	Not significant

Table 27: Serum HDL levels between different groups

One way ANOVA was used to compare the serum LDL between the different groups and a statistically significant increase was seen in the prediabetic group compared to the prediabetic group (Table 28).

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	88.98	.013	**Significant**
	Group 3 (Control)	105.34	.338	Not significant
Group 2	Group 1	116.41	.013	**Significant**
	Group 3 (Control)	105.34	.135	Not significant
Group 3 (Control)	Group 1	116.41	.338	Not significant
	Group 2	88.98	.135	Not significant

Table 28: Serum LDL levels between different groups

One way ANOVA was used to compare the serum VLDL between the different groups and no statistically significant difference was seen between the groups (Table 28).

Group	**Comparison group**	**Mean**	**p-value**	**Significance**
Group 1	Group 2	36.53	.283	Not significant
	Group 3 (Control)	42.76	.841	Not significant
Group 2	Group 1	31.12	.283	Not significant
	Group 3 (Control)	42.76	.402	Not significant
Group 3 (Control)	Group 1	31.12	.841	Not significant
	Group 2	36.53	.402	Not significant

Table 29: Serum VLDL levels between different groups

DISCUSSION

Type II Diabetes Mellitus is the most prevalent chronic disease across the world. Termed 'The Silent Killer', Type II Diabetes Mellitus exerts a great toll on the health infrastructure of all nations across the globe. The long-term complications of Type II Diabetes Mellitus, including cardiovascular, renal, neurological, and metabolic disturbances greatly increase the mortality and morbidity of patients (41). The extent of damage that may be caused by Type II Diabetes Mellitus results in the need for developing newer tools for predicting the extent of complications effectively and cost efficiently. This study was such an attempt to develop a tool for accurate and economical prediction of the disease and its complications.

In this study, there was no statistically significant difference between the total leukocyte count between the three different groups. This contrasts with the observation made by Barbora et. al and Kayo et. al (54) (55) (56) (57), where they noticed an association between increased WBC count and deteriorating action of insulin, tipping the scale towards the development of Diabetes Mellitus. Their findings strengthened the notion that development of Type II Diabetes Mellitus may be associated with the chronic activation of immune system.

In this study, Absolute Neutrophil Count (ANC) is found to be indicator for Type II Diabetes Mellitus, with a statistically significant increase seen in ANC in those with Diabetes Mellitus compared to Impaired Glucose Tolerance and the Control group. These findings were corroborated by various other authors including Fatih et al (2).

Absolute Lymphocyte Count was found to be statistically significant among the three different groups in our study. The absolute lymphocyte count was the lowest for the Diabetic group in comparison to pre-diabetic and control group, whereas it was lower for the pre-diabetic group in

comparison to the control group. Similar findings were obtained by Fatih et. al in his work on Neutrophil-Lymphocyte Ratio and diabetes mellitus (2).

The Neutrophil-Lymphocyte Ratio (NLR) is found to be significantly different in diabetics in comparison to both pre-diabetics and control group. Similar findings have been obtained by various other authors across the world (58) (2) (59) (60) (1) (61). In addition to Diabetes Mellitus, NLR has also been attributed in morbidly obese patients, as shown in a study by Yilmaz et al. His data proved that increase in NLR is a strong predictor of development of Type II Diabetes Mellitus in morbidly obese individuals (62). On top of being a predictor for Type II Diabetes Mellitus, an increase in NLR has also been noticed in cases of Diabetic Retinopathy, as shown by Wang et al (63). The use of NLR as a prognostic markers extends beyond Diabetic Retinopathy, but may be used for other diabetic complications like neuropathy, peripheral vascular disease, and nephropathy (64).

Our study also proves that the Platelet-Lymphocyte Ratio (PLR) also increases in the diabetic group in comparison to the pre-diabetic and control group. This is an expected finding since a decrease in lymphocytes is seen in Type II Diabetes Mellitus. This contrasts with the findings by Metroglau et al, who concluded that PLR decreases in the pre-diabetic stages, but increases in diabetic group (58). Demirtas et al concluded that leukocyte count, platelet distribution width, and PLR may serve as independent predictor of diabetes, while leukocyte count, mean platelet volume, neutrophil-lymphocyte ratio and platelet-lymphocyte ratio may be independent predictors of impaired glucose tolerance (47).

The serum LDL and VLDL levels were found to be significantly elevated in the diabetic group in comparison to the pre-diabetic group. Surprisingly, no statistically significant difference was

observed between the diabetic and control group. This is in contrast to the conclusion from Mertoglu et al, who found no statistically significant difference among any of the groups (58).

The conclusions derived from our study is compared and contrasted with other studies on similar parameters in the table given below (Table 30):

Parameter	**Relation to blood glucose levels**	**Concordant studies**	**Discordant studies**
Total leukocyte count	No statistically significant difference	None	Vozaroa et al; Kayo et al; Tong et al
Absolute Neutrophil Count	Increased ANC in diabetics in comparison to control Increased ANC in pre-diabetics in comparison to control Increased ANC in pre-diabetics in comparison to control	Fatih et al	None
Absolute lymphocyte count	Significantly lowered in the diabetic group compared to prediabetic and control	Fatih et al	None
Neutrophil-Lymphocyte ratio	Increased NLR in diabetics in comparison to control Increased NLR in pre-diabetics in comparison to control Increased NLR in diabetics in comparison to pre-diabetics	Sefil et al Merteglu and Gunay Azab B et al Devamsh et al Hussain et al Lou et al	None
Platelet-Lymphocyte	Increased PLR in	Demitras et al	Mertoglu and Gunay

Ratio	diabetics in comparison to control Increased PLR in pre-diabetics in comparison to control Increased PLR in diabetics in comparison to pre-diabetics		
LDL	Statistically significant increase in the diabetic group in comparison to the pre-diabetic group	None	Mertoglu and Gunay
VLDL	Statistically significant increase in the diabetic group in comparison to the pre-diabetic group	None	Mertoglu and Gunay

Table 30: Comparison of the conclusions of our study to other studies on the same parameters

Summary

Type II Diabetes Mellitus is one of the most important chronic diseases prevalent in the world in terms of morbidity and mortality. In developing countries, the effect of diabetes mellitus and its complications proves to be a huge burden on the healthcare infrastructure. Even though there are a lot of tests available for the diagnosis and follow-up of diabetes mellitus, these may not be available in all health care centers, particularly in the rural areas. This study was undertaken with the objective of developing an affordable and reliable test for follow up as well as prediction of glycemic control.

Research has proven that neutrophil-lymphocyte ratio and platelet-lymphocyte ratio is altered during different stages of Type II Diabetes Mellitus. Building upon that, we divided our participants into three groups, (Group 1 (prediabetic with HbA_{1c} between 5.7-6.4%), Group 2 (diabetes mellitus with HbA_{1c} more than 6.4%), and Group 3 (control with HbA_{1c} value less than 5.7%). Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio (among other parameters) were compared among the three groups and the statistical significance determined using ANOVA.

We found a statistically significant association for NLR and PLR between the participants in the diabetic group and the control group. Hence, we conclude that NLR and PLR may be used as a more economical alternative to glycated hemoglobin for monitoring glycemic control in those areas with minimal health infrastructure. Further research needs to be undertaken to establish the NLR/PLR values against the corresponding HbA_{1c} values.

LIMITATIONS OF THE STUDY

1. Our study is a cross-sectional study, and the parameters are measured on that specific day. If any pre-analytical errors/variables have crept in on the day of analysis, the values might be biased. Conducting serial measurements of these parameters would have provided a better picture.
2. Lipid profiles of all patients could not be collected, thereby potentially biasing the analysis of lipid profile parameters.
3. Patients with anemia of unknown causes/unknown hemoglobinopathies were not excluded. This may potentially cause false high/low values of HbA_{1c}.
4. The current coronavirus pandemic may have introduced bias into the study since patients recovered from COVID were not a part of the exclusion criteria.

CONCLUSIONS

- Neutrophil-Lymphocyte Ratio and Platelet-Lymphocyte Ratio may be used as economical and efficient screening tests for the monitoring of patients with Type II Diabetes Mellitus.
- Being a routine investigation and being something that may be done in any basic hematology laboratory, NLR and PLR has the potential to be of great use for follow-up and prediction of complications in cases of Type II Diabetes Mellitus.
- Further studies conducted in more controlled conditions will definitely pave way for various advances in the diagnosis, follow-up, and management of Type II Diabetes Mellitus across the globe.

References

1. Hussain M, Babar MZM, Akhtar L, Hussain MS. Neutrophil lymphocyte ratio (NLR): A well assessment tool of glycemic control in Type-2 diabetic patients. Pakistan J Med Sci. 2017;33(6):1366–70.

2. Sefil F, Ulutas KT, Dokuyucu R, Sumbul AT, Yengil E, Yagiz AE, et al. Investigation of neutrophil lymphocyte ratio and blood glucose regulation in patients with type 2 diabetes mellitus. 2014;

3. Alberti KGMM, Zimmet PZ. Definition , Diagnosis and Classification of Diabetes Mellitus and its Complications Part 1 : Diagnosis and Classification of Diabetes Mellitus Provisional Report of a WHO Consultation. 1998;539–53.

4. Diagnosis and classification of diabetes mellitus. Diabetes Care [Internet]. 2014;37(SUPPL.1):81–90. Available from: https://care.diabetesjournals.org/content/27/suppl_1/s5

5. Report A, Consultation WHO. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. :1–25.

6. Atlas IDFD. Idf diabetes atlas. 2019.

7. R.M.Anjana RP. Prevalence of diabetes and prediabetes (impaired fasting glucose and / or impaired glucose tolerance) in urban and rural India : Phase I results of the Indian Council of Medical Research – INdia DIABetes (ICMR – INDIAB) study. Diabetologia. 2011;3022–7.

8. Hwang CK, Han P V, Zabetian A, Ali MK, Narayan KMV. Rural diabetes prevalence quintuples over twenty-five years in low- and middle-income countries : A systematic review and meta-analysis. Diabetes Res Clin Pract [Internet]. 2012;96(3):271–85. Available from: http://dx.doi.org/10.1016/j.diabres.2011.12.001

9. Fathallah, Neila, Raoudha Slim, Sofien Larif, Houssem Hmouda CBS. Drug-induced Hyperglycemia and Diabetes. Drug Saf. 2015;38:1153–68.

10. Of S, Carediabetes M. STANDARDS OF MEDICAL CARE Standards of Medical Care in Diabetes d 2016. 2016;39(January). Available from: https://care.diabetesjournals.org/content/suppl/2015/12/21/39.Supplement_1.DC2/2016-Standards-of-Care.pdf

11. Home P. Contributions of basal and post-prandial hyperglycaemia to micro- and macrovascular complications in people with type 2 diabetes Contributions of basal and post-prandial hyperglycaemia to micro- and macrovascular complications in people with type 2 diabet. Curr Med Res Opin. 2015;7995(October).

12. Little M, Humphries S, Patel K, Dodd W, Dewey C. Factors associated with glucose tolerance , pre - diabetes , and type 2 diabetes in a rural community of south India : a cross - sectional study. Diabetol Metab Syndr. 2016;1–11.

13. Shera AS, Jawad F, Maqsood A, Jamal S. Prevalence of Chronic Complications and Associated Factors in Type 2 Diabetes. J Pak Med Assoc. 2004;(54):54–9.

14. Agrawal RP, Ranka M, Beniwal R, Sharma S, K. Prevalence of micro and macro vascular complications in Type 3 Diabetes Mellitus and their risk factors. Vol. 24, Int. J. Diab.

Dev. Countries. 2004.

15. Venguidesvarane AG, Jasmine A, Varadarajan S, Shriraam V, Muthuthandavan AR, Durai V, et al. Prevalence of Vascular Complications Among Type 2 Diabetic Patients in a Rural Health Center in South India. 2020;

16. Gerstein HC. Dysglycaemia as a cause of cardiovascular outcomes. Nat Publ Gr [Internet]. 2015;1–2. Available from: http://dx.doi.org/10.1038/nrendo.2015.118

17. Emerging T, Factors R. Diabetes Mellitus, Fasting Glucose, and Risk of Cause-Specific Death. 2011;

18. Diabetes mellitus , fasting blood glucose concentration , and risk of vascular disease : a collaborative meta-analysis of 102 prospective studies. Lancet [Internet]. 2010;375(9733):2215–22. Available from: http://dx.doi.org/10.1016/S0140-6736(10)60484-9

19. Krauss RM. Lipids and Lipoproteins in Patients With Type 2 Diabetes. Diabetes Care. 2004;27(6):1496–500.

20. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. 2009;5(3):150–9.

21. Andersson C, Gaal L Van, Caterson ID, Weeke P. Relationship between HbA 1c levels and risk of cardiovascular adverse outcomes and all-cause mortality in overweight and obese cardiovascular high-risk women and men with type 2 diabetes. 2012;2348–55.

22. Low-Grade Chronic Inflammation in the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) Population Associations with insulin resistance and cardiometabolic risk profile. 2009;32(7).

23. Akbas EM, Timuroglu A, Ozcicek A, Ozcicek F, Demirtas L, Gungor A. Association of uric acid , atherogenic index of plasma and albuminuria in diabetes mellitus. 2014;7(12):5737–43.

24. Buyukkaya E, Karakas MF, Karakas E, Akc AB. Correlation of Neutrophil to Lymphocyte Ratio With the Presence and Severity of Metabolic Syndrome. 2014;

25. Lewis MR, Tracy RP. The Role of the Immune System in the Insulin Resistance Syndrome. 2002;

26. Ferna M. Insulin Resistance and Chronic Cardiovascular Inflammatory Syndrome. Endocr Rev. 2015;24(July):278–301.

27. Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. JCI insight. 2017;2(14):1–13.

28. Lim AKH. Diabetic nephropathy – Complications and treatment. Int J Nephrol Renovasc Dis. 2014;7:361–81.

29. Thomas MC. Anemia in diabetes : marker or mediator of microvascular disease ? 2007;3(1):20–30.

30. Stevens PE, Donoghue DJO, Lameire NR. Anaemia in patients with diabetes : unrecognised , undetected and untreated ? 2003;19(5):395–401.

31. Yu Z, Zhang J, Li X, Wang Y, Fu Y, Gao X. Jo ur na l P re of. Life Sci [Internet]. 2019;117138. Available from: https://doi.org/10.1016/j.lfs.2019.117138

32. Bansal V, Kalita J, Misra UK. Diabetic neuropathy. Postgrad Med J. 2006;82(964):95–

100.

33. T.H.J H. High-performance chromatography as a method to identify hemoglobin abnormalities. Acta Hematol. 1987;78:123–6.

34. Peterson KP, Pavlovich JG, Goldstein D, Little R, England J, Peterson CM. What is hemoglobin Alc? An analysis of glycated hemoglobins by electrospray ionization mass spectrometry. Clin Chem. 1998;44(9):1951–8.

35. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and prospective observational study. 2000;(Ukpds 35):405–12.

36. Eckerbom S, Bergqvist Y, Jeppsson JO. Improved method for analysis of glycated haemoglobin by ion exchange chromatography. Ann Clin Biochem. 1994;31(4):355–60.

37. D Groche, W. Hoeno, G Hoss, B Vogt, Z Hermann AV. Standardization of two immunological HbA1c routine assays according to the new IFCC reference method. Clin Lab. 2003;49(11):657–61.

38. Shaivya Gupta UJ. Laboratory Diagnosis of HbA1c : A Review. 2017;5(4):1–10.

39. Lois Jovanovic HS. Frequent Monitoring of A1C During Pregnancy as a Treatment Tool To Guide Therapy. 2011;34(1):0–1.

40. Kenneth Kaushansky, Marshall A Lichtman, Josef T Prchal, Marcel M Levi, Oliver W Press, Linda J Burns MAC. Williams Hematology. 9th ed. McGraw Hill; 2016. 925–935 p.

41. Cheekurthy AJ. Bioanalysis & Biomedicine Biochemical Biomarkers-Independent Predictors of Type 2 Diabetes Mellitus. 2018;(April 2015):6–11.

42. Imtiaz F, Shafique K, Mirza SS, Ayoob Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. 2012;1–6.

43. Atak B. Diabetes control could through platelet-to-lymphocyte ratio in hemograms. 2019;65(1):38–42.

44. Qin B, Ma N, Tang Q, Wei T, Yang M, Fu H. Neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) were Useful Markers in Assessment of Inflammatory Response and Disease Activity in SLE patients. 2015;7595(September).

45. Lee G, Lee L, Chong E, Lee C, Teo S, Chia B. The long-term predictive value of the neutrophil-to- lymphocyte ratio in Type 2 diabetic patients presenting with acute myocardial infarction. 2012;(July):1075–82.

46. Garcia C, Feve B, Ferré P, Halimi S, Baizri H, Bordier L, et al. Diabetes and inflammation : Fundamental aspects and clinical implications. Diabetes Metab [Internet]. 2010;36(5):327–38. Available from: http://dx.doi.org/10.1016/j.diabet.2010.07.001

47. Turkmen K, Erdur FM, Ozcicek F, Ozcicek A, Akbas EM, Ozbicer A, et al. Platelet-to-lymphocyte ratio better predicts inflammation than neutrophil-to-lymphocyte ratio in end-stage renal disease patients. 2013;391–6.

48. Huang W, Huang J, Liu Q, Lin F, He Z, Zeng Z, et al. Neutrophil – lymphocyte ratio is a reliable predictive marker for early-stage diabetic nephropathy. 2015;229–33.

49. George RM, Inamadar AC, Janagond AB. Neutrophil-to-lymphocyte ratio : A biomarker for predicting systemic involvement in iga vasculitis Neutrophil - to - Lymphocyte Ratio : A Biomarker for Predicting Systemic Involvement in IgA Vasculitis. 2020;(June).

50. Bhat T, Bhat H, Raza M, Khoueiry G, Meghani M, Akhtar M. Neutrophil to lymphocyte ratio and cardiovascular diseases : a review. 2013;55–9.

51. Verdoia M, Schaffer A, Barbieri L, Aimaretti G, Marino P, Sinigaglia F. Impact of diabetes on neutrophil-to-lymphocyte ratio and its relationship to coronary artery disease. Diabetes Metab [Internet]. 2015;1–8. Available from: http://dx.doi.org/10.1016/j.diabet.2015.01.001

52. Christoforaki V, Zafeiriou Z, Daskalakis G, Siristatidis C. First trimester neutrophil to lymphocyte ratio (NLR) and pregnancy outcome. J Obstet Gynaecol (Lahore). 2019;0(0):1–6.

53. Vinay Kumar, Abul Abbas JA. Pathologic basis of disease. 10th editi. Elsevier Inc; 2021. 583–590 p.

54. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High White Blood Cell Count Is Associated With a Worsening of Insulin Sensitivity and Predicts the Development of Type 2 Diabetes. 2002;51(October 2001).

55. Kayo Ohshita, Kiminori Yamane, Miki Hanafusa, Hiroshi Mori, Kazuyo Mito, Massamichi Okubo, Hitoshi Hara NK. Elevated White Blood Cell Count in subjects with impaired glucose tolerance. Diabetes Care. 2004;27(2):491–6.

56. Peter C Tong, Ka-Fai Lee, Wing-Yee So, Margaret H. Ng, Wing-Bun CHan MK Lo.

White Blood Cell Count is Associated With Macro- and Microvscular Complications in CHinese Patients With Type 2 Diabetes. Diabetes Care. 2004;27:216–22.

57. Duman TT, Aktas G, Atak BM, Kocak MZ, Erkus E, Savli H. Neutrophil to lymphocyte ratio as an indicative of diabetic control level in type 2 diabetes mellitus. 2019;19(1):1602–6.

58. Mertoğlu C, Günay M. Neutrophil-Lymphocyte Ratio and Platelet-Lymphocyte Ratio as Useful Predictive Markers of Prediabetes and Diabetes Mellitus. Diabetes Metab Syndr Clin Res Rev [Internet]. 2016; Available from: http://dx.doi.org/10.1016/j.dsx.2016.12.021

59. Azab B, Chainani V, Shah N MJ. Neutrophil–Lymphocyte Ratio as a Predictor of Major Adverse Cardiac Events Among Diabetic Population: A 4-Year Follow-Up Study. Angiology. 2013;64(6):456–65.

60. Devamsh GN, Parvathi M, Madhumathi R, Raghavan L. Study of neutrophil lymphocyte ratio in patients with type 2 diabetes mellitus and its correlation with glycemic control. 2019;6(5):1637–41.

61. Lou M, Luo P, Tang R, Peng Y, Yu S, Huang W, et al. Relationship between neutrophil-lymphocyte ratio and insulin resistance in newly diagnosed type 2 diabetes mellitus patients. 2015;4–9.

62. Yilmaz H, Ucan B, Sayki M, Unsal I, Sahin M, Ozbek M, et al. Diabetes & Metabolic Syndrome : Clinical Research & Reviews Usefulness of the neutrophil-to-lymphocyte ratio to prediction of type 2 diabetes mellitus in morbid obesity. Diabetes Metab Syndr

Clin Res Rev [Internet]. 2014;2–7. Available from: http://dx.doi.org/10.1016/j.dsx.2014.04.009

63. Wang R, Zhang J, Li Y, Liu T, Yu K. Journal of Diabetes and Its Complications Neutrophil – Lymphocyte ratio is associated with arterial stiffness in diabetic retinopathy in type 2 diabetes. J Diabetes Complications [Internet]. 2015;29(2):245–9. Available from: http://dx.doi.org/10.1016/j.jdiacomp.2014.11.006

64. Shiny A, Bibin YS, Shanthirani CS. Association of Neutrophil-Lymphocyte Ratio with Glucose Intolerance : An Indicator of Systemic Inflammation in Patients with Type 2 Diabetes. 2014;16(8):524–30.

Annexure I

IEC/131/2019
22-11-2019

B.L.D.E. (DEEMED TO BE UNIVERSITY)

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The ethical committee of this college met on 13-11-2019 at 3-15 pm to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: A study on neutrophil-lymphocyte ratio as an assessment tool of glycemic control in type 2 diabetes mellitus patients

Name of PG student: Dr. Veena Varier, Department of Pathology

Name of Guide/Co-investigator: Dr. Prakash M. Patil, Associate Professor Department of Pathology

DR RAGHVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

Annexure II

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, _____ ,S/O D/O W/O _____,age ____years, ordinarily resident of _____do hereby state/declare that Dr. _____

of _____ Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases. Further Doctor informed me that he/she is conducting dissertation/research titled _____under the guidance of Dr _____requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative, and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future and, also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time, I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place

Annexure III

PROFORMA

NAME**:** OP/IP No.**:**

AGE**:**

SEX**:** D.O.A**:**

RELIGION**:** D.O.D**:**

OCCUPATION**:**

RESIDENCE**:**

Presenting Complaints :

Past history :

Personal history :

Family history :

Treatment history :

General physical examination:

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

VITALS: PR: RR:

BP: TEMPERATURE: WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system:

Respiratory system:

Per Abdomen:

Central nervous system:

Renal System:

Fundus examination:

Clinical Diagnosis:

INVESTIGATIONS

Parameters	*Group 1*	*Group 2*	*p-value*
Total WBC count(10^3/µl)			
Absolute Neutrophil count(10^3/µl)			
Absolute Lymphocyte count(10^3/µl)			
Absolute Eosinophil count(10^3/µl)			
Absolute Monocyte count(10^3/µl)			
Absolute Basophil count(10^3/µl)			
NLR			
Platelet(10^3/µl)			
PLR			
RBC(10^6/µl)			
Hb(gm%)			
HCT/PCV(%)			

Key to master chart

OP	Outpatient
IP	Inpatient
HbA_{1c}	Glycated Hemoglobin A_{1c}.
NLR	Neutrophil Lymphocyte Ratio
PLR	Platelet Lymphocyte Ratio
WBC	White Blood Cells
RBC	Red Blood Cells
ANC	Absolute Neutrophil Count
ALC	Absolute Lymphocyte Count
AEC	Absolute Eosinophil Count
AMC	Absolute Monocyte Count
ABC	Absolute Basophil Count
PCV	Packed cell volume
S.TG	Serum Triglycerides
S. Chl	Serum Cholestrol
S.HDL	Serum High density lipoprotein
S.LDL	Serum Low density lipoprotein
S.VLDL	Serum Very low density lipoprotein

Master Chart

9	8	7	6	5	4	3	2	1		Case no.
I-1735	O-20909	I-1609	I-1114	O-9056	O-7815	I-43875	I-371	I-85		IP/OPD
68	25	34	45	73	65	33	53	45		Age
M	M	M	M	M	F	M	M	M		Sex
5.7	6.3	6	5.9	5.9	6.3	6.3	5.9	6.4		HbA1C
9000	5560	4700	7050	11630	9390	7590	8930	10280		Total WBC
5526	3424.96	1851.8	4949.1	6024.34	5277.18	5738.04	5625.9	6168		ANC
2601	1590.16	1771.9	1276.05	4547.33	2957.85	1609.08	2598.63	3289.6		ALC
414	250.2	719.1	310.2	558.24	751.2	7.59	312.55	308.4		AEC
441	272.44	347.8	500.55	430.31	347.43	227.7	357.2	308.4		AMC
18	22.24	9.4	7.05	69.78	56.34	7.59	35.72	20.56	GROUP 1	ABC
2.124567	2.153846	1.045093	3.878453	1.324808	1.784127	3.566038	2.164948	1.875		NLR
420000	256000	310000	234000	335000	340000	490000	337000	400000		Platelet
161.4764	160.9901	174.9534	183.3784	73.6696	114.9484	304.5218	129.6837	121.5953		PLR
3.73	5.41	4.23	3.22	5.22	4.19	3.69	5.17	3.14		RBC
9.4	16.5	11	9.4	14.7	11.4	8	16.5	7.6		Hb
30.9	47.3	33.4	28.4	44.3	36.7	25.9	47.6	22.5		PCV
	144						209			S. TG
	162						144			S. Chl Total
	29						50			S. HDL
	104						52			S.LDL
	29						42			S.VLDL

20	19	18	17	16	15	14	13	12	11	10
I-2328	I-2578	I-2585	O-31196	O-30993	O-29151	I-2365	I-2310	I-2227	I-2217	O-21695
52	70	40	70	40	47	60	25	35	78	52
M	F	F	F	M	F	M	F	F	M	M
6.4	6.1	5.8	6.1	6.1	5.9	6.1	5.7	6	6	6.2
6040	7430	3300	11010	8210	10520	7910	11750	10280	10620	9210
3636.08	5550.21	2148.3	6826.2	4187.1	6859.04	4746	7050	6168	6722.46	5986.5
1739.52	1500.86	920.7	3303	3226.53	2345.96	2373	4112.5	3048	2909.88	3131.4
241.6	126.31	9.9	440.4	287.35	915.24	395.5	352.5	514	127.44	92.1
362.4	222.9	211.2	330.33	500.81	326.12	237.3	117.5	514	796.5	92.1
60.4	29.72	9.9	110.1	8.21	73.64	158.2	117.5	205.6	63.72	0
2.090278	3.69802	2.333333	2.066667	1.29771	2.923767	2	1.714286	2.023622	2.310219	1.911765
301000	277000	343000	477000	298000	568000	274000	277000	308000	544000	289000
173.0362	184.5609	372.5426	144.4142	92.35928	242.1184	115.4657	67.35562	101.0499	186.9493	92.29099
3.9	4.56	3.68	4.17	5.65	4.34	3.03	4.1	3.27	3.42	5.92
9.4	13.2	7.8	9.3	13.8	9.8	9.8	10.7	9.3	10.6	15.6
30.7	40.6	24.3	29.1	42.6	30.8	27.6	31.5	29	30.7	45.3
				107	100					225
				137	194					217
				44	41					34
				72	133					138
				21	20					45

31	30	29	28	27	26	25	24	23	22	21
O-66637	I-4892	O-50634	I-3888	I-4084	O-45852	I-3727	O-41027	I-3317	I-3164	O-39213
37	55	50	35	66	40	58	41	33	32	35
F	F	F	M	M	M	M	M	F	M	F
5.7	6.4	6.3	6.4	6.3	5.8	6	6	5.7	5.8	5.8
6060	8400	7440	5830	5930	13820	7060	11270	11660	8520	9230
3363.3	5090.4	4434.24	4180.11	3807.06	10655.22	4236	5894.21	5247	5810.64	4864.21
2327.04	2839.2	2477.52	1317.58	1470.64	2694.9	2118	4541.81	4081	2070.36	3821.22
121.2	67.2	111.6	52.47	302.43	96.74	353	247.94	932.8	93.72	203.06
230.28	386.4	386.88	268.18	290.57	317.86	211.8	495.88	1166	502.68	323.05
18.18	16.8	29.76	11.66	59.3	55.28	141.2	90.16	233.2	42.6	18.46
1.445313	1.792899	1.78979	3.172566	2.58871	3.953846	2	1.297767	1.285714	2.806584	1.272947
307000	278000	208000	223000	289000	266000	321000	465000	506000	338000	249000
131.9273	97.91491	83.95492	169.2497	196.5131	98.70496	151.5581	102.3821	123.9892	163.2566	65.16244
3.98	5.3	3.29	4.25	4.26	5.12	4.61	5.52	4.29	5.32	5.11
10.4	12	9	9.5	10	13.8	15.5	16.2	11.4	14.6	13.4
31.4	36.9	27.4	28.6	30.8	40.2	42.7	46.4	34.3	43.8	40.6
162	119			123	178	101				
159	274			135	159	270				
43	45			33	29	41				
84	205			77	95	209				
32	24			25	36	20				

42	41	40	39	38	37	36	35	34	33	32
O-91709	O-89844	O-81340	O-81235	I-6872	O-76641	I-6403	I-6351	I-6202	I-6087	O-70619
30	58	58	60	30	54	45	77	54	38	52
F	M	F	F	M	F	M	M	M	F	M
6	6.3	6.2	5.7	6	5.7	6.1	6.2	6.2	6.1	6.4
7620	12550	8750	11600	8900	5300	10290	9150	11450	11780	9680
4815.84	8195.15	4305	7145.6	5793.9	3227.7	5361.09	5883.45	6641	7068	6776
2476.5	3288.1	3771.25	3538	2500.9	1558.2	2202.06	2415.6	3435	3534	2226.4
137.16	514.55	148.75	324.8	62.3	280.9	2315.25	228.75	572.5	589	242
182.88	514.55	498.75	545.2	489.5	212	380.73	549	458	353.4	406.56
7.62	37.65	26.25	46.4	53.4	21.2	30.87	73.2	229	235.6	29.04
1.944615	2.492366	1.141531	2.019672	2.316726	2.071429	2.434579	2.435606	1.933333	2	3.043478
261000	270000	383000	359000	329000	267000	325000	214000	172000	246000	293000
105.3907	82.11429	101.5578	101.4698	131.5526	171.3516	147.5891	88.59083	50.07278	69.60951	131.6026
4.35	5.12	4.68	4.89	4.54	4.16	4.56	5.06	5.23	4.24	5.77
11.3	14.2	12.3	13.7	14.1	11.6	13.4	16.5	15.5	12.1	15.4
32	41.6	37.2	41.3	40.7	34.4	40.5	49.3	43.8	37.4	43.1
										277
										211
										33
										122
										55

53	52	51	50	49	48	47	46	45	44	43
I-10426	O-116965	O-107431	O-115241	O-113587	O-108775	O-108756	O-109270	O-108934	O-95674	I-8094
25	38	27	40	41	75	65	47	49	45	67
F	M	M	M	M	M	M	M	M	M	F
6.3	5.7	6	6	6	5.7	5.8	6.3	5.9	5.9	6.4
10970	6700	8560	10320	7520	13250	6180	8070	6710	7520	5520
6450.36	3061.9	3946.16	5758.56	4421.76	6890	4017	4656.39	4052.84	3978.08	3797.76
3674.95	3001.6	3424	3890.64	2519.2	5644.5	1854	2929.41	1919.06	2917.76	1247.52
296.19	341.7	915.92	268.32	203.04	185.5	61.8	137.19	322.08	278.24	182.16
493.65	268	256.8	371.52	338.4	490.25	247.2	306.66	369.05	308.32	281.52
54.85	26.8	17.12	30.96	37.6	39.75	0	40.35	46.97	37.6	11.04
1.755224	1.020089	1.1525	1.480106	1.755224	1.220657	2.166667	1.589532	2.111888	1.363402	3.044248
170000	254000	263001	263000	301000	221000	232000	211000	284000	277000	270000
46.25913	84.62154	76.81104	67.59813	119.4824	39.15316	125.1348	72.02816	147.9891	94.93584	216.4294
5.64	5.78	5.72	5.04	5.62	5.67	3.95	4.6	5.49	5.47	3.46
10.4	16	14.2	13.8	16.3	16	13.9	13.7	11.6	17	9.2
32.9	46	42.8	39.8	45.3	47.1	38.1	38.7	38.3	48.7	27.7
	495		183	261	152	100	95	137		
	263		164	276	228	164	132	183		
	40		32	33	33	31	25	33		
	124		95	190	165	113	88	122		
	99		37	52	30	20	19	27		

64	63	62	61	60	59	58	57	56	55	54
I-21826	I-20690	I-19372	I-3183	I-1325	O-19830	I-19157	I-12351	I-11323	O-121644	I-10497
59	65	84	55	60	46	60	36	43	63	60
M	M	F	F	M	M	F	M	M	F	M
6.3	6.1	6.3	6.3	5.7	6.1	6.4	5.9	6.1	6	6.2
8810	8820	5850	10090	13320	8020	9540	10800	5960	4200	11810
5550.3	5733	3510	6659.4	7992	5213	5609.52	6804	2914.44	2503.2	7440.3
2466.8	2822.4	2047.5	2926.1	4662	2406	3195.9	3024	2080.04	1478.4	3543
176.2	176.4	58.5	302.7	399.6	240.6	257.58	324	238.4	42	472.4
616.7	88.2	175.5	201.8	133.2	80.2	429.3	216	679.44	168	236.2
0	0	58.5	0	133.2	80.2	47.7	324	47.68	8.4	118.1
2.25	2.03125	1.714286	2.275862	1.714286	2.166667	1.755224	2.25	1.401146	1.693182	2.1
367000	230000	360000	325000	147000	290000	251000	116000	176000	190000	299000
148.7757	81.49093	175.8242	111.0693	31.53153	120.532	78.53813	38.35979	84.61376	128.5173	84.39176
4.13	4.48	3.99	3.44	5.83	3.36	4.3	3.02	3.31	3.46	4.37
10.2	9.7	11.7	8.5	11.5	15	13.8	8.9	10.6	5.4	14
34.9	31.2	35.2	25.8	36.2	45.8	41.5	27.2	29.4	18.9	39.6
114						79				
83						243				
12						50				
48						177				
23						16				

75	74	73	72	71	70	69	68	67	66	65
O-24738	I-24228	I-23651	I-21109	O-20134	I-23677	I-23340	I-22638	I-21055	I-21894	O-14029
50	55	48	60	48	70	60	38	25	67	54
M	M	M	M	M	F	M	M	M	M	F
6.1	6.2	6.1	5.8	6.4	5.8	6.2	6.4	6.1	6.3	6.1
6650	11450	9130	8550	10050	11290	12260	11440	5850	10330	4600
4189.5	7786	5368.44	4446	4592.85	7530.43	7208.88	7630.48	3802.5	6198	2704.8
1928.5	3206	3058.55	3642.3	4502.4	3195.07	4107.1	3237.52	1872	2995.7	1541
199.5	229	246.51	119.7	512.55	270.96	331.02	274.56	117	516.5	124.2
332.5	114.5	410.85	316.35	402	270.96	551.7	274.56	58.5	619.8	207
0	114.5	45.65	25.65	40.2	22.58	61.3	22.88	0	0	23
2.172414	2.428571	1.755224	1.220657	1.020089	2.35689	1.755224	2.35689	2.03125	2.068966	1.755224
290000	282000	219000	221000	180000	206000	303000	376000	225000	218000	200000
150.3759	87.96007	71.60256	60.67595	39.97868	64.47433	73.77468	116.1383	120.1923	72.77097	129.7859
4.66	5.86	4.63	5.67	4.24	4.42	5.54	5.3	3.96	4.79	5.63
13.7	17.8	13.9	16	10	12.4	14.6	12.7	11.2	14	10.5
39.7	52.5	40.5	47.1	31.3	39.2	43.7	41	40	41.1	36.3
199	90	192							124	198
227	177	135							142	218
40	31	45							40	39
147	128	52							77	139
40	18	38							25	40

86	85	84	83	82	81	80	79	78	77	76
O-26448	O-01376	O-26516	I-26085	I-25865	I-25727	I-24987	I-25258	I-23928	I-24542	I-24565
65	38	60	50	65	65	26	82	51	28	43
M	M	M	M	F	M	F	F	M	M	M
6.4	6.2	6	6	6.4	5.9	5.8	6	5.7	6	6
13620	9230	6310	12960	14350	10180	9580	8320	10250	8680	14350
9125.4	5722.6	4290.8	8164.8	9758	7879.32	5077.4	5549.44	6970	5381.6	9758
4086	2769	1262	4276.8	4018	1822.22	3353	2354.56	2870	2604	4018
272.4	369.2	63.1	259.2	430.5	101.8	670.6	199.68	307.5	347.2	430.5
136.2	276.9	567.9	129.6	143.5	356.3	383.2	199.68	102.5	260.4	143.5
0	92.3	0	129.6	0	20.36	95.8	16.64	0	86.8	0
2.233333	2.066667	3.4	1.909091	2.428571	4.324022	1.514286	2.35689	2.428571	2.066667	2.428571
223000	182000	260000	357000	300000	251000	268000	298000	198000	182000	198000
54.5766	65.7277	206.0222	83.47363	74.66401	137.7441	79.92842	126.5629	68.98955	69.89247	49.27825
3.65	5.39	5.43	4.88	3.84	4.31	4.15	3.97	2.11	5.39	2.11
9.6	16.3	15.7	13.6	10.1	13.6	9.9	10.6	12.3	16.3	8
28.4	46.8	46.7	42.8	31.9	40.2	31.6	32.7	36.9	46.8	22.9
			109							
			179							
			52							
			105							
			22							

97	96	95	94	93	92	91	90	89	88	87
I-28668	I-28245	O-28602	I-28001	I-25460	O-27663	I-27028	O-26775	I-26372	I-25336	I-26403
50	50	50	47	36	51	54	55	80	30	50
M	F	F	F	M	M	M	M	M	F	M
5.9	6.4	6.4	6.1	6.1	5.7	6.2	6	6.1	5.9	6.2
12050	6650	7860	12330	7250	7860	12430	8550	3230	9450	9300
7712	4189.5	5344.8	8150.13	4785	5344.8	7955.2	5814	1485.8	6303.15	5859
3976.5	1928.5	2200.8	3464.73	2030	2200.8	3729	2394	1356.6	2674.35	3255
241	199.5	157.2	197.28	290	157.2	372.9	256.5	193.8	226.8	186
120.5	332.5	78.6	394.56	145	78.6	248.6	85.5	161.5	226.8	0
0	0	78.6	49.32	0	78.6	124.3	0	32.3	18.9	0
1.939394	2.172414	2.428571	2.352313	2.357143	2.428571	2.133333	2.428571	1.095238	2.35689	1.8
368000	290000	255000	363000	268000	255000	255000	198000	138000	298000	162000
92.54369	150.3759	115.867	104.7701	132.0197	115.867	68.38294	82.70677	101.7249	111.4289	49.76959
5.65	4.66	4.63	5.08	3.86	4.63	4.53	2.11	4.21	3.97	3.32
14.1	13.7	12.1	13.6	12.5	12.1	14.1	12.3	11.7	10.6	8.7
47.1	39.7	36.9	42.1	40.5	36.9	40.8	36.9	35.4	32.7	26.5
			76							
			220							
			46							
			159							
			15							

7	6	5	4	3	2	1	GROUP 2
I-514	I-452	O-3847	I-257	I-267	I-115	I-108	
45	51	50	45	80	65	53	
M	M	M	F	F	M	M	
11.2	8.1	10.6	9.3	13.3	14.2	7	
11390	4660	7700	8580	6480	11140	8200	
9556.21	3527.62	4620	7619.04	5417.28	8154.48	6814.2	
1287.07	671.04	1925	789.36	997.92	2272.56	992.2	
205.02	321.54	462	0	12.96	33.42	90.2	
318.92	130.48	462	171.6	38.88	646.12	270.6	
22.78	9.32	231	0	12.96	33.42	32.8	
7.424779	5.256944	2.4	9.652174	5.428571	3.588235	6.867769	
288000	286000	251000	182000	233000	85000	180000	
223.7641	426.2041	130.3896	230.5665	233.4857	37.40275	181.415	
6.34	5.04	5.31	4.02	5.7	4.8	4.38	
16	9.4	14.3	11.8	15.7	13.8	12.5	
48.7	32	42.4	32.2	45.3	40.9	37.2	
	64			388			
	139			167			
	32			41			
	95			108			
	13			17			

100	99	98
I-28397	O-28336	O-28741
82	74	71
F	M	F
6.3	5.9	6.1
8060	6120	5710
5424.38	3672	2906.39
2168.14	1836	2489.56
88.66	367.2	51.39
362.7	183.6	222.69
16.12	61.2	39.97
2.501859	2	1.167431
280000	150000	295000
129.143	81.69935	118.4948
4.59	3.2	4.74
12.6	10.1	13.8
38.6	30.5	41.6
	111	139
	102	180
	28	46
	52	106
	22	28

18	17	16	15	14	13	12	11	10	9	8
I-1391	I-1279	I-1234	I-1371	I-1132	O-14045	I-5408	I-605	O-7969	I-556	O-7290
55	66	55	50	41	75	68	45	62	50	66
F	M	M	F	M	M	F	M	M	M	M
7	10.1	10.6	6.9	15.3	14	7.6	10.1	9.1	7.5	10.5
12190	8500	13340	5230	10040	8550	9460	7720	7080	6430	7640
9069.36	7539.5	9818.24	4367.05	6807.12	5788.35	7000.4	4987.12	4467.48	4719.62	5661.24
2596.36	918	2734.7	611.91	2329.28	1957.95	1598.74	2323.72	1677.96	1157.4	1451.6
85.33	0	200.1	31.38	130.52	239.4	340.56	77.2	346.91	199.33	38.2
414.46	34	560.28	219.66	753	495.9	510.84	301.08	559.32	321.5	450.76
24.38	8.5	26.68	0	20.08	68.4	9.46	30.88	28.32	32.15	38.2
3.493106	8.212963	3.590244	7.136752	2.922414	2.956332	4.378698	2.146179	2.662447	4.077778	3.9
391000	245000	283000	70000	225000	372000	286000	374000	383000	203000	273000
150.5954	266.8845	103.4848	114.3959	96.59637	189.9946	178.8909	160.9488	228.2534	175.3931	188.0683
4.82	4.15	4.76	1.92	3.05	3.9	5.42	5.38	4	4.54	4.16
13.1	11.6	15.1	5.1	8	10.8	14.5	13.6	10.6	14.9	12.4
39	35.7	43.3	15.5	23.4	32.3	47	41.1	32.3	44.7	34.9
										144
										105
										30
										46
										29

29	28	27	26	25	24	23	22	21	20	19
O-23556	I-1669	I-1843	I-1922	I-1817	I-1545	I-1475	I-1311	I-1546	O-18091	O-10863
37	74	64	79	58	38	65	37	77	62	70
M	M	M	M	F	M	M	M	M	M	M
10.4	9.9	9.9	11	9.2	7.2	6.7	11.2	10.7	8.4	7.4
7280	6170	9500	7170	12710	7100	8860	6770	9700	6890	8220
4732	4004.33	7001.5	5090.7	9481.66	4260	6884.22	4732.23	5917	5139.94	4389.48
1820	1591.86	1463	1340.79	2681.81	2627	1497.34	1428.47	3298	1123.07	2252.28
364	172.76	114	186.42	114.39	71	26.58	230.18	194	158.47	1200.12
218.4	382.54	912	523.41	406.72	142	451.86	358.81	291	440.96	336.2
145.6	18.51	9.5	28.68	25.42	0	0	20.31	0	0	41.1
2.6	2.515504	4.785714	3.796791	3.535545	1.621622	4.597633	3.312796	1.794118	4.576687	1.948905
251000	300000	120000	243000	238000	188000	47000	148000	261000	307000	176000
137.9121	188.4588	82.02324	181.2364	88.74603	71.56452	31.389	103.6074	79.13887	273.3578	78.14304
5.79	5.19	2.22	4.17	3.18	4.97	2.56	4.27	4.3	3.89	4.54
16.1	14.3	5.8	14.4	8.8	13.9	7.5	12.3	11.9	11	12.5
43.7	43.4	18.5	38.8	26.2	41.1	21.7	35	35.2	34	36.2
525	182							354	196	
222	160							157	79	
30	26							22	13	
90	98							65	26	
105	36							71	39	

40	39	38	37	36	35	34	33	32	31	30
I-2324	I-2329	I-2372	O-28337	O-23777	O-27268	I-2206	O-24131	I-192	I-1698	I-550
50	40	68	65	64	62	60	46	42	55	18
F	F	M	M	F	M	M	M	F	F	M
9	7.2	8	8.6	7.3	11.9	9.9	8.9	10.9	6.7	7.3
12850	4480	8830	5960	8130	6440	11040	9420	13820	13530	12080
7350.2	2746.24	5615.88	3826.32	5406.45	4546.64	6900	5670.84	7849.76	11243.43	8770.08
4561.75	1191.68	2242.82	1722.44	2081.28	1416.8	2848.32	3202.8	4215.1	1461.24	2343.52
424.05	112	273.73	107.28	243.9	128.8	794.88	141.3	1202.34	392.37	24.16
462.6	421.12	679.91	292.04	357.72	309.12	474.72	386.22	525.16	419.43	930.16
51.4	8.96	17.66	11.92	40.65	38.64	22.08	18.84	27.64	13.53	12.08
1.611268	2.304511	2.503937	2.221453	2.597656	3.209091	2.422481	1.770588	1.862295	7.694444	3.742268
327000	282000	232000	227000	306000	370000	269000	273000	446000	385000	155000
71.68302	236.6407	103.4412	131.7898	147.0249	261.1519	94.44164	85.23792	105.8101	263.4749	66.13982
5.54	2.56	4.46	4.44	4.93	4.74	5.02	4.17	4.96	5.04	2.22
12.5	8.6	13.5	13.4	12.2	14	14	10.4	12	13.5	5
40	23.7	40.8	40.2	38.4	40.4	41.5	32.7	38.5	40.2	16.7
				192	174				137	
				119	206				180	
				52	32				39	
				29	139				114	
				38	35				27	

51	50	49	48	47	46	45	44	43	42	41
I-2793	O-32901	O-32953	O-32511	I-2602	O-32300	I-2517	I-2676	O-29236	O-20059	O-29174
57	60	65	58	65	56	68	59	55	65	52
F	F	F	F	M	F	F	M	M	M	M
13.8	9.1	6.6	7.7	13.4	7.4	8.2	10.5	9.6	7.4	6.5
14670	9590	6690	4660	8040	10760	9350	12740	9290	6700	8270
9887.58	5964.98	3746.4	2959.1	5491.32	8575.72	7180.8	10204.74	6372.94	5004.9	4515.42
4063.59	2483.81	2461.92	1351.4	2042.16	1689.32	1299.65	1490.58	2471.14	1259.6	2902.77
205.38	604.17	160.56	130.48	160.8	139.88	46.75	445.9	83.61	100.5	421.77
484.11	479.5	301.05	209.7	337.68	33.56	804.1	573.3	353.02	328.3	339.07
29.34	57.54	20.07	9.32	8.04	21.52	18.7	25.48	9.29	6.7	90.97
2.433213	2.401544	1.521739	2.189655	2.688976	5.076433	5.52518	6.846154	2.578947	3.973404	1.555556
367000	306000	345000	214000	177000	236000	261000	244000	235000	285000	323000
90.31423	123.1978	140.1345	158.3543	86.67293	139.7012	200.8233	163.6947	95.09781	226.2623	111.273
4.35	4.44	4.54	4.34	4.27	4.39	4.16	4.49	4.65	5.25	4.67
11.2	12.2	11.4	12.7	13.1	10.8	10.4	13	15.1	14.2	14.1
34.5	35.4	34.8	37.3	36.8	32.7	31.7	37.9	41.6	42.6	40.6
		160	159		100		115			235
		215	175		113		78			231
		68	34		20		27			37
		115	108		73		28			147
		32	32		20		23			47

62	61	60	59	58	57	56	55	54	53	52
I-3634	O-43550	O-40323	O-39887	I-3252	I-3192	I-3117	O-37177	I-2799	I-2651	O-32940
74	35	50	35	23	28	56	47	42	50	42
M	M	M	M	M	F	M	F	F	F	M
7.7	12.4	8	13.5	15	15.1	9.3	6.6	10.8	10.9	8.4
11630	10940	8260	6140	10850	10190	8690	9720	11180	10110	12660
7292.01	7865.86	4452.14	3487.52	8300.25	7693.45	5978.72	6036.12	8496.8	6895.02	8140.38
3489	2724.06	2271.5	1940.24	1627.5	1783.25	2042.15	2945.16	1978.86	2770.14	3823.32
232.6	54.7	1098.58	392.96	130.2	234.37	252.01	379.08	268.32	11.21	88.62
593.13	273.5	388.22	288.58	770.35	448.36	399.74	311.04	424.84	313.41	582.36
23.26	21.88	49.56	30.7	21.7	30.57	17.38	48.6	11.18	20.22	25.32
2.09	2.88755	1.96	1.797468	5.1	4.314286	2.92766	2.049505	4.293785	2.489051	2.129139
113000	340000	273000	227000	360000	302000	176000	494000	281000	375000	251000
32.3875	124.8137	120.1849	116.9958	221.1982	169.3537	86.18368	167.7328	142.001	135.3722	65.64975
4.48	5.3	6.09	5.94	4.93	5.35	4.66	4.07	3.68	4.16	5.73
11.6	15.7	13.7	17.6	12.3	11	13.4	9.5	9.3	11	16
35.1	44.7	42.9	49.6	37.5	36.3	38.2	29.6	28.6	33.9	45.5
195			380				112			
107			184				196			
26			32				36			
39			76				137			
43			76				22			

73	72	71	70	69	68	67	66	65	64	63
I-10470	I-10417	I-10356	I-10318	I-10363	I-10258	I-10240	I-10156	O-61091	I-3192	I-3615
66	62	57	54	60	60	57	33	65	28	45
F	F	M	M	M	M	F	M	F	F	F
8.2	14.1	11.7	10.1	6.5	8.6	13.1	10.6	6.5	14.8	8.1
9220	10830	8690	5190	6920	11460	14040	12890	10110	10190	9010
6417.12	8349.93	7568.99	2719.56	5439.12	7861.56	11189.88	10028.42	7471.29	7693.45	5541.15
2406.42	1732.8	842.93	2138.28	913.44	2876.46	2344.68	1997.95	2022	1783.25	2730.03
64.54	43.32	17.38	171.27	69.2	206.28	84.24	425.37	232.53	234.37	333.37
285.82	682.29	243.32	140.13	477.48	481.32	393.12	373.81	353.85	448.36	378.42
46.1	21.66	17.38	20.76	20.76	34.38	28.08	64.45	30.33	30.57	27.03
2.666667	4.81875	8.979381	1.271845	5.954545	2.733068	4.772455	5.019355	3.695	4.314286	2.029703
343000	178000	183000	166000	334000	399000	222000	217000	284000	302000	277000
142.5354	102.7239	217.0999	77.63249	365.6507	138.7122	94.68243	108.6113	140.455	169.3537	101.4641
4.62	4.12	5.91	6.01	3.73	5	3.77	4.61	4.32	5.35	4.32
11.4	10.9	16.2	17.9	10.3	14.3	11.5	15.3	12.1	11	10.8
34.2	31.8	44.9	49.7	30.9	42.6	33.2	42.4	36.4	36.3	33.2
			306	58				172		
			144	149				259		
			28	51				50		
			55	86				175		
			61	12				34		

84	83	82	81	80	79	78	77	76	75	74
O-124003	I-11095	O-123519	I-11053	I-11036	I-10776	O-121089	O-121130	I-10618	O-120640	I-10502
40	55	56	42	50	40	48	60	65	42	40
F	M	F	M	M	M	F	M	M	F	F
8	8.5	6.9	7.3	14.1	6.9	7	10.1	9	9.1	14.3
7960	11080	8990	11390	8810	12510	6830	7260	6670	10160	11620
6368	9296.12	7120.08	9795.4	6924.66	10933.74	3415	5641.02	5482.74	7559.04	8563.94
1194	975.04	1438.4	1480.7	1312.69	1150.92	2868.6	1110.78	1007.17	1940.56	2463.44
238.8	354.56	152.83	0	96.91	75.06	307.35	43.56	0	416.56	46.48
79.6	409.96	242.73	113.9	458.12	337.77	218.56	442.86	160.08	223.52	522.9
79.6	44.32	35.96	0	17.62	12.51	20.49	21.78	20.01	20.32	23.24
5.333333	9.534091	4.95	6.615385	5.275168	9.5	1.190476	5.078431	5.443709	3.895288	3.476415
292000	186000	257000	121000	368000	155000	252000	237000	169000	320000	565000
244.5561	190.7614	178.6707	81.71811	280.3404	134.6749	87.84773	213.3636	167.7969	164.9009	229.3541
4.23	4.57	4.52	2.85	3.91	4.92	5.24	4.81	4.8	3.63	4.3
13.6	13.2	8.8	8.2	11.3	15.1	13.1	14.4	13.7	10.2	12.2
37.6	36.6	27.5	24.4	31.5	41.5	39.4	41.8	40.1	29.9	34.7
						332				
						205				
						30				
						108				
						66				

95	94	93	92	91	90	89	88	87	86	85
O-127958	I-11928	O-127124	I-11921	I-11816	I-11463	I-11467	O-125075	I-11333	I-11310	I-11142
45	50	42	61	30	65	60	52	47	22	23
F	M	F	M	F	F	M	M	M	F	F
6.9	9	8.6	8.1	7.3	7.9	7.7	9.8	7.3	9.5	6.9
10070	13100	6560	10500	9210	9570	13240	9580	5600	6230	13000
7552.5	11475.6	4618.24	7276.5	7331.16	7282.77	8036.68	4952.86	4435.2	5121.06	8983
2114.7	1296.9	1679.36	2908.5	1399.92	1464.21	3204.08	3774.52	896	940.73	3224
201.4	0	19.68	73.5	248.67	354.09	1244.56	421.52	95.2	0	182
201.4	327.5	229.6	315	221.04	430.65	662	411.94	151.2	149.52	585
0	0	13.12	52.5	9.21	38.28	92.68	19.16	22.4	18.69	26
3.571429	8.848485	2.75	2.501805	5.236842	4.973856	2.508264	1.312183	4.95	5.443709	2.78629
255000	410000	216000	361000	280000	203000	257000	328000	175000	232000	226000
120.5845	316.1385	128.6204	124.119	200.0114	138.6413	80.21023	86.89847	195.3125	246.617	70.09926
3.95	2.58	4.06	4.65	4.6	4.05	4.68	4.63	4.05	4.56	3.81
9.5	7.6	12.9	12.8	10.4	11.6	12.4	13.1	15	12.2	11
35.7	23.1	35.2	38.4	32	33.3	37.6	39	40.4	33.4	32.9
140				122			178			
148				174			183			
59				45			37			
61				105			110			
28				24			36			

5	4	3	2	1	
I-196	I-278	I-242	I-55	I-3	
34	48	60	44	30	
M	F	M	M	F	
5.2	5.3	4.7	4.4	4.5	
8720	6440	3840	6220	11170	
4534.4	3220	2726.4	4291.8	8209.95	
3627.52	2576	806.4	1399.5	2468.57	
139.52	322	0	118.18	44.68	
392.4	193.2	153.6	391.86	424.46	
26.16	128.8	0	18.66	22.34	
1.25	1.25	3.380952	3.066667	3.325792	
250000	308000	46000	311000	119000	
68.91761	119.5652	57.04365	222.2222	48.20604	
3.5	4.08	2.65	3.19	4.68	
10.5	12.9	7.7	10.5	13.2	
36.6	37.8	21.9	30.7	40.7	

GROUP 3

100	99	98	97	96
O-20496	O-23070	I-21806	O-22151	I-12215
50	67	35	60	36
M	M	F	M	F
9.9	8.2	10.3	9.5	13.1
6670	9570	5330	11440	11900
5482.74	7282.77	4210.7	7630.48	9079.7
1007.17	1464.21	799.5	3237.52	2558.5
0	354.09	106.6	274.56	0
160.08	430.65	213.2	274.56	261.8
20.01	38.28	0	22.88	0
5.443709	4.973856	5.266667	2.35689	3.548837
169000	203000	174000	376000	552000
167.7969	138.6413	217.636	116.1383	215.7514
4.8	4.05	5.19	5.3	3.96
13.7	11.6	12.6	12.7	9.8
40.1	33.3	36	41	31.2
332	249			
197	145			
27	33			
104	62			
66	50			

16	15	14	13	12	11	10	9	8	7	6
I-2747	O-31305	O-31386	O-28151	O-27590	I-2178	O-21302	I-1601	O-15396	I-636	I-596
30	65	23	61	29	30	42	88	25	28	60
M	M	F	M	M	F	M	F	F	F	M
5.6	5.4	5.4	5.3	4.6	5.6	5.1	5.3	4.6	5.2	5.1
13070	12840	12580	7880	5120	8700	6230	6500	11830	11620	6370
6535	6420	6290	3869.08	3056.64	5976.9	3893.75	3445	7098	5810	3185
5881.5	5778	5661	3081.08	1602.56	2148.9	1968.68	2795	3785.6	4648	2548
392.1	256.8	377.4	543.72	220.16	313.2	87.22	130	354.9	348.6	318.5
261.4	256.8	125.8	338.84	220.16	252.3	261.66	65	354.9	581	254.8
130.7	128.4	125.8	47.28	20.48	8.7	18.69	65	236.6	232.4	63.7
1.111111	1.111111	1.111111	1.255754	1.907348	2.781377	1.977848	1.232558	1.875	1.25	1.25
289000	319000	325000	205000	147000	332000	232000	156000	253000	229000	299000
49.13712	55.20942	57.41035	66.53511	91.72823	154.4976	117.8455	55.81395	66.83221	49.2685	117.3469
5.26	5.55	4.56	4.71	2.54	4.45	5.62	3.98	4.43	3.35	4.18
14.2	16.9	13	15.1	16.3	11.4	16.8	9.3	13.1	10	12.5
41	48.4	33	41.5	47.2	34.4	47.6	29.3	37.5	28.8	37.9
			136			326				
			187			128				
			43			26				
			118			36				
			27			65				

27	26	25	24	23	22	21	20	19	18	17
O-69091	I-5841	I-5204	O-59426	O-57174	O-51557	I-4213	I-3853	I-3545	O-42020	O-35462
45	18	12	35	41	65	32	60	45	46	67
M	F	F	M	F	F	M	M	M	M	F
5.2	5.4	5.3	5.6	5.2	5.2	4.8	5.3	4.8	5.5	5.4
7030	5160	9880	4370	8200	3010	7370	8630	4430	9940	7710
3072.11	2667.72	5266.04	1896.58	5313.6	1802.99	5033.71	5505.94	3101	5834.78	5127.15
2418.32	1991.76	3299.92	1931.54	2296	848.8	1842.5	2571.74	1138.51	2803.08	1811.85
1159.95	417.96	790.4	218.5	131.2	159.53	198.99	172.6	39.87	815.08	416.34
337.44	82.56	494	301.53	451	168.56	257.95	353.83	141.76	417.48	346.95
42.18	0	29.64	21.85	8.2	30.1	36.85	25.89	8.86	69.58	7.71
1.270349	1.339378	1.595808	0.9819	2.314286	2.124164	2.732	2.14094	2.723735	2.08156	2.829787
222000	235000	413000	249000	228000	83000	245000	217000	229000	276000	270000
91.79927	117.9861	125.1545	128.9127	99.30314	97.78511	132.9715	84.37867	201.1401	98.46312	149.019
5.68	3.25	3.94	5.37	3.75	4.07	5	4.52	3.58	4.44	4.37
16.5	8.1	10.9	12	11.6	12.8	15.4	16	13.6	16.1	12
46.6	25.1	32.4	37.9	32.8	38	43.6	42.1	37.4	43.6	36.2
119					172		187		170	
185					171		171		178	
36					44		33		35	
125					93		100.6		109	
24					34		37.4		34	

38	37	36	35	34	33	32	31	30	29	28
O-41173	O-94039	O-88652	I-7651	O-89740	I-6757	I-6751	I-6561	I-6095	O-70571	O-69041
35	44	40	69	37	60	75	32	26	45	45
M	M	F	F	M	F	F	M	F	M	M
5.1	5.6	5.2	5.1	5.2	4.5	5.5	5.6	5.2	5.4	5.4
11770	9830	10730	770	5240	4230	9050	10160	11670	7160	9660
5885	5406.5	5901.5	5216.76	3164.96	2000.79	6298.8	7396.48	8694.15	4482.16	4781.7
4708	3440.5	3755.5	1919.52	1257.6	1730.07	2398.25	2255.52	2345.67	1969	3788.42
588.5	393.2	643.8	193.5	628.8	139.59	36.2	81.28	256.74	350.84	647.22
588.5	393.2	321.9	387	178.16	351.09	307.7	406.4	338.43	307.88	415.38
0	196.6	107.3	23.22	10.48	8.46	9.05	20.32	35.01	50.12	77.28
1.25	1.571429	1.571429	2.717742	2.516667	1.156479	2.626415	3.279279	3.706468	2.276364	1.262188
435000	301000	448000	256000	206000	80000	181000	206000	293000	274000	352000
92.39592	87.48728	119.2917	133.3667	163.8041	46.2409	75.4717	91.33149	124.911	139.1569	92.91472
5.66	5.53	4.91	3.88	3.28	2.85	5.1	5.53	4.07	5.48	4.74
17.1	16.7	10.9	10.5	11.1	8.5	14.4	16.9	11.4	13.8	15.2
47.5	47.2	35	32.5	31.8	25.2	41.2	48.1	34.1	41.3	44.1
	182						252		308	157
	239						204		160	169
	39						38		29	41
	163						116		89	97
	36						50		62	31

49	48	47	46	45	44	43	42	41	40	39
I-9872	O-113347	I-9448	O-109797	O-108597	O-108913	O-108921	O-108929	O-105290	O-102030	I-8831
78	47	35	45	45	47	29	52	36	41	52
M	M	F	F	F	M	F	M	M	M	F
5	4.8	5.5	5.1	5.5	5.3	5.2	5.5	4.3	5.2	5.6
11430	7030	9350	9960	6710	6800	10190	6780	6890	6740	8600
6835.14	3515	4675	7021.8	3737.47	4073.2	5095	3532.38	3830.84	3370	5762
3794.76	2812	3740	2380.44	2449.15	2318.8	4076	2847.6	2390.83	3033	3182
594.36	351.5	467.5	129.48	288.53	149.6	713.3	88.14	303.16	202.2	86
194.31	281.2	280.5	398.4	208.01	231.2	305.7	271.2	358.28	134.8	430
11.43	70.3	187	29.88	26.84	27.2	0	40.68	6.89	0	0
1.801205	1.25	1.25	2.949791	1.526027	1.756598	1.25	1.240476	1.602305	1.111111	1.810811
70000	191000	259000	252000	260000	285000	322000	220000	289000	199000	488000
18.44649	67.92319	69.25134	105.8628	106.1593	122.9084	78.99902	77.25804	120.8785	65.61161	153.3627
1.38	5.81	4.15	4.99	4.08	4.67	5.3	5.24	4.73	5.19	4.74
4.4	18.7	10.4	15.1	12	15.2	15.5	15.4	15.4	16.5	12.6
11.7	52.6	32.2	41.6	34.3	43.3	42.7	42.8	39.8	45.9	35
					276	225	172	74		
					234	220	182	148		
					39	35	30	45		
					140	140	117	88		
					55	45	34	15		

60	59	58	57	56	55	54	53	52	51	50
I-11457	O-125840	O-125610	I-11376	I-10961	I-11067	O-121404	I-9740	I-10218	O-117043	O-114935
32	30	50	86	24	35	25	90	26	30	32
F	F	M	M	F	F	F	M	F	F	M
4.9	5	5.5	5.6	5.4	5.4	5.1	4.5	5	5.1	5.3
5070	8230	6580	8230	8440	9230	9820	6910	10770	9440	4730
3229.59	3826.95	4323.06	3538.9	4836.12	5639.53	4566.3	2971.3	4631.1	5314.72	2199.45
1510.86	3514.21	1960.84	3785.8	3156.56	3276.65	4193.14	3040.4	4846.5	3417.28	2019.71
141.96	419.73	78.96	411.5	126.6	18.46	500.82	483.7	646.2	283.2	241.23
187.59	436.19	197.4	411.5	320.72	286.13	520.46	276.4	538.5	396.48	250.69
0	32.92	19.74	82.3	0	9.23	39.28	138.2	107.7	28.32	18.92
2.137584	1.088993	2.204698	0.934783	1.532086	1.721127	1.088993	0.977273	0.955556	1.555249	1.088993
237000	378000	234000	218000	194000	266000	335000	230000	278000	371000	202000
156.8643	107.5633	119.3366	57.5836	61.45931	81.18047	79.8924	75.64794	57.36098	108.5659	100.0144
3.91	4.49	5.75			4.97	4.33	3.39	4.21	4.85	5.08
10.8	13.1	14.8			13.5	8.5	11.4	11.5	13.3	15.8
31	38.6	43.3			39.7	26.2	31.6	35.2	39.1	43.6
		115								215
		186								177
		33								26
		130								109
		23								43

71	70	69	68	67	66	65	64	63	62	61
O-23056	O-22585	I-15038	O-21602	I-21133	I-20089	I-19494	I-19399	I-12359	O-129815	I-12129
46	38	65	60	27	48	38	50	22	53	25
M	M	M	M	M	M	M	M	F	M	F
5.2	5.6	4.6	5.4	5.6	5.1	3.9	4.6	4.5	4.6	5.5
7300	6880	5820	6100	10660	11090	11310	12840	9840	9290	7400
4577.1	5022.4	3666.6	3843	6108.18	6166.04	4750.2	6856.56	4526.4	5165.24	3182
1949.1	1720	1920.6	1708	3986.84	4114.39	5089.5	5097.48	4231.2	3446.59	3404
481.8	68.8	116.4	244	159.9	365.97	678.6	359.52	492	306.57	370
270.1	68.8	116.4	244	405.08	377.06	565.5	487.92	393.6	315.86	370
21.9	0	0	61	0	66.54	226.2	38.52	98.4	55.74	74
2.348315	2.92	1.909091	2.25	1.532086	1.498652	0.933333	1.345088	1.069767	1.498652	0.934783
235000	360000	225000	156000	283000	228000	176000	518000	251000	308000	215000
120.5685	209.3023	117.1509	91.33489	70.98354	55.41526	34.581	101.6188	59.32123	89.36369	63.16099
4.2	5.19	3.95	4.65	5.43	4.49	2.79	2.25	3.71	4.48	1.96
13.5	15.7	13.4	13.9	14.8	14.4	10.1	8.6	10.9	15.7	4.5
39.7	46.5	36.1	39	46.8	43.5	27.3	24.9	30.7	44.6	14.5
	201			94						
	232			171						
	43			65						
	149			87						
	40			19						

82	81	80	79	78	77	76	75	74	73	72
O-24679	I-24460	I-24605	I-23352	I-23664	O-24118	O-20053	O-23228	O-23376	I-21914	I-22735
19	67	60	75	30	66	29	28	28	56	18
F	M	M	F	F	M	F	F	F	M	M
4.6	4.3	5.3	5.6	5.5	5.3	5.6	5.3	5	5.5	5.3
6940	11080	14250	5820	10550	6870	8320	9820	11390	8200	7880
4372.2	6648	7125	2444.4	6119	2954.1	5432.96	4566.3	4783.8	5248	4980.16
2359.6	3878	6127.5	2502.6	3692.5	3435	2337.92	4193.14	5125.5	2870	2537.36
138.8	332.4	570	349.2	422	274.8	116.48	500.82	683.4	82	78.8
69.4	221.6	285	465.6	211	206.1	399.36	520.46	569.5	0	260.04
0	0	142.5	58.2	105.5	0	33.28	39.28	227.8	0	23.64
1.852941	1.714286	1.162791	0.976744	1.657143	0.86	2.323843	1.088993	0.933333	1.828571	1.962733
271000	122000	248000	365000	284000	395000	394000	335000	329000	195000	233000
114.85	31.45952	40.47328	145.8483	76.91266	114.9927	168.5259	79.8924	64.18886	67.94425	91.82773
4.33	4.63	4.87	4.93	3.06	5.18	5.62	4.33	4.2	4.93	5.17
12.9	9	12.5	7.7	9.9	12.3	14.4	8.5	12.3	12.1	13.2
36.2	30.1	38.2	45.6	39	45.6	45.8	36.2	36.7	37.8	40.6
	38	70								
	55	158								
	21	31								
	26	113								
	8	14								

93	92	91	90	89	88	87	86	85	84	83
O-27677	I-27646	O-27306	O-27153	I-26462	I-26713	I-26736	O-25761	O-09670	I-25596	I-24625
42	27	50	27	87	43	40	70	75	92	55
F	F	M	F	F	M	M	M	M	M	M
5.6	4.8	5.6	5	5.4	5.4	5.6	5.1	5.1	5.5	5.4
9840	13440	6620	13830	11760	14090	9660	6870	10400	8110	11760
4526.4	7257.6	3773.4	6776.7	6115.2	5636	3767.4	2748	5408	4460.5	6115.2
4231.2	5376	2184.6	5532	5409.6	6058.7	5313	3778.5	3120	3244	5409.6
492	403.2	19.86	829.8	0	986.3	386.4	274.8	728	243.3	0
492	268.8	529.6	553.2	235.2	1268.1	193.2	68.7	936	162.2	235.2
98.4	0	13.24	138.3	0	140.9	0	0	208	0	0
1.069767	1.35	1.727273	1.225	1.130435	0.930233	0.709091	0.727273	1.733333	1.375	1.130435
251000	180000	243000	328000	351000	236000	272000	395000	296000	372000	351000
59.32123	33.48214	111.2332	59.2914	64.88465	38.95225	51.19518	104.5388	94.87179	114.6732	64.88465
3.71	2.11	4.98	3.44	4.85	4.81	4.86	5.18	5.08	3.31	4.85
10.9	5.2	15.7	10	13.3	15.6	15.3	12.3	15.4	8.2	13.3
30.7	36.3	45.5	30	40.3	43.3	44.6	45.6	46	36.9	40.3
					81					151
					138					266
					36					51
					86					185
					16					30

100	99	98	97	96	95	94
I-1004	I-28267	I-30404	I-30340	I-28339	O-28017	I-26726
26	70	42	25	76	51	44
F	F	M	F	M	M	M
5	5	5.5	5.5	5.5	4.8	5.2
3450	10870	7020	14180	7110	5220	8650
1380	4348	4141.8	10890.24	3981.6	2829.24	4238.5
1828.5	4891.5	2667.6	2467.32	2559.6	1738.26	3719.5
0	326.1	140.4	269.42	355.5	370.62	432.5
241.5	217.4	70.2	496.3	213.3	229.68	173
0	0	0	56.72	0	52.2	86.5
0.754717	0.888889	1.552632	4.413793	1.555556	1.627628	1.139535
240000	303000	259000	361000	206000	254000	248000
131.2551	61.94419	97.09102	146.3126	80.48133	146.1231	66.67563
4.91	4.29	6.37	3.98	3.17	4.86	4.87
12.7	13.2	16.6	10.6	11	14.9	12.5
38.7	37.8	50.3	32.2	30.7	43.8	38.2
	120			109	142	
	170			138	138	
	43			38	61	
	103			78	49	
	24			22	28	