"STUDY OF NEUTROPHIL LYMPHOCYTE RATIO AS A NOVEL MARKER OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETES MELLITUS"

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Endorsement

This is to certify that the dissertation entitled **"STUDY OF NEUTROPHIL LYMPHOCYTE RATIO AS A NOVEL MARKER OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETIC NEPHROPATHY"** is a bonafide research work done by **(REG. NO. BG0117011).**

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Sub: Acceptance Letter

Sir/Madam,

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LIST OF ABBREVIATIONS USED

WBC	-	WHITE BLOOD CELLS
WHO	-	WORLD HEALTH ORGANISATION
GDM	-	GESTATIONAL DIABETES MELLITUS
MODY	-	MATURITY ONSET DIABETES OF YOUNG
ATP	-	ADENOSINE TRIPHOSPHATE
HLA	-	HUMAN LEUKOCYTE ANTIGEN
DM	-	DIABETES MELLITUS
OGTT	-	ORAL GLUCOSE TOLERANCE TEST
IGT	-	IMPAIRED GLUCOSE TOLERANCE
IFG	-	IMPAIRED FASTING GLUCOSE
LADA	-	LATENT AUTOIMMUNE DIABETES IN ADULTS
BP	-	BLOOD PRESSURE
BMI	-	BODY MASS INDEX
CVD	-	CEREBRO VASCULAR DISEASE
AGE	-	ADVANCED GLYCOSATION END PRODUCTS
VEGF	-	VASCULAR ENDOTHELIUM GROWTH FACTOR
PAI	-	PLASMINOGEN ACTIVATOR INHIBITOR
DN	-	DIABETIC NEPHROPATHY
ESRD	-	END STAGE RENAL DISEASE
GFR	-	GLOMERULAR FILTRATION RATE
TGF	-	TUMOUR GROWTH FACTOR
GBM	-	GLOMERULAR BASEMENT MEMBRANE
IL	-	INTERLEUKINE

DCCT	-	DIABETES CONTROL AND COMPLICATION TRIAL
UKPDS	-	UNITED KINGDOM PROSPECTIVE DIABETES STUDY
NLR	-	NEUTROPHIL TO LYMPHOCYTE RATIO
ROS	-	REACTIVE OXYGEN SPECIES
PLR	-	PLATELET TO LYMPHOCYTE RATIO
eGFR	-	ESTIMATED GLOMERULAR FILTRATION RATE
CKD	-	CHRONIC KIDNEY DISEASE

ABSTRACT

BACKGROUND AND OBJECTIVES: Diabetic nephropathy is the leading cause of chronic kidney disease and ESRD leading to renal replacement therapy. Neutrophil to Lymphocyte ratio (N:L ratio) was found to be a useful inflammatory marker which predicts the adverse outcomes in many conditions . Diabetic nephropathy has an inflammatory pathology but not much information regarding the relationship between diabetic nephropathy and N:L ratio is available.

METHODS : 100 consecutive patients with diabetic nephropathy admitted in the wards of JN Medical College and Hospital between the time period of January 2018 – December 2018 were included in this cross sectional study according to the inclusion and exclusion criteria. Relevant history , clinical examination and investigations were recorded. All diabetic nephropathy patients were investigated for N:L ratio , proteinuria , creatinine , eGFR ,CKD stage and a correlation between N:L ratio and these variables were done. Statistical analysis was done using SPSS version 23.

RESULT : The mean age of patients was 59.6±11.4 years (mean±sd).56% of patients were in 50 to 70 year age group. 68% of subjects in the study were male whereas the remaining 32% were females. The mean NLR in this study was 10.8 (SD - 9.05).The mean NLR in males was 9.53±8.42 (mean±sd) whereas 11.54±10.2 (mean±sd) in females which was statistically not significant ($p>0.05$) . The mean eGFR was 16.5 ml/min (SD - 16.9 ml/min). eGFR range was from 2 – 85 ml/min ..The mean serum creatinine was 5.93 mg/dl with standard deviation of 3.66 mg/dl. . There were 5 , 3, 8, 16, and 68 diabetic nephropathy patients in CKD stage 2, 3A,3B, 4 and 5 respectively. Patients with proteinuria grade 1+ was 31 %, 2+ was 31% , 3+

was 36% and 4+ was 2%. In our study 28 patients were on hemodialysis support while 72 patients were not dependant on HD .NLR had statistically significant co-relation with higher CKD stages, lower EGFR, and higher levels of serum creatinine levels in diabetic nephropathy patients. There was no co-relation between NLR with grade of proteinuria, sex, and age.

CONCLUSION : From our study we conclude that NLR can be used a novel marker for diabetic nephropathy patients.

KEYWORDS: Diabetic nephropathy, Neutrophil lymphocyte ratio, Chronic kidney disease.

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INTRODUCTION

Diabetes mellitus is a condition in which there is a chronically raised blood glucose concentration. It is caused by an absolute or relative lack of the hormone insulin, i.e. insulin is not being produced from the pancreas or there is insufficient production of insulin or impaired insulin action for the body's needs.

The two main types of diabetes are Type 1 which presents mainly in childhood and early adult life and accounts for about 20% of cases in Europe and North America. It is thought to be caused by autoimmune destruction of insulin producing islet cells of pancreas.

Type 2 Diabetes usually starts in middle age or in the elderly. It is more common accounting for 80% of cases. It is due to either impaired insulin secretion or resistance to the action of insulin at its targets cells. Most of these patients are obese.

One of the most important clinical features of diabetes is its association with chronic tissue complications that occurs after several years of diabetes and affect both small blood vessel (microangiopathy) in the eye, kidney and nerves and large vessels[1].

The frequency of arterial disease (atherosclerosis or microangiopathy) is also markedly increased. Microangiopathy is thought to be related to the duration and severity of hyperglycemia.

Diabetic nephropathy is a serious complication of diabetes mellitus and it is considered as a major cause of kidney disease in working population[2].

Its pathogenesis is complicated and it is related to many factors, but many groups have described the role of inflammatory markers in the development of diabetic nephropathy.

The WBC and its sub types Neutrophil lymphocyte ratio, Monocyte lymphocyte ratio and platelet lymphocyte ratio are all novel markers of inflammation[3]. Till date, only few articles have studied the relationship between the occurrence of diabetic microvascular complication and the markers of inflammation.

AIMS AND OBJECTIVES

To study the Neutrophil : Lymphocyte ratio as a novel marker of Diabetic Nephropathy in type 2 Diabetes Mellitus.

REVIEW OF LITERATURE

DIABETES MELLLITUS[4]

"Diabetes mellitus is a metabolic disorder involving carbohydrate, protein and fat metabolism resulting from absolute or relative insulin deficiency and resulting in chronic hyperglycemia manifesting with its microvascular and macrovascular complications. In fact diabetes can have a long variable asymptomatic period of 5 years to 15 years and may be diagnosed for the first time because of its comorbidities or complications.

The long term effects of diabetes include damage, dysfunction and failure of various other organs which includes progressive development of retinopathy with potential blindness, nephropathy that may lead to renal failure, neuropathy with risk of foot ulcers, amputation, charcot joints and features of autonomic dysfunction including sexual dysfunction. People with diabetes are at increased risk of macrovascular complications like cardiovascular, peripheral vascular and cerebrovascular diseases.

EPIDEMIOLOGY

According to the data collected by World Health Organization (WHO) in 2016, it is estimated that around 422 million of adults are living with diabetes mellitus. It is mainly due to increase in risk factors for type 2 diabetes mellitus notably sedentary lifestyle, obesity and increased longevity.

TYPE 1 DIABETES

Type 1 diabetes may present at any age, but most typically presents in early life with peak incidence around the time of puberty. Its incidence varies from 50 -100 fold around the world, with the highest among the Northern Europe and in individuals of European extraction. Both the sexes are equally affected in childhood, but men are more commonly affected in early adult life. This distinction between type 1 and type 2 varies in later life and thus true life incidence is unknown. According to DIAMOND study, Finland has the maximum number of patients with Diabetes.

TYPE 2 DIABETES

The World Health Organization estimated that 9% of the population suffered from Diabetes in 2014, over 90% of them had type 2 Diabetes. Moreover type 2 diabetes caused 5 million deaths per year , mostly from cardiovascular disease, and type 2 diabetes is expected to become 7 th leading cause of death globally by 2030. Type 2 diabetes is strongly associated with obesity and as such the major burden is now in the middle income and developing countries where urbanization and recent affluence has rapidly changed the lifestyle.

The large population of the Western pacific contributed most to the absolute numbers, while the % prevalence is highest in the Middle East and North Africa. The larger number of diabetics is in the 40-59 age groups (132 million in 2010) which are expected to rise further. By 2030, there will be more diabetic population in the 60 to 79 age groups (196 million).

GESTATIONAL DIABETES

GDM is common in many populations including Asian Indians. Pregnant women should be tested for GDM at 24 weeks to 28 weeks of gestation. Gestational diabetes is a pre diabetic state with an increased risk of development of the disorder in subsequent pregnancies, in 60 to 90%. It is also known that women with GDM have a high risk up to 30% of developing diabetes within [7 10] years of index pregnancy.

PREVALENCE IN INDIA

The prevalence of diabetes in India in 1970s was 2.3% in urban and 1.5% in rural areas as shown by study conducted by ICMR. In 2000s the prevalence gradually increased. India which has a large pool of pre diabetics shows rapid conversion of these high risk subjects to diabetes. The Indian Diabetic Prevention Programme 1 (IDPP 1) has shown an annual incidence of approximately 18% among subjects with IGT.

National Studies or Population Based Studies on Diabetic complications are sparse in India. A few population based studies indicate that the prevalence of retinopathy to be 27% and overt nephropathy is 2.2%. Peripheral vascular disease is prevalent in 6.3%, peripheral neuropathy in 25% and coronary artery disease is seen in 21%.

The major contributory factors for the Prevalence of the complications are: delayed diagnosis of diabetes, inadequate control of diabetes, hypertension and lack of awareness about the disease among majority of the public.

WHO CRITERIA:

Criteria for Diabetes diagnosis: 4 options

- Fasting plasma glucose ≥ 126 mg/dl (7.0mmol/L)

  Fasting is defined as no caloric intake for ≥ 8 hours

- 2 hour post prandial glucose ≥ 200mg/dl (11.1mmol/L) during OGTT (75g) Using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.

- HbA1C ≥ 6.5% (48mmol/L)

- Random plasma glucose ≥ 200mg/dl (11.1 mmol/L) with symptoms of hyperglycemia.[26]

CLINICAL CLASSIFICATION

Classically, the clinical presentation of Diabetes was the basis of its classification into Insulin dependent and non insulin dependent diabetes. Insulin dependent diabetes term was used to refer an early onset diabetes (affecting children and adolescents and adults < 30 years of age) , affected patients are lean and thin presenting with classical osmotic symptoms (polyuria, polydipsia, weight loss) and are ketosis prone and requiring insulin i.e.,insulin was needed to prevent ketosis for their survival.

Similarly, non insulin dependent diabetes refers to adult onset diabetes (>40 years age) , in overweight / obese individuals who were not insulin requiring but were insulin resistant and could be controlled on diet, exercise and medications in combination or alone. However at the turn of the twenty first century this nomenclature was replaced and diabetes was classified into type 1 and type 2 diabetes , this indicated a paradigm shift in the basis of classification from a clinical basis to pathogenic basis.

PATHOGENIC CLASSIFICATION

Type 1 diabetes mellitus referring to immune destruction of pancreatic islets etiology and type 2 diabetes referring to non immune etiology were enunciated.

Diabetes can be classified into four clinical categories:

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

– Immune mediated

– Idiopathic.

2. **Type 2 diabetes** (due to a progressive insulin secretory defect on the background of insulin deficiency) .

3. **Other specific types of diabetes due to other causes** ;

Genetic defects in beta cell function

MODY 1- Mutation in Hepatocyte nuclear transcription factor

(HNF) 4 alpha.

MODY 2 Mutation in Glucokinase gene

MODY 3 Mutation in HNF 1 alpha

MODY 4 Mutation in Insulin promoter factor-1 gene

MODY 5 Mutation in HNF 1D gene

MODY 6

Mutation in neurogenic differentiation -1 transcription

factor

(Neuro D1/Beta 2)

Mitochondrial deoxyribonucleic acid

Subunits of ATP sensitive potassium channel

Mutations in Proinsulin or insulin

Genetic defects in insulin action

Type A insulin resistance

Leprechaunism

Rabson Mendelhall syndrome

Lipodystrophy syndromes

Diseases of the exocrine pancreas:

Cystic fibrosis,

chronic pancreatitis,

pancreatectomy,

hemochromatosis,

neoplasia, fibrocalculous pancreatopathy, mutations in carboxy esteryl lipase.

Endocrinopathies:

Acromegaly,

glucoganoma,

pheochromocytoma,

hyperthyroidism, somatostatinoma, aldosteronoma.

Drug or chemical induced :

Such as in the treatment of HIV / AIDS or after organ transplantation,viz., glucocorticoids,

pentamidine,

protease inhibitors,

nicotinic acid,

diazoxide, epinephrine, beta adrenergic agonists, thiazides, hydantoin,

asparaginase, antipsychotics, etc.,

Infections:

Congenital rubella, cytomegalovirus, coxsackie virus

Uncommon forms of immune mediated diabetes:

Stiff person syndrome, insulin receptor antibodies

Other ;

Genetic syndromes associated with diabetes:

Wolfram's syndrome, downs syndrome, klinefelters syndrome, turners syndrome, friedrich's ataxia, huntingtons chorea , Laurence moon biedl syndrome, myotonic dystrophy, porphyria, Prader willi syndrome.

4. **<u>Gestational diabetes mellitus.</u>**

ETIOLOGY AND RISK FACTORS:

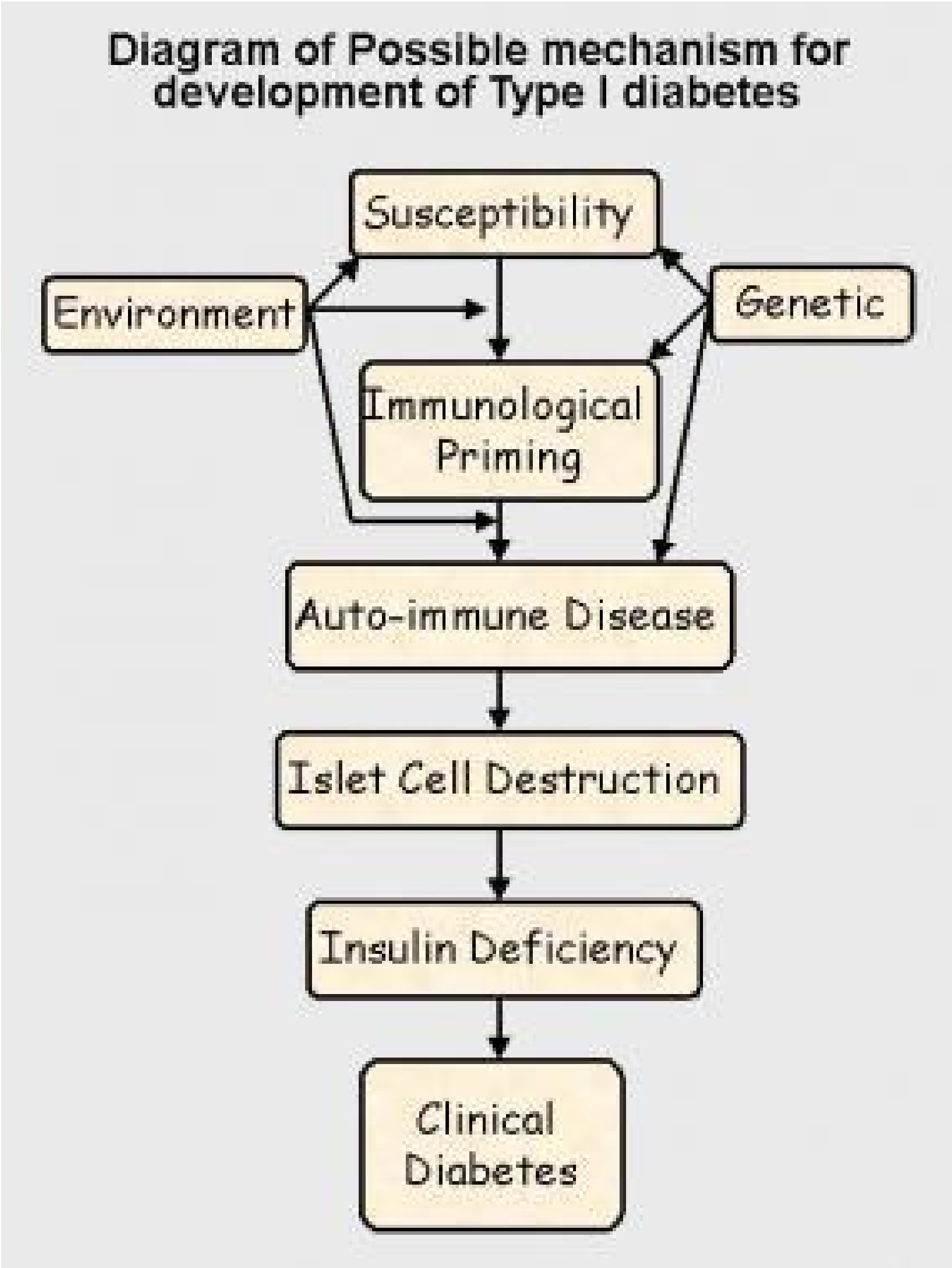
Diabetes both type 1 or type 2 has equally strong genetic and environmental risk factors an interaction of which leads to clinical expression of the disease. The genetic susceptibility for type 1 is associated with certain Human leucocyte antigen (HLA) combinations DR3, DR4 and the environmental insults are rather ill defined. Possibility of some aspects of diet and viral infections triggering an autoimmune exposure causing specific destruction of beta cells of pancreas has been proposed.

Type 2 Diabetes mellitus has a more complex etiopathology. Though it has strong genetic basis as shown by its hereditary nature, the major susceptibility genes have not yet been identified. Racial predisposition as seen in Asian population is also common. The environmental factors showing strong association with diabetes are increasing age, family history of diabetes, obesity, unhealthy diet, physical inactivity, insulin resistance, adverse intrauterine, environmental and stress factors.

PATHOPHYSIOLOGY

In Type 1 diabetes there is autoimmune destruction of pancreatic islet cells following interaction between environmental and genetic factors. The major antibodies detected are insulin autoantibody, glutamic acid decarboxylase autoantibody, insulinoma associated autoantigen 2 autoantibody and zinc transporter 8 autoantibody. Indeed, immunological studies revealed the essential involvement of the adaptive immune system in the pathogenesis of T1DM. It is found that CD8+ T cells are the most predominant in the insulitis lesion, followed by CD68+ macrophages, CD4+ T cells, CD20+ B lymphocytes and CD138+ plasma cells.

Figure 1. Pathophysiology of Type 1 DM

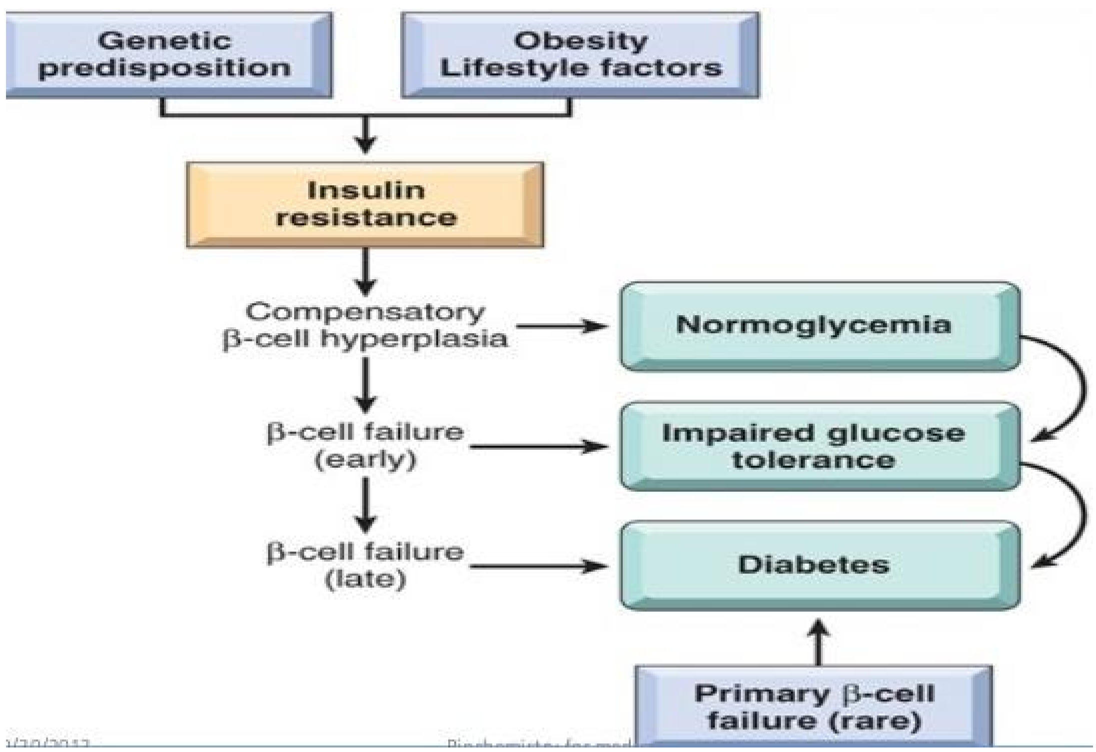


The pathophysiology of type 2 diabetes includes impaired insulin secretion, impaired insulin action, insulin resistance and impaired incretin effect on the beta cell function and non suppression of the action of alpha cells with rising blood glucose levels. In the last two decades the role of adipokines as regulators of beta cell function and insulin sensitivity has been demonstrated in number of studies.

Type 2 Diabetes is a life style disorder and an interaction of genetic and environmental factors precipitates the metabolic abnormalities existing in prediabetic subjects to the clinical stage of diabetes. There is a long asymptomatic pre-diabetic stage before the development Of diabetes. These stages are easily identifiable by OGTT.

For the development of diabetes both the basic pathophysiological defects ,i.e., insulin resistance and beta cell secretory defect have to coexist .

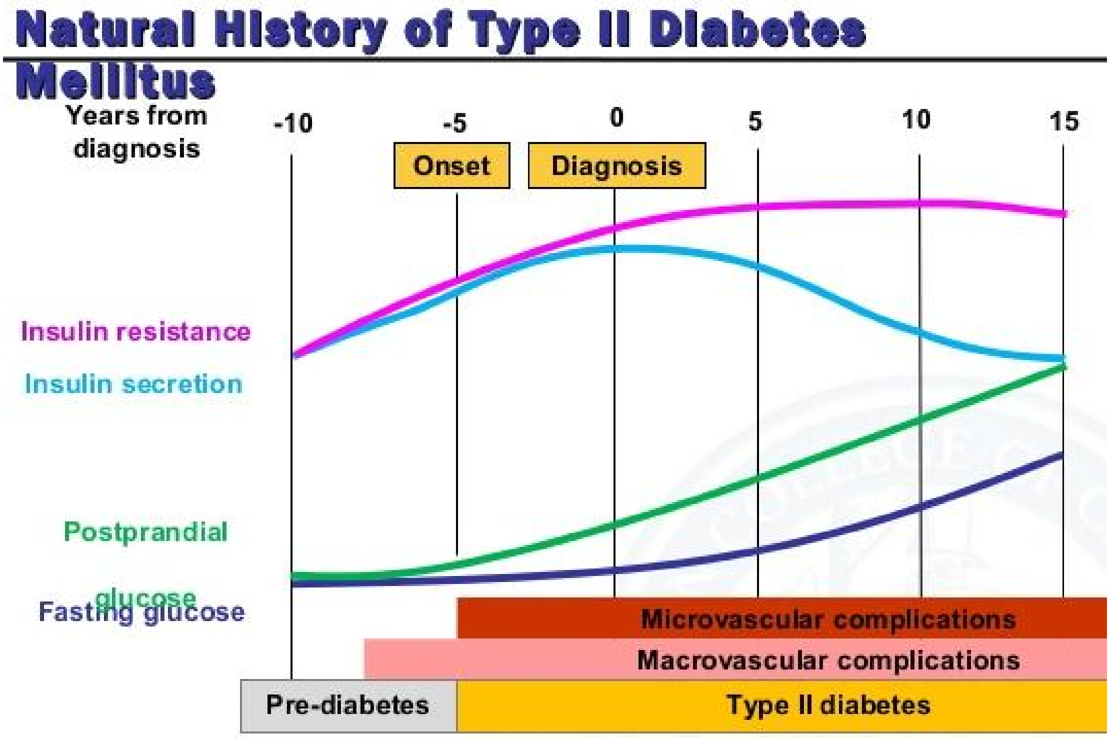
Figure 2: Pathophysiology of Type 2 Diabetes Mellitus



PRE DIABETES

The definition of IGT has been stable. The American Diabetes Association recommends the normal cut off for fasting plasma glucose as <100mg/dl. Both IGT and IFG have heterogenous pathogenesis and hence may have different rates of progression to diabetes. People with combined IFG and IGT have approximately double the rate of conversion to diabetes than those with any one of the abnormalities. Both the states are associated with insulin resistance and other cardiovascular risk factors such as dyslipidemia and hypertension. IGT is shown to be stronger predictor than IFG.

Figure 3: Natural history of type 2 diabetes



CLINICAL FEATURES

The clinical features depend upon the type of Diabetes, the stage in the natural history of diabetes and on the presence of its attendant complications or comorbidities. Type 1 diabetes constitutes <5% of all diabetes while Type 2 diabetes contributes to 95% of diabetes in the world including in India. Type 1 diabetes results in near total destruction of beta cells of islets of Langerhans in the pancreas resulting in absolute insulin deficiency resulting in hyperglycemia which presents with osmotic symptoms like polyuria, polydipsia and unexplained weight loss and with diabetic ketoacidosis and intercurrent infections.

Type 2 Diabetes due to environmental and genetic factors result in insulin resistance (relative insulin deficiency). Hyperinsulinemia in initial stages keeps fasting plasma glucose normal but in response to glucose load, post prandial hyperglycemia occurs. During the period of worsening insulin resistance there will be loss of first phase insulin release followed by hyperglycemia in both fasting and post prandial state. This continued hyperglycemia will result in both microvascular and macrovascular complication after a variable period of asymptomatic phase.

LATENT AUTOIMMUNE DIABETES OF ADULTS (LADA)

It is a peculiar type of diabetes of adults wherein patients harbor autoantibodies to islet cells as seen in type 1 diabetes, but it occurs in adults and is phenotypically similar to type 2 diabetes i.e., somewhere between type 1 and type 2 diabetes. Age of onset 30years, no insulin requirement for at least 6 months after diagnosis and presence of one or more of the antibodies (anti-GAD, IAA OR ICA) are the criteria for diagnosis of LADA. Latent autoimmune diabetes of adults in turn has been classified into LADA type 1 and LADA type 2.

The LADA type 1 has two or more antibodies present in high titres and has a phenotype closer to one described for classical type 1 diabetes. On the contrary, LADA type 2 has only one antibody present in low titres and the phenotype is similar to that for type 2 diabetes.

MODY

Maturity onset diabetes of Young is diabetes with a monogenic inheritance pattern. It has a autosomal dominant inheritance and has early age of onset and the genetic defect results in diminished insulin secretion. Any person with diabetes in three successive generations with the age of diabetes < 25 years without any classical features of insulin resistance should be suspected to have MODY.

Diabetes mellitus can come to our notice with one or more of the following clinical features:

- Asymptomatically diagnosed on routine screening in health camps or preventive health check ups.
- Noticing ants in the toilet after urination because of melituria- sweet urine Unexplained weight loss despite normal appetite
- Recurrent bacterial skin infection - boils, carbuncles, cellulitis
- Recurrent urinary tract infections
- Recurrent or difficult to treat fungal infections of skin and its appendages tinea corporis / pedis / cruris / candidial intertrigo or paronychia.

- Chronic vaginal discharge or vulval pruritis in females and recurrent balanoposthitis in males.
- Tuberculosis -diabetics with uncontrolled hyperglycemia have a greater predisposition to develop tuberculosis
- Polyuria and polydipsia
- Overt renal failure or microalbuminuria (diabetic nephropathy)
- Diabetic neuropathy -variants Impotence, symmetrical peripheral neuropathy or other loss of libido, dyspareunia and loss of sudomotor functions secondary to autonomic dysfunction
- Vision disturbances-rapidly changing glasses because of osmotic changes in the lens secondary to glycemic fluctuations, early onsetcataract, secondary glaucoma and diabetic retinopathy.
- Atherosclerotic vascular disease-coronary, cerebral or peripheral vascular disease
- Acute Complications Diabetic Ketoacidosis and non ketotic hyperosmolar state. “

RISK FACTORS TO SCREEN

Risk factors for diabetes which mandates screening in asymptomatic adult individual:

Over weight (BMI ≥23kg/m²) with any one of the the following features:

Physical inactivity

Diabetes in first degree relative

History of gestational diabetes mellitus or gave birth to a baby weighing >4kg

Hypertension (BP 140/90mmHg)

High density Lipoprotein cholesterol <35mg/dl

Serum triglycerides >250mg/dl

High risk ethic groups like pima Indians or races Pre diabetes

Women with polycystic ovarian syndrome

Clinical conditions associated with insulin resistance ,acanthosis nigricans

History of cardiovascular disease and age >45 years-severe obesity,

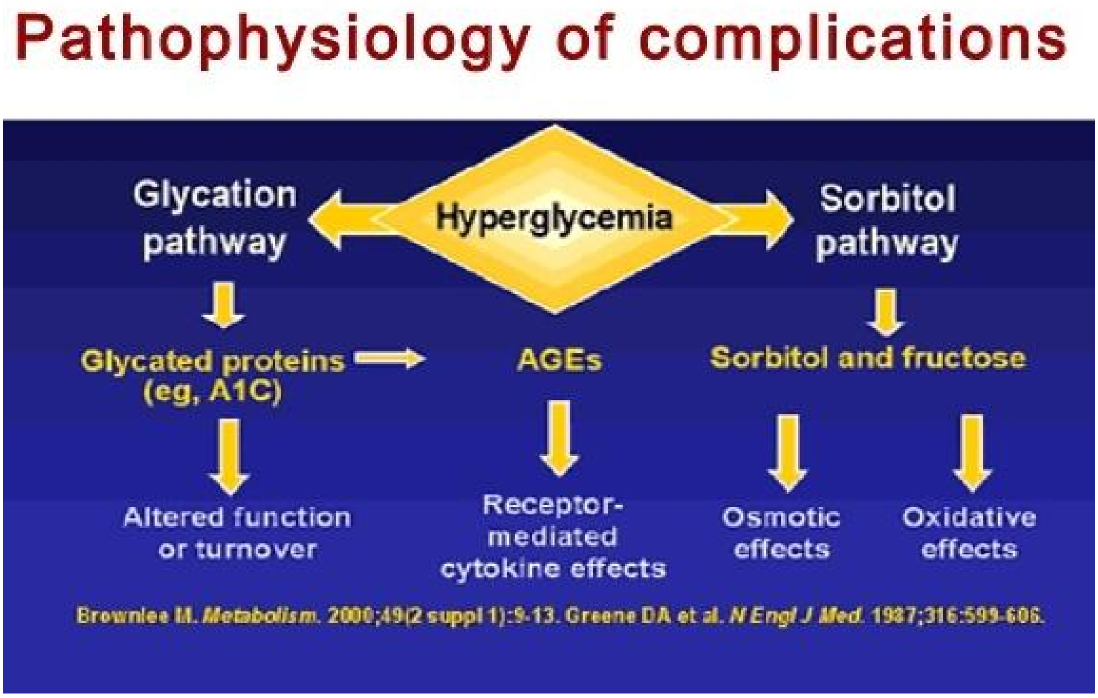
COMPLICATIONS

Unlike the microvascular disease, which starts with the onset of diabetes, the macrovascular disease antedates the development of overt diabetes by several years. Around 75% to 80% of all diabetic patients will die prematurely of cardiovascular (macrovascular) disease, particularly coronary heart disease. Diabetic foot problems (gangrene, large non healing infected ulcers) are the commonest cause of non traumatic lower limb amputation. In one Indian study done at Chennai, the prevalence of coronary heart disease was 21.4% among diabetic patients, 14.9% among impaired glucose tolerance subjects and 11% among non diabetic patients.The prevalence of peripheral arterial disease in the same population was 6.3%.

There is a close relationship existing between prediabetes, Diabetes and macrovscular disease throughout life and the substantial body of evidence supports the concept that increased morbidity and mortality due to cardiovascular disease is associated with abnormalities in glucose metabolism across the entire continuum of glucose intolerance Ranging from normal to clinical diabetes. While the interplay between diabetes and cardiovascular disease has been recognized for many decades, recent data analysis of the existing studies has helped to redefine several aspects of the relationship with relevance to clinical practice.

Meta analysis and systemic reviews have confirmed that DM increases CVD risk by around two fold on an average and this risk is subject to wide variation being lowest in the newly diagnosed and highest in those with existing vascular disease, proteinuria or renal disease.

Figure 4: Pathophysiology of Complications



The effects of diabetes on pathogenesis of atherosclerosis is due to endothelial dysfunction, oxidative stress, activation of polyol pathways, metabolic factors, coagulation and inflammatory factors and vascular related factors.

Chronic microvascular complications of diabetes mellitus are retinopathy, neuropathy, and nephropathy. Three fourth of cases develop retinopathy after more than 15 years of diabetes, half the diabetics have neuropathy and one third have nephropathy in larger population study in US. Poor glycemic control, long duration of diabetes and systolic blood pressure are risk factors for microvascular complications.

Risk of chronic complication in type 1 and 2 diabetes results from chronic hyperglycemia. It has been conclusively demonstrated in type 1 and type 2 diabetes microvascular complications can be prevented or delayed if chronic hyperglycemia is reduced. Other incompletely defined factors may moderately reduce development of complications. For instance, some individuals never develop nephropathy or retinopathy despite long standing diabetes though they may have similar glycemic control to patients with microvascular complications suggesting genetic susceptibility for developing particular complications.

There is now strong correlation between the occurrence and severity of microvascular complications in both type 1 and type 2 with duration and degree of hyperglycemia. Glucose appears to damage tissues by acute reversible changes in metabolism (e.g. sorbitol accumulation, increased NADH/NAD+ ratio, decreased myoinositol, early glycation) and by cumulative, irreversible alterations in stable macromolecules forming advanced End Glycation products. Genetic susceptibility And other accelerating factors such as hypertension and hyperlipidemia, smoking also play a role.

Hyperglycemia causes increase in intracellular glucose in insulin independent tissue like nerves, lens, retina, glomerulus. In these tissues glucose is converted by aldose reductase to sugar alcohol, sorbitol. In many tissues it is subsequently oxidixed to fructose with the help of sorbitol dehydrogenase with NAD+ as cofactor. This sorbitol does not cross the cell membrane easily and gets accumulated intracellularly. It causes damage to the tissues through its osmotic effects, by increasing NADH/NAD+ ratio inducing pseudohypoxia and by depleting intracellular Myoinositol.

Myoinositol is structurally related to glucose and it helps in activating Na+ -K+- ATPase for maintaining nerve conduction. Its depletion leads to impaired nerve function in diabetes.

In other tissues hyperglycemia leads to de novo synthesis of diacylglycerol and activation of the enzyme protein kinase C. Protein kinse c pathway is a family of serine threonine kinase that change the transcription of genes for fibronectin, type 4 collagen, contractile proteins and extracellular matrix protein in endothelial cells and neurons. This enzyme is implicated in several process causing diabetic complications such as increased capillary permeability and contractility, blood flow, cellular proliferation, basement membrane thickening. Protein kinase c mediates TGF-angiotensin 2 and vascular endothelial growth factor and modulates mitogen activated protein kinase which mediates sclerosis. Inhibitors of PKC like ruboxistuarin mesylate that reduce the direct cellular actions of AGES, VEGF, endothelin-1 reduce oxidized lipids and oxidant production are being studied in clinical trials in DM for retinopathy and neuropathy.

Next is the Hexosamine pathway where Fructose 6 phosphate, a substrate for O linked glycosylation and proteoglycan production is generated through the hexosamine pathway when hyperglycemia increases the flux. Hexosamine may alter function by glycosylation of proteins such as endothelial nitric oxide synthase or by changes in gene expression of TGF- or plasminogen activator inhibitor-1 (PAI-1).

With chronic hyperglycemia Amadori products in long lived molecules like collagen and DNA combine to form cross linked irreversible structures called advanced end glycation products (AGE).Early non enzymatic glycation products are reversible as hyperglycemia continues intermediate poorly reversible products are formed and later irreversible AGEs are formed. There is a correlation between serum levels of AGEs and the level of glycemia; these products accumulate as the glomerular filtration rate declines.

AGE accumulation leads to binding of LDL and other proteins to collagen in blood vessel walls predisposing to atherosclerosis and disruption of structure and impaired enzymatic turn over of matrix proteins leading to basement membrane permeability and thickening. It also causes endothelial cells to release cytokines and growth factors for cell proliferation.

Diabetic nephropathy

Historical facts –

Kimmelstiel and Wilson described the renal pathology of DN by autopsy as early as 1936. Eight cases were found to have the typical nodular glomerulosclerosis of diabetic nephropathy. These findings were confirmed by others also. Diffuse glomerulosclerosis was described and differentiated from the nodular forms. [5-6]

Later studies involving percutaneous renal biopsy and electron microscopy showed the earliest changes of diabetic nephropathy to be thickening of the basement membrane (GBM) and mesangial expansion. [6]

Epidemiology

DN is well studied in patients with type 1 diabetes. The onset of disease is usually known in them.25% to 45% of these patients develop nephropathy in their lifespan. [7] The peak time to develop nephropathy is 10 to 15 years from onset. In patients with type 2 diabetes, the prevalence of nephropathy is reported to be lower. Nephropathy developed in 50% of type 2 diabetic Pima Indians. [8] This was 20 years after diagnosis and 15% progressed to ESRD. Proteinuria is known to predispose for cardiovascular disease. It is thought that earlier studies underestimated the prevalence of DN. The actual prevalence of DN is difficult to determine , as early cardiovascular mortality would have preceded the development of ESRD. Recent data shows the risk of nephropathy to be same in both types of diabetes . The time to develop proteinuria from the onset and to progress to ESRD once proteinuria develops, were same in types 1 and 2 diabetes. [9]

Natural history of Diabetic nephropathy –

Mogensen et al proposed five stages for renal involvement. [10]

Stage 1: glomerular hyperfiltration.

Even with optimal blood glucose, GFR remains above normal in 25% to 40% of patients. In this group fall in GFR developed at a faster rate, compared to controls with normal GFR. This raised GFR is due to increased glomerular capillary filtration surface. Higher GFR early in the course of disease makes it more likely to develop DN. [11]

Stage 2: Early lesions

Mild thickening of glomerular basement membrane takes place 18 to 24 months from the start of type 1 diabetes [10]. Glycosylation of the basement membrane causes an increase in filtration of proteins.The negative charge of the basement membrane is reduced which causes increased loss of albumin as it is no longer repelled by basement membrane. [11]

Stage 3: incipient diabetic nephropathy—stage of microalbuminuria.

Microalbuminuria is the urine albumin excretion of 20 to 200 g/min per day. It is associated with loss of renal function and poor outcomes . Associated with vascular injury in other organs also[10].

Stage 4: clinical nephropathy — macroalbuminuria, decline in GFR .

Usually manifests in patients who have diabetes for 15 to 20 years. Without intervention, the GFR in these patients, falls at about 1 mL/min/month.

Stage 5: End-stage renal disease-

ESRD occurs after 20 to 30 years of diabetes. This is seen in 30% to 40% with type 1 diabetes. Uremic symptoms and signs are seen at much higher creatinine clearances than nondiabetics[11].

PATHOLOGY

The 3 main pathological changes in the glomeruli of DN:

1) Mesangial expansion.
2) Glomerular basement membrane (GBM) thickening.
3) Glomerular sclerosis. [12]

Glomerular sclerosis with a nodular appearance is called the ‘‘Kimmelstiel-Wilson lesion’’. Hyaline deposits in the glomerular arterioles reflects the leakage of proteins. These are namely fibrin, immunoglobulins, and complement . [13]

The mesangial expansion and glomerulosclerosis do not always develop together . This means that they have different pathogenetic mechanisms. [12] Mesangial expansion is caused by hyperglycemia which causes an increased formation or glycosylation of mesangial proteins. [13]

Pathogenesis of Diabetic nephropathy-

Factors involved -

- Hemodynamic pathways
- Hyperglycemia and Advanced glcosylation end products
- Protein kinase C pathways
- Aldose reductase pathways
- Prorenin
- Cytokines
- Oxidative stress
- Genetic susceptibility

Hemodynamic factors –

Glomerular hyperfiltration is because of decreased resistance in both the afferent and efferent arterioles.The afferent arteriole has lesser resistance than the efferent. [12] Factors involved in this defective autoregulation are prostanoids,nitric oxide,vascular endothelial growth factor VEGF. TGF-β1, and the renin–angiotensin system mainly angiotensin II are the other factors mainly seen. [13] The hemodynamic factors result in albumin to leak from the glomerular capillaries. Increased production of mesangial cells, thickening of the GBM and injury to podocytes are due to hemodynamic factors. Glomerular hypertension and hyperfiltration play an important role in the pathogenesis diabetic nephropathy as use of angiotensin blockers prevents loss of nephrons. Blocking the angiotensin system prevents the fibrosis inducing effects of angiotensin II on TGF -β. [15]

Hyperglycemia and advanced glycosylation end products

Hyperglycemia causes an increase in mesangial cell proliferation and hypertrophy. [16] Hyperglycemia is associated with an increased matrix production and basement membrane thickening. Mesangial cell expansion is due to an increase in the mesangial cell glucose concentration. [17] Hyperglycemia Causes the Upregulation Of VEGF Expression In podocytes.This markedly increases vascular permeability. [18]

Advanced glycosylation end products

Glycosylation of proteins is one of the causes of nephropathy. Long standing increased sugars, causes glucose to combine with free amino acids on tissues. [17] This nonenzymatic process has an affect on the glomerular basement membrane [18] .Other matrix components in the glomerulus are also affected.The early formation of reversible glycation products are replaced later by irreversible advanced glycation products. The levels of AGE are increased in the diabetics. This is more in renal failure,as it is excreted in the urine. [18] This leads to accumulation of advanced glycosylation end products which has a tendency to cross-link with collagen. This contributes to development of DN.

Protein kinase C

Hyperglycemia causes diabetic nephropathy by activation of PKC. Activation of this enzyme causes the increased production of prostanoids which cause vasodilatation.This contributes to glomerular hyperfiltration. PKC activation causes the formation of diacylglycerol which leads to oxidative stress. [19] PKC activation also increases the activity of mitogen- activated protein kinases (MAPK).

Aldose reductase pathway

This enzyme helps in changing sugars into alcohols. By this enzyme glucose is converted into sorbitol and galactose into galactitol.These alcohols ,fail to diffuse out from the cells .Their accumulation intracellularly causes osmosis thereby allowing entry of water into the cell. [20] This results in electrolyte imbalances and also the depletion of myoinositol. This causes tissue damage to occur. [21]

Prorenin

Increased prorenin activity in the plasma was found to be a risk factor for the development of diabetic nephropathy. [21] The prorenin receptors are located in the mesangium and podocytes of the kidney. Prorenin binding to specific tissue receptors promotes the activation of p44/p42 MAPK. [22] The blocking of Prorenin receptors prevented MAPK activation which blocked nephropathy from progressing.

Cytokines

Activation of cytokines, enzymes causing profibrosis, inflammation and VEGF are some of the factors involved in increased matrix formation in diabetic nephropathy.[23.] Hyperglycemia increases VEGF expression acts to cause endothelial injury in diabetes. [22]

The VEGF and angiopoetin cause vessel leakage of proteins .These two factors play important roles in development of retinopathy and nephropathy. VEGF stimulates the formation of α3 chain of collagen which is an important component of the GBM. It is seen that increased formation of collagen leads to the thickening of the GBM. [22]

Some studies however do not show VEGF as a causative factor , Baelde et al demonstrated VEGF mRNA levels,to be low in the glomeruli for some cases of diabetic nephropathy.This was associated with reductions in podocytes number and development of nephropathy. [24]

Hyperglycemia can increase the production of TGF-β1 in the glomeruli. [21]

Inflammatory cytokines are responsible for the progression of diabetic nephropathy.They are interleukins 1,6,8 (IL- 1,6,8) and tumor necrosis factor.Increased levels of interleukins correlate well with development of nephropathy. IL-1 acts on chemotactic factors and adhesion molecules altering their expression. It also causes intraglomerular hypertension. Increases vascular endothelial permeability. It also has an effect to increase hyaluron production by renal tubular epithelial cells. [22] IL- 6 acts likely by increasing the glomerular basement membrane thickening. It also has a possible role to increase endothelial permeability and mesangial proliferation. IL-18 increases the production of other inflammatory cytokines.

Oxidative stress

Reactive oxygen species are produced in the nephrons during cellular metabolism. These molecules are countered by a large number of antioxidants and free radical scavengers. Reactive oxygen species have negative effects. They cause peroxidation of cell membranes lipids.They also cause renal vasoconstriction. Hyperglycemia shifts the balance towards formation of reactive oxygen species.Most of these are generated in the mitochondria. [21] .Concentrations of the reactive oxygen species are higher in those with more severe nephropathy.

Genetic susceptibility

As mentioned earlier increased production of angiotensin II determines the initiation and progression of DN. Angiotensin II mainly acts by affecting Hemodynamic and Nonhemodynamic mechanisms. [24] Polymorphism of the ACE gene due to inversion or deletion (ACE/ID) occurs. This is associated with varying levels of circulating ACE. The probability of developing DN is more for patients,having a sibling or parent with history of DN. Patients with DD polymorphism of the ACE gene have a high risk of developing diabetic nephropathy and progressive renal failure.

Clinical predictors and risk factors for diabetic nephropathy and Retinopathy

1) **Glycemic control -**

Diabetic nephropathy develops more often in patients with long standing uncontrolled hyperglycemia. Diabetics with a hemoglobin A1c level less than 8.1% are at much lower risk for DN 25 .Randomized clinical trials have confirmed this association both for nephropathy and retinopathy. The United Kingdom Prospective Diabetes Study (UKPDS) in type 2 diabetics, showed fewer patients of the tight blood sugar control to progress to microalbuminuria. Less in the intensive blood sugar group compared to conventional group progressed to microalbuminuria (27% versus 39%) and proteinuria (7% versus 13%)over a 15 year followup period [26] . The Diabetes Control and Complications Trial (DCCT) highlighted the benefits of intensive therapy for glycemic control. They showed a reduction in microalbuminuria by 39% and albuminuria by 54% respectively [27] .The DCCT showed that in the intensive arm there was a 76% reduction in the development of retinopathy [28] .Even in those with established retinopathy there was a 80% reduction in progression,compared to those with conventional control. These targets were achieved by maintaining a median hemoglobin A1c level of 9.1% and 7.3 % in the conventional and intensive treatment group respectively [28] .These benefits persisted even after 7 years of continued follow-up. This finding was demonstrated by Epidemiology of diabetes Interventions and Complications study. The difference in HbA1c levels between two groups was 0.4% .Though this difference appears small it still provided benefit I year after completion of the DCCT. Wisconsin study of DR was a population based study. This study was done to determine the prevalence and severity of DR in their study population.

The study concluded that there is a strong association between severity of retinopathy and high levels of HBA1C. [27] For patients with already existing advanced retinopathy, strict control of blood glucose will not be helpful in preventing the progression of retinopathy. [27]

2) Duration of disease -

The total duration of disease was found to be an important risk factor for the development and progression of retinopathy [26] .The Wisconsin epidemiologic study of retinopathy, showed that the longer the duration of diabetes more the prevalence of retinopathy in the study population .[27]

3) Blood pressure -

Studies show increased systolic blood pressure to be a strong risk factor for both diabetic nephropathy and retinopathy. This risk was more for retinopathy [26] . In UKPDS trial it was seen that good blood pressure control led to decreased progression of retinopathy. A 10/5 reduction of blood pressure caused a 34% reduction in progression of retinopathy and 47% decreased risk of deterioration in visual acuity. [27]

4) Glomerular filtration rate –

Patients with glomerular hyper filtration had an increased risk for developing diabetic nephropathy .The glomerular hyper filtration and hypertrophy are due to intraglomerular hypertension and capillary wall stress. [29]

In type 2 diabetes , 45% have a GFR that is significantly more than age matched nondiabetics and obese controls The amount of hyperfiltration is lesser in type 2 diabetics compared to type 1. Type 2 diabetics being older are more likely to have atherosclerotic vascular disease, this factor probably limits the increase in glomerular filtration. [30]

DIAGNOSIS OF DIABETIC NEPHROPATHY

The techniques available for detecting diabetic nephropathy are the albumin : creatinine ratio or a protein:creatinine ratio from a random spot urine or a 24-hour urine collection [37] . Positive results need a second measurement to reconfirm. In diabetics protein excretion Varies considerably. Urine dipsticks for microalbuminuria (on fresh morning specimens) are used only for initial screening. A positive test should be followed-up by a 24-hour urine collection. 38 Microalbuminuria is defined as the urine protein : creatinine ratio of 30- 300 mg/g and macroalbuminuria is defined as a protein : creatinine ratio > 300 mg/g in a spot or 24 hours timed specimen

Short-term hyperglycemia, exercise, urinary tract infections, marked hypertension, heart failure, and acute febrile illness can cause transient elevations in urinary albumin excretion [38] . As there is a marked variability in the day to day excretion of albumin, at least 3 samples are required over a 3 month period ,before confirming the presence of microalbuminuria . [36]

INFLAMMATION AND DIABETES:[3,37,38]

Some studies defined a clear cut relation between systemic inflammation and insulin resistance in Type 2 diabetes mellitus suggesting that altered immune system plays a decisive role in the pathogenesis of DM. Due to increased delivery of glucose to adipose tissue in DM, endothelial cells in the fat pad may take up increasing amounts of glucose through their constitutive glucose transporters. Increased glucose uptake by endothelial cells in hyperglycemic conditions causes excess production of

ROS in mitochondria, which inflicts oxidative damage and activates inflammatory signaling cascades inside endothelial cells.

Endothelial injury in the adipose tissue might attract inflammatory cells such as macrophages to this site and further exacerbate the local inflammation. Hyperglycemia also stimulates ROS production in adipocytes, which leads to increased production of proinflammatory cytokines.

Studies have proposed excessive oxidation that hyperglycemia can lead to an reaction in the tricarboxylic acid cycle leading to an increase in the generation of reactive oxygen species (ROS). As a result, mitochondrial function is impaired during the production of ROS. Studies have reported that leukocytes in subjects with diabetes mellitus generates more ROS, resulting in elevated oxidative DNA damage of lymphocytes in the hyperglycemic state.

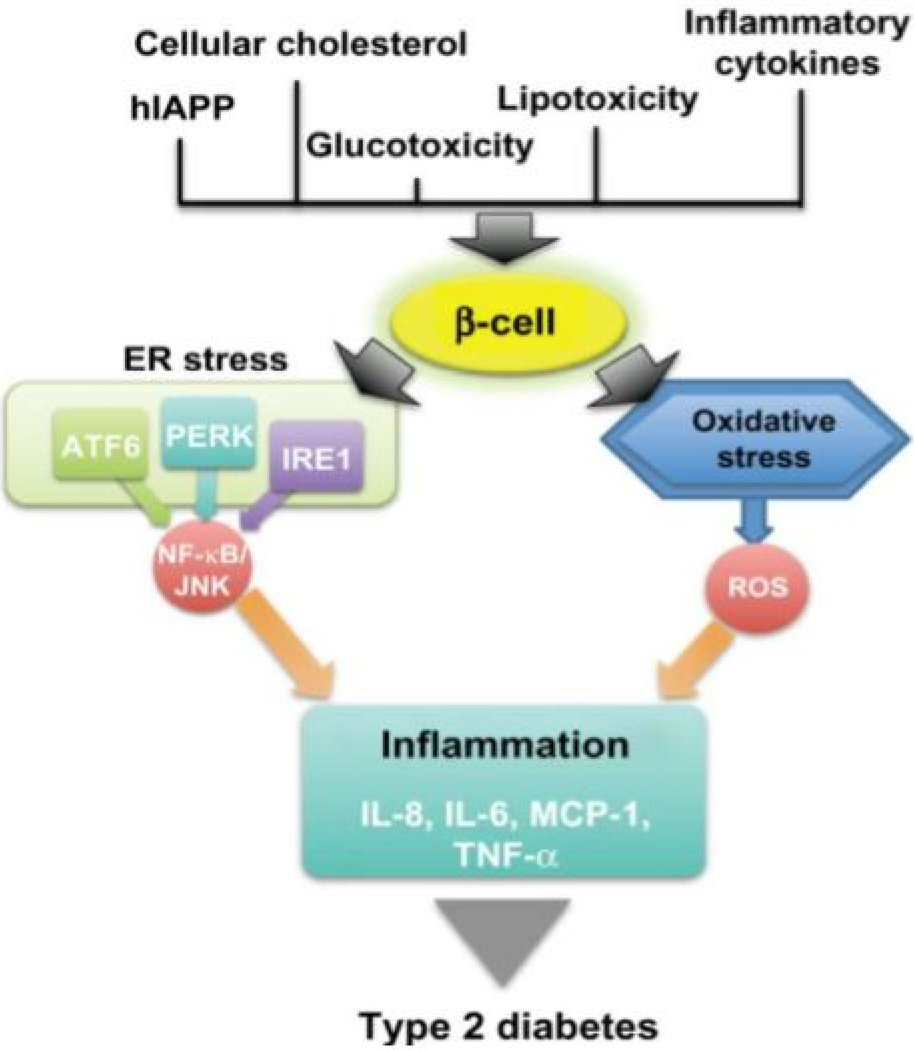
Insulin surface of exerts its action through binding to its receptor on the insulin-responsive cells. The stimulated insulin receptor phosphorylates itself and several substrates, including members of the insulin receptor substrate (IRS) family, thus initiating downstream signaling events. The inhibition of signaling downstream of the insulin receptor is a primary mechanism through which inflammatory signaling leads to insulin resistance.

Exposure of cells to TNF- or elevated levels of free fatty acids stimulates inhibitory phosphorylation of serine residues of IRS-1. This phosphorylation reduces both tyrosine phosphorylation of IRS-1 in response to insulin and the ability of IRS-1 to associate with the insulin receptor and thereby inhibits downstream signaling and insulin action.

Hence, it has become clear that inflammatory signaling pathways can also become activated by metabolic stresses originating from inside the cell as well as by extracellular signaling molecules. It has been demonstrated that obesity overloads the functional capacity of the ER and that this ER stress leads to the activation of inflammatory signaling pathways and thus contributes to insulin resistance. Additionally, increased glucose metabolism can lead to a rise in mitochondrial production of ROS. ROS production is elevated in obesity, which causes enhanced activation of inflammatory pathways.

Several serine / threonine kinases are activated by inflammatory or stressful stimuli and contribute to inhibition of insulin signaling, including JNK, inhibitor of NF- kinase (NKK). Again, the activation of these kinases in obesity highlights the overlap of metabolic and immune pathways. Inflammatory cytokine stimulation can also lead to induction of iNOS. Overproduction of nitric oxide also appears to contribute to impairment of both muscle cell insulin action and cell function in obesity. Deletion of iNOS prevents impairment of insulin signaling in muscle caused by a high-fat diet. Thus, induction of such proteins and iNOS represent two additional and potentially important mechanisms that contribute to cytokine-mediated insulin resistance. It is likely that additional mechanisms linking inflammation with insulin resistance remain to be uncovered.

Figure 5. Inflammation and Diabetes



The role of lipids in metabolic disease is complex. As discussed above, hyperlipidemia leads to increased uptake of fatty acids by muscle cells and production of fatty acid metabolites that stimulate inflammatory state. In patients with diabetes, several studies explored connection between systemic inflammation and vascular disease and found that chronic inflammation promotes the development and acceleration of microvascular and macrovascular complications.

Many inflammatory markers have been found to be related to DM, such as interleukin-1 (IL1), IL6, IL8, transforming growth factor beta 1, Tumor necrosis factor- alpha (TNF- measurement is not used routinely as it is not easy to do it. Total white blood cell (TWBC) count is a crude but sensitive indicator of inflammation which can be done easily in laboratory routinely. It is a cost-effective investigation. Besides WBC counts, the Platelet Lymphocyte Ratio (PLR), Monocyte Lymphocyte Ratio (MLR), and Neutrophil Lymphocyte Ratio (NLR) are potential biomarkers reflecting inflammation and immune responses. Many studies have reported positive correlations of conventional inflammatory markers with the PLR and NLR.

NEUTROPHIL LYMPHOCYTE RATIO:

More importantly, a large number of studies found predictive effects of the PLR and NLR, particularly in DM[39-42.] The neutrophil-lymphocyte ratio (NLR) in complete blood count is studied in many diseases as an inflammatory marker and is used to predict the prognosis of diseases. Interestingly, NLR has been found to have a positive corelation with metabolic syndrome.

A study by Imtiaz et al.[3] has suggested that chronic diseases such as hypertension and diabetes have a significant association with systemic inflammation, reflected by NLR.

Shiny et al.[43] have shown that NLR is correlated with increasing severity of glucose intolerance and insulin resistance and can be used as a prognostic marker for macro- and micro-vascular complications in patients with glucose intolerance. Initially, NLR was recognized as a predictive marker in multiple types of cancer that might assist in patient stratification and individual risk. In this respect, NLR has emerged as a novel surrogate marker. The immune response to various physiological challenges is characterized by increased neutrophil and decreased lymphocyte counts, and NLR is often recognized as an inflammatory marker to assess the severity of the disease. NLR represents a combination of two markers where neutrophils represent the active nonspecific inflammatory mediator initiating the first line of defense, whereas lymphocytes represent the regulatory or protective component of inflammation. But recently, multiple other studies have indicated that NLR might be a predictive marker for vascular diseases also. Recently, several studies have suggested that NLR could play a predictive role for assessing the development of microvascular complications of diabetes.

In a study, Ulu et al. [41] demonstrated NLR to be a quick and reliable prognostic marker for diabetic retinopathy and its severity.

A study conducted in geriatric population also suggested that increased NLR levels were in itself an independent predictor for microvascular complications of DM[44]. The exact molecular action leading to IR is not yet understood, but several studies have confirmed NLR is superior to other leukocyte parameters (e.g., neutrophil, lymphocyte, and total leukocyte counts) because of its better stability compared with the other parameters that can be altered by various physiological, pathological, and physical factors. Thus, as a simple clinical indicator of IR, NLR is more sensitive compared with the neutrophilic granulocyte count and CRP levels.

PLATELET LYMPHOCYTE RATIO:

Some angiogenesis factors such as vascular endothelial growth factor (VEGF) are key protein modulators expressed by platelets. Notably, high VEGF levels can stimulate the development of proliferative diabetic retinopathy (PDR)[45]. This would suggest that there is association between the PLR and DR progression. Data generated from research has supported a close association of systemic inflammatory processes with oxidative stress, leading to alterations of platelet and lymphocyte levels. Thus, the underlying mechanism of up-regulated PLR may also be based on the dysfunction of the inflammatory response.

MATERIALS AND METHODS

Source of Data :

Patients with Type 2 Diabetes Mellitus with nephropathy admitted in the wards of KLE Dr. Prabhakar Kore Hospital , Belgaum that fulfill the inclusion criteria.

Study period :

January 2018 – December 2018 – One year.

Study design :

Cross – sectional study.

Sample Method :

All consecutive patients fulfilling the inclusion criteria were included in the study.

Statistical analysis was done by chi- square test.

Sample size :

Sample size calculation is done using the formula

$N= z_a^2 P(1-P) / d^2$

Where z=1.96 for 95% Confidence interval,

p=Prevalence, and

d= acceptable margin of error

From the previous study done in Gujarat by Vitan Patel at el., prevalence of Diabetic Nephropathy among Diabetic patients in the study was 43%. We took the values for the calculation

≈94.16 94 is the minimum sample size required for the study.

Sample size was taken as 100

Inclusion criteria :

All patients in the wards in KLE Hospital & Research Centre with Type 2 Diabetes Mellitus with Diabetic Nephropathy.

Exclusion criteria:

Type 1 Diabetes Mellitus .
HIV positive patients .

Patients on steroids .

Malignancy .

Patients with Liver disease .

Patients with TB.

Patients with active infection .

Patients with Myeloproliferative disease and Leukemia .

UTIs

Methodology

After obtaining institutional ethical committee clearance 100 diabetic nephropathy patients who met inclusion and exclusion criteria were included in the study. Informed consent was obtained from patients.

All patients who were included in study were evaluated with following investigations:

Complete blood count

Urine routine and microscopy

Serum creatinine

HbA1c

Random blood sugar

Neutrophil lymphocyte ratio (NLR):

NLR was calculated by dividing absolute neutrophil count by absolute lymphocyte count

Estimated glomerular filtration rate (EGFR):

EGFR was calculated using Modified diet renal disease (MDRD) equation

$$EGFR = 1.86 \times (S.cr)^{-1.154} \times (age)^{-0.203}$$

Multiply 0.742 for woman

Multiply 1.21 for african origin

EGFR - ml/min/1.73㎡

Serum creatinine (S.cr) - mg/dl

Age in years

CKD staging:

Table 1 : CKD Staging

CKD STAGING	**GFR (Ml/min/1.73㎡)**
Stage 1	90
Stage 2	60-89
Stage 3A	45-59
Stage 3B	30-44
Stage 4	15-29
Stage 5	<15

Creatinine clearance:

Cockcroft-Gault formula

Creatinineclearance

$$CrCl = ([140 - age] \times weight) \div (SerumCreatinine \times 72)$$

Multiply by 0.85 for females

Age in years

Weight in kilogram

Data analysis

Data was collected using predisigned proforma. The collected data were analysed with IBM-SPSS software (version 23).Here continous variables are represented by mean ± sd form and categorical variables by frequency table. Comparison is done using Mann Whitney U-test. .Spearman rank and rank biserial correlation is used to study the correlation of NL ratio with different factors

RESULTS

Methods Used:

Here continous variables are represented by mean ± sd form and categorical variables by frequency table. Comparison is done using Mann Whitney U-test.Spearman rank and rank biserial correlation is used to study the correlation of NL ratio with different factors.

Summary:

Here 100 observations of age 59.59 ± 11.44(years) with range [33,88] were included in the sample. Summary statistics are given in following tables

Table 2: Summary Statistics Of The Data

Factor		Count	Percentage
Gender	**Male**	68	68%
	Female	32	32%
Age		59.59±11.44	
Age group	**30-40**	5	5%
	40-50	17	17%
	50-60	26	26%
	60-70	30	30%
	70-80	19	19%
	80-90	3	03%

From table 1, we observe that 68 subjects in the sample are male and the remaining 32% are female subjects. Majority (30%) of the subjects in the sample are of the age group ”60-70” followed by “50-60” (26%). In figure 1 and figure 2, we visualize the results.

Figure 6: Distribution Of Subjects By Gender

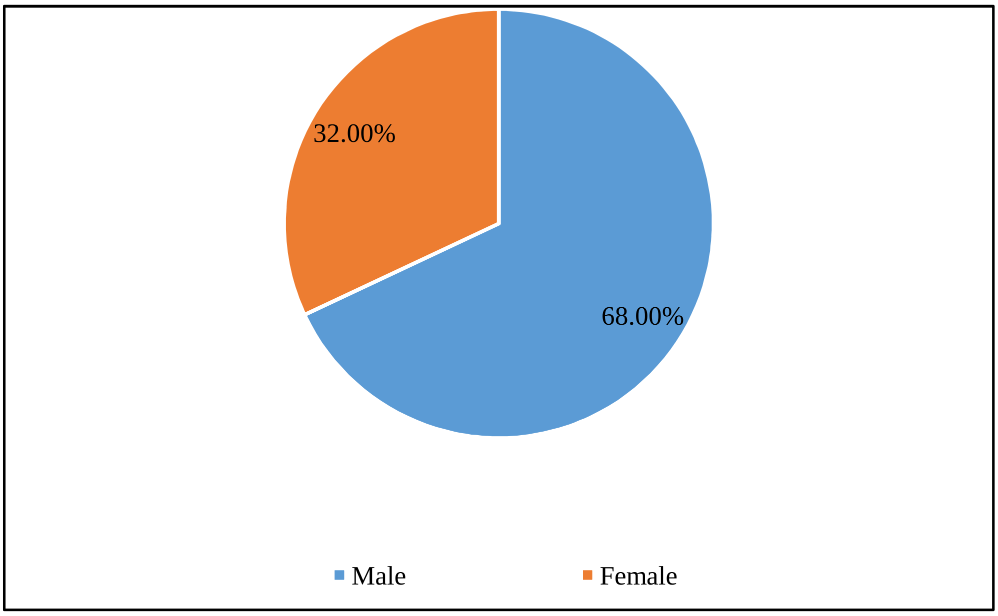


Figure 7: Distribution of subjects by Age group

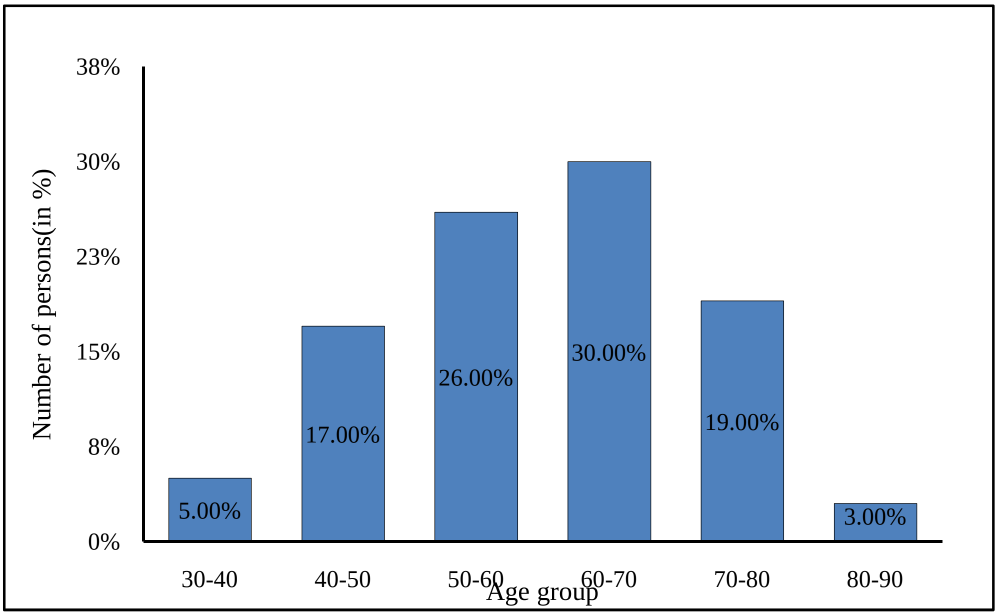


Table 3: CORRELATION OF NL RATIO WITH DIFFERENT FACTORS.

Factor	Rho	p-value
Age	-0.1749	0.0818
Sex	0.0356	0.7247[a]
Proteinuria	-0.0326	0.7474
EGFR	-0.2247	0.0012*
Maintenance HD	0.1597	.1124[a]
Creatinine	0.3175	0.0013*
CKD stage	0.2873	.0037*

[a]*indicates rank biserial correlation; * indicates significance*

From table 2, using spearman rank correlation, we concludethat there is a significant negative correlation between NL ratio and EGFR while CKD stage as well as Creatinine significantly positively correlated with NL ratio. following figure visualise the results.

Figure 8: Relationship Between Creatinine and N:L Ratio

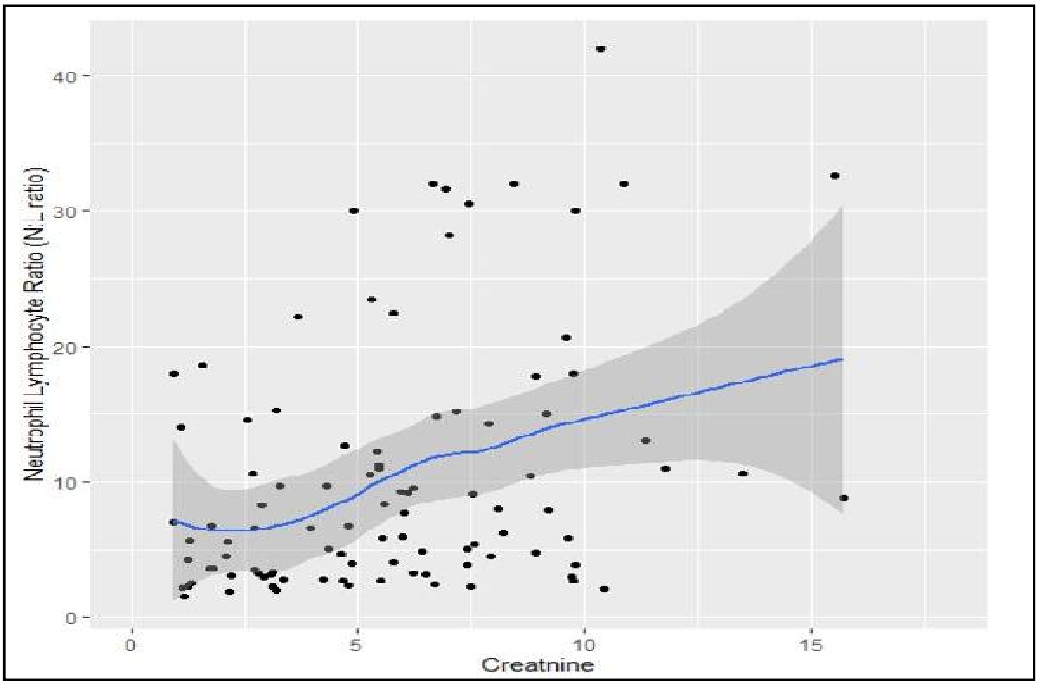


Figure 9: Relationship Between EGFR and N:L Ratio

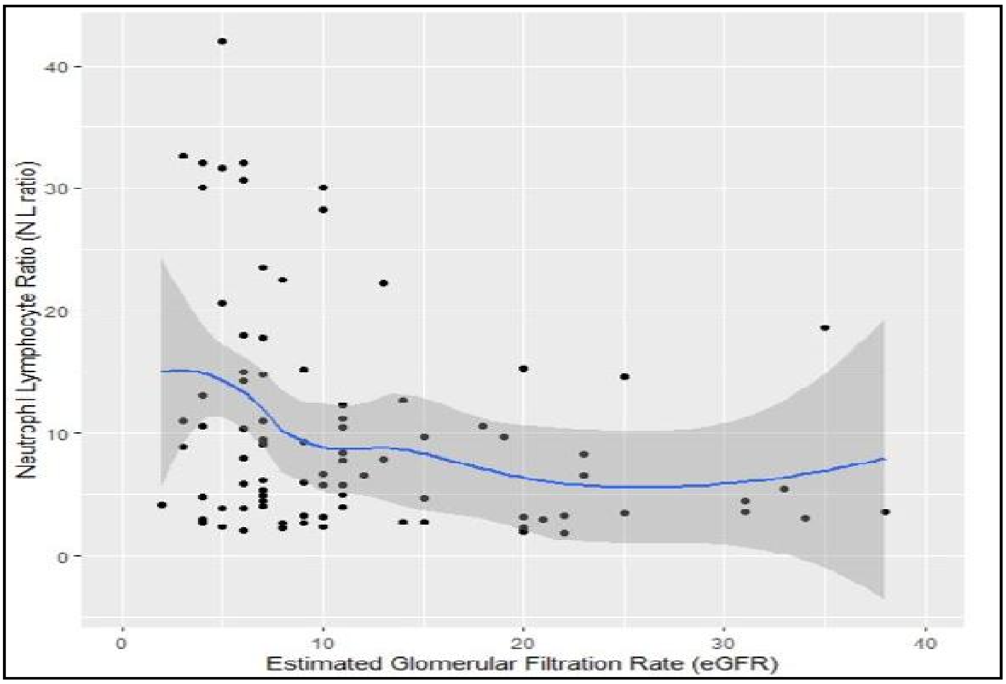


Figure 10: Relationship Between Proteinuria and N:L Ratio

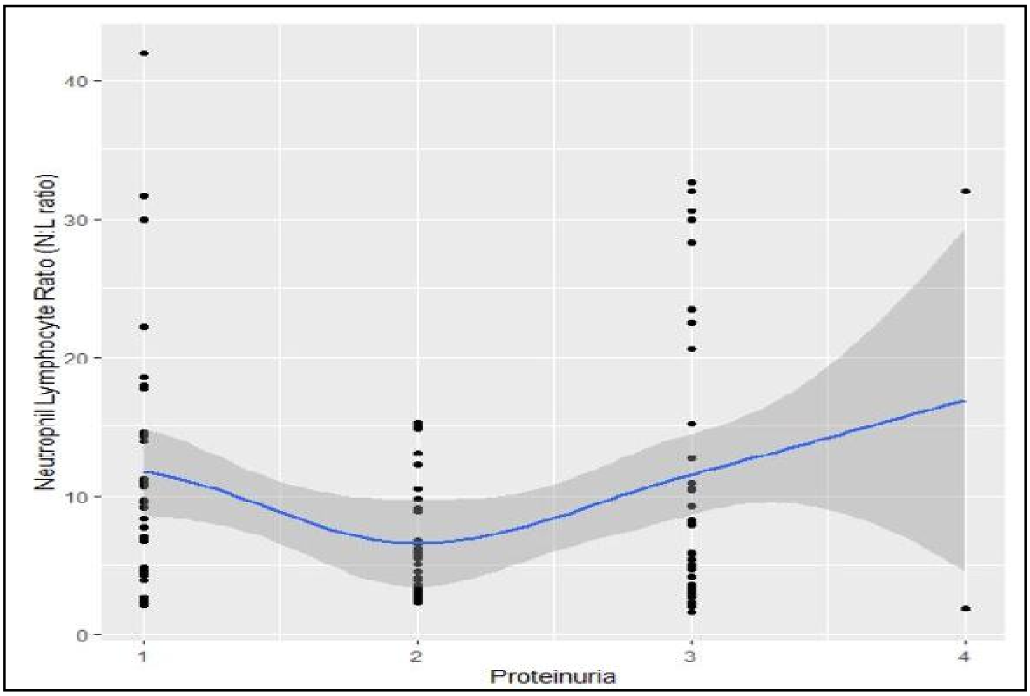


Figure 11: Relationship Between CKD Stage and N:L Ratio

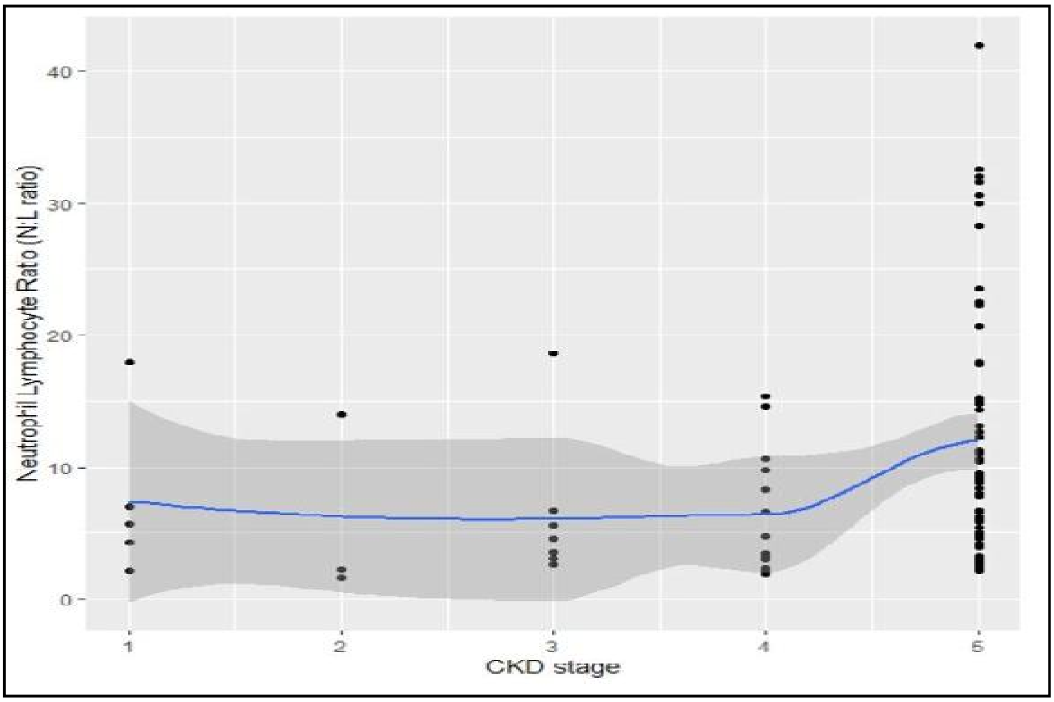


Table 4: Comparison Of Nl Ratio Between Gender

	Gender		P-value
	Male	Female	
NL ratio	9.5338 ± 8.4229	11. 54718 ± 10.2609	.7256#

 # indicates Mann Whitney U-test

From table 3, using Mann-Whitney U-test, we conclude that there is no significant difference in median of NL ratio between male and female subjects.

Table 5: Comparison of NL Ratio Between Maintenance HD

	Maintenance HD		P-value
	Yes	No	
NL ratio	13.30±11.51	8.96±7.64	0.1129#

From table 4, using Mann Whitney U-test, we conclude that there is no significant difference in median of NL ratio between maintenance HD.

Inférences:

- There is a significant negative correlation between EGFR and NL ratio.
- There is a significant positive correlation between NL ratio and CKD stage.
- There is a significant positive correlation between NL ratio and Creatinine
- Median of NL ratio for male subjects is not significantly different from female subjects.

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DISCUSSION

Complete blood count is a cheap, routine and practical laboratory test that gives us important information about the patient’s formed blood components[31]. Routine complete blood counts may be useful in diagnosis and prognosis of many diseases. NLR is measured by dividing the number of neutrophils by the number of lymphocytes. NLR is an indicator of systemic inflammation. Inflammation plays a significant role in the body's combat against various noxious insults and is important in the development and progression of various diseases. Even when the total WBC count is in the normal range, NLR has been demonstrated to play a predictive role in the prognosis of chronic and acute inflammatory processes. A recent meta-analysis depicts that an elevated NLR is an independent factor associated with poorer overall survival in many solid tumors.

An elevated NLR may be associated with [43,47-51,52]

Renal or hepatic dysfunction

Diabetes mellitus

Abnormal thyroid function

Hypertension

Metabolic syndrome

Hematological malignancies

Malignancy

Preceding history of local or systemic infection

Inflammatory diseases

Some medications

In this study 100 patients with diabetic nephropathy were included , factors like age, duration of diabetes, random blood sugar, HbA1C, serum creatinine , urine examination, creatinine clearance ,TLC, were assessed. NLR ratio was derived by dividing absolute neutrophil count by absolute lymphocyte count .

In this study majority of them , 56% lie in the age group of 50 to 70 years . The mean age of patients was 59.6±11.4 years (mean±sd). 68% of subjects in the study were male whereas the remaining 32% were females. Median of NL ratio for male subjects is not significantly different from female subjects. The mean NLR in males was 9.53±8.42 (mean±sd) whereas 11.54±10.2 (mean±sd) in females which was statistically not significant ($p>0.05$) . The mean NLR in this study was 10.8 with standard deviation of 9.05.

Using the modified MDRD equation we estimated glomerular filtration rate (eGFR) in all diabetic nephropathy patients included in the study .The mean eGFR was 16.5 ml/min with a standard deviation of 16.9 ml/min. eGFR range was from 2 – 85 ml/min .There was significant negative correlation between EGFR and NLR (p 0.001).The mean serum creatinine was 5.93 mg/dl with standard deviation of 3.66 mg/dl. There was significant positive correlation between serum creatinine and NLR (p 0.001). There were 5 , 3, 8, 16, and 68 diabetic nephropathy patients in CKD stage 2, 3A,3B, 4 and 5 respectively.There was significant positive correlation between CKD stage and NLR (p 0.004)

Patients with proteinuria grade 1+ was 31 %, 2+ was 31% , 3+ was 36% and 4+ was 2%. Proteinuria grade and NLR had no significant correlation ($p>0.05$). In our study 28 patients were on hemodialysis support while 72 patients were not dependant on HD .There was no statistically significant difference in patients with or without hemodialysis and NLR ($p>0.05$).

Goldberg [53]showed that complications like nephropathy , retinopathy and cardiovascular disease seen in type 1 and 2 diabetes were associated with increased inflammatory molecules and cytokine molecules (CRP, IL-6, TNFA , Neutrophilia and relative lymphocytopenia).

Shiny et al [43]in their study found that there was significant co-relation between NLR with severity of glucose intolerance and resistance . Their study concluded that it can be used as a adjuvent prognostic marker for micro and macro vascular complications in such patients.

Sagar ashokrao khandare et.al.[54] conducted a cross sectional study on 115 diabetic patient in 2016. here the patients were divided into two groups - patients with diabetic nephropathy and patients with normal urine albumin. Here the mean age of DN patients was 50.05+/- 11.29 years and patients with albuminuria had significantly low eGFR (85.71+/-27.72 , mean sd) as compared to those patients with normal urine albumin (96..2+/-28.23 , mean+/-sd) which was statistically significant (p 0.0470). The NLR in diabetic nephropathy patient group (2.83+/-0.85, mean+/- sd) and that of the other group patient was 1.94+/-0.65 (mean+/- sd) was statistically significantly. In a study done by Hung et al.,55 it was found that NLR values were significantly higher in diabetic nephropathy patients compared to diabetic patients without nephropathy.

Moursy et al., [56] conducted a case control study on 280 patients to access the relationship between NLR and microvascular complications in type 2 diabetes of which 200 were diabetic patients and 80 healthy subjects . This study concluded that NLR can be used as a marker of inflammation and there was statistically significant co-relation between NLR with presence of microvascular complications seen in diabetes.

The NLR normal value is 1.8-3.5 as in numerous previous studies[57] . In our study the mean NLR was 10.8 with standard deviation of 9.05 . The lowest NLR was 1.61 and 42 was the highest value. NLR in our study was higher compared to above studies owing to majority of diabetic nephropathy patients in our study falling into CKD 4 and 5 (ESRD).

CONCLUSION

The results of our study show that NLR had statistically significant co-relation with reduced GFR, higher CKD stages and higher serum creatinine levels. But there was no co-relation between NLR with proteinuria grade.

From our study we conclude that NLR can be used as a novel prognostic marker for diabetic nephropathy patients.

SUMMARY

We conducted a observational cross sectional study on 100 patients of diabetic nephropathy admitted at KLE's Dr.Prabhakar kore's hospital from january 2018 to december 2018 after fulfilling the inclusion and exclusion critera.

In this study 100 patients with diabetic nephropathy were included , factors like age, duration of diabetes, random blood sugar, HbA1C, serum creatinine , urine examination, creatinine clearance ,TLC, were assessed. NLR ratio was derived by dividing absolute neutrophil count by absolute lymphocyte count .

The mean age of patients was 59.6±11.4 years (mean ±sd).56% of patients were in 50 to 70 year age group. 68% of subjects in the study were male whereas the remaining 32% were females. The mean NLR in this study was 10.8 with standard deviation of 9.05.The mean NLR in males was 9.53±8.42 (mean±sd) whereas 11.54±10.2 (mean±sd) in females which was statistically not significant ($p>0.05$) .

Using the modified MDRD equation we estimated glomerular filtration rate (eGFR) in all diabetic nephropathy patients included in the study .The mean eGFR was 16.5 ml/min with a standard deviation of 16.9 ml/min. eGFR range was from 2 – 85 ml/min.

The mean serum creatinine was 5.93 mg/dl with standard deviation of 3.66 mg/dl. . There were 5 , 3, 8, 16, and 68 diabetic nephropathy patients in CKD stage 2, 3A,3B, 4 and 5 respectively. Patients with proteinuria grade 1+ was 31 %, 2+ was 31% , 3+ was 36% and 4+ was 2%.. In our study 28 patients were on hemodialysis support while 72 patients were not dependant on hemodialysis .

NLR had statistically significant co-relation with higher CKD stages, lower EGFR, and higher levels of serum creatinine levels in diabetic nephropathy patients. There was no co-relation between NLR with grade of proteinuria, sex, and age.

NLR can be used as a reliable prognostic marker in patients of diabetic nephropathy.

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ANNEXURE I – CONSENT FORM

INFORMED CONSENT

Title Of Research Study: STUDY OF NEUTROPHIL LYMPHOCYTE RATIO AS A NOVEL MARKER OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETES MELLITUS:A ONE YEAR HOSPITAL BASED CROSS SECTIONAL STUDY .

Principal Investigator:- **Guide:-**

- **Introduction and Purpose:**

Diabetic Nephropathy is a well recognised clinical complication of type 2 Diabetes mellitus and the presence and prompt identification of well defined precipitating factors is extremely important in diagnosis and treatment of this condition.

- **Procedure**:

If you agree to be part of the research study, you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood and urine samples for the necessary investigations.

- **Risk and Benefits:**

The only risk and possible discomfort you might get is while taking blood from your arm for the investigations. It may cause swelling, pain, redness (rarely happens) at the site from where the blood is drawn.You may not be benefitted by these investigations but you will be part of this study which is going to be useful to others in the future.

- **Alternatives:**

Taking part in this study is voluntary. You may choose not to take part in this study.

If you decide to take part you can later change your mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may stop your participation in this study at any time. If you choose not to take part in the study, you will receive the standard treatment for patients with your condition.

- **Privacy and Confidentiality:**

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

- **Institution / Sponsor's policy:**

Does not apply to this research

- **Financial incentives for participation:**

You will not be paid / offered any gifts /incentives for participating in the study.

- **Authorisation to publish the results:**

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MD degree, review and publishing.

In case of the queries during study or in future you may contact following persons,

1. Dr. Roopa M Bellad Chairman JNMC Ethical Committee for Human Research, JNMC , Belgaum. 9448113403

CONSENT FORM

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read this consent form, or it has Been read to me, this consent form and have had all the questions answered

Signature / Left Thumb print of the Participant or legally authorised representative

Participant’s name :……………………………...

Signature / Left thumb impression: ……………………………...

of the participant

Name of the legally authorised :……………………………...

representative / guardian

Signature / Left thumb impression :……………………………...

Witness’ name :……………………………...

Signature / Left thumb impression :……………………………...

Investigator’s name and signature :……………………………...

Date:

Place:

ANNEXURE-II

PROFORMA

CASE NO:

NAME:

AGE/SEX:

IP NO.:

ADDRESS:

OCCUPATION

COMPLAINTS AT PRESENTATION:

Past history:

Family history:

Personal history:

Treatment history:

PHYSICAL EXAMINATION:

General Condition:

Pallor- Yes/No
Icterus-Yes/No
Lymphadenopathy- Yes/No
Cyanosis-Yes/No
Clubbing-Yes/No
Edema-Yes/No

VITALS :

Pulse Rate :

Blood Pressure:

Respiratory Rate :

Temperature :

Weight :

SYSTEMIC EXAMINATION:

Respiratory System

Cardiovascular System

Per Abdominal

Central Nervous System

INVESTIGATIONS

CBC:
NL RATIO:

Urine Routine and MicroscopicExamination:

Renal Function Test:

Liver Function Test:

Creatinine Clearance:
eGFR :

HbA1C :

ANNEXURE-III- ETHICAL CLEARANCE LETTER

K.L.E.UNIVERSITY'S
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
(Accredited 'A' Grade by NAAC)

Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+ 91-(0)831 Office : 2471350
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 44 **Date: 22/11/2017**

To,

REG. NO. BG0117011

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"STUDY OF NEUTROPHILS: LYMPHOCYTE RATIO AS A NOVEL MARKER OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETES MELLITUS – ONE YEAR HOSPITAL BASED CROSS SECTIONAL STUDY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURES IV - MASTER CHART

SL NO	NAME	AGE	SEX	IP NO	DIAGNOSIS	VITALS	TLC	N:L RATIO	CREATININE	PROTEINURIA	CREAT CLEARANCE	EGFR	HBA1C	MAINTANENCE HD	RBS	CKD STAGE
1	LAXMAVVA WALIKER	65	F	911362	DIABETIC NEPHROPATHY	STABLE	4,800	14	1.09	1+	37.35	54	6.5	NO	234	3A
2	RAMZAAN NADAF	47	M	909521	DIABETIC NEPHROPATHY	STABLE	9,300	15.2	7.17	3+	13	9	7.5	YES	267	5
3	MADHURI SATWANI	73	F	904323	DIABETIC NEPHROPATHY	STABLE	10,800	6.6	3.94	2+	14	12	NOT DONE	NO	100	5
4	RAFIQ ATTAR	45	M	884067	DIABETIC NEPHROPATHY	STABLE	14,000	7.8	6.01	1+	17	11	NOTDONE	NO	163	5
5	NEELAKANTHA DUNDANATTI	42	F	886833	DIABETIC NEPHROPATHY	STABLE	13,000	30.6	7.44	3+	7	6	NOT DONE	YES	370	5
6	CHANDRASHEKHAR BADIGER	64	M	883802	DIABETIC NEPHROPATHY	STABLE	10,000	5.84	5.52	3+	11	11	NOT DONE	NO	184	5
7	MANU KORAV	51	M	869846	DIABETIC NEPHROPATHY	STABLE	8,600	2.14	10.44	1+	7	6	NOT DONE	NO	110	5
8	MEGHANA KHOT	65	F	876096	DIABETIC NEPHROPATHY	STABLE	6,300	8.4	5.56	1+	14	11	NOT DONE	YES	127	5
9	ARCHANA TUMARI	60	F	872769	DIABETIC NEPHROPATHY	STABLE	7,900	11	11.78	3+	4	3	5.8	NO	273	5
10	RAJAMMA MATHEW	62	F	870971	DIABETIC NEPHROPATHY	STABLE	8,700	2.6	1.31	2+	56	44	13.6	NO	200	3B
11	GUDAPPA SANNAMMANAVAR	62	M	870195	DIABETIC NEPHROPATHY	STABLE	9,200	7.9	9.21	3+	6	13	NOT DONE	NO	133	5
12	SHUSHILA DODABHANGI	55	F	854613	DIABETIC NEPHROPATHY	STABLE	13,600	6.75	4.8	2+	13	10	NOT DONE	NO	140	5
13	PRABHULINGSWAMU CHOUKIMATU	50	M	854470	DIABETIC NEPHROPATHY	STABLE	2,520	3.18	6.49	3+	14	10	5	NO	256	5
14	SAVITRI DANDIN	68	F	854196	DIABETIC NEPHROPATHY	STABLE	9,400	2.37	4.78	2+	13	10	NOT DONE	YES	140	5
15	SANGAMESH KOTI	55	M	861147	DIABETIC NEPHROPATHY	STABLE	10,600	5.43	7.56	3+	14	7	7	NO	200	5
16	SHOBHA RANBHASE	67	F	817321	DIABETIC NEPHROPATHY	STABLE	10,000	10.66	2.67	1+	28	18	NOT DONE	NO	146	4
17	DILAWAR BUDHIHAL	63	M	857229	DIABETIC NEPHROPATHY	STABLE	9,200	10.42	8.8	3+	7	6	NOT DONE	NO	260	5
18	SHOBHA MEGDEKATTE	54	F	933235	DIABETIC NEPHROPATHY	STABLE	6,900	4.93	6.42	3+	16	7	NOT DONE	YES	124	5
19	KAMALLAVA KAMBLE	50	F	870018	DIABETIC NEPHROPATHY	STABLE	10,600	9.55	6.22	1+	12	7	NOT DONE	NO	100	5
20	YALLAPPA SHIRAPUR	70	M	868208	DIABETIC NEPHROPATHY	STABLE	9,100	32	10.88	4+	4	4	NOT DONE	NO	246	5
21	SADASHIV HANJAGI	56	M	855472	DIABETIC NEPHROPATHY	STABLE	13,400	3.5	2.73	3+	29	25	NOT DONE	NO	313	4

22	NEELAMBIA PATIL	65	F	868524	DIABETIC NEPHROPATHY	STABLE	11,600	4.1	5.76	2+	7	7	NOT DONE	NO	120	5
23	NAMDEO KAMBLE	75	M	85775	DIABETIC NEPHROPATHY	STABLE	9,000	4	4.86	2+	10	11	NOT DONE	NO	90	5
24	BASAPPA ASUNDI	53	M	857759	DIABETIC NEPHROPATHY	STABLE	7,800	3.33	3.13	3+	28	22	NOT DONE	NO	148	4
25	RAMANGOUDA CHONCHANNAVAR	40	M	858232	DIABETIC NEPHROPATHY	STABLE	7,900	6.25	8.22	2+	10	7	NOT DONE	YES	121	5
26	RACHAYYA HIREMATH	78	M	857179	DIABETIC NEPHROPATHY	STABLE	11,500	3.94	7.4	1+	6	6	NOT DONE	NO	154	5
27	SANJEEV TALLUR	48	M	869234	DIABETIC NEPHROPATHY	STABLE	9,700	4.23	22.56	3+	9	2	NOT DONE	YES	124	5
28	SHRIKANTH MASAJI	54	M	857055	DIABETIC NEPHROPATHY	STABLE	7,100	2.86	4.24	2+	11	15	NOT DONE	NO	89	5
29	ANUSAYYA SAPTASAGAR	65	F	856721	DIABETIC NEPHROPATHY	STABLE	14,000	18.6	1.55	1+	27	35	7.5	NO	218	3B
30	ISHWAR NAIK	38	M	891739	DIABETIC NEPHROPATHY	STABLE	10,100	20.66	9.58	3+	7	5	NOT DONE	NO	146	5
31	VASHALI PRABHU	64	F	851045	DIABETIC NEPHROPATHY	STABLE	7,200	2.72	5.5	3+	11	8	NOT DONE	NO	200	5
32	PRAMOD BASTAWADI	35	M	851809	DIABETIC NEPHROPATHY	STABLE	9,500	4.75	4.62	3+	7.16	15	NOT DONE	NO	184	4
33	ZUBEIDA KAKATI	80	F	939873	DIABETIC NEPHROPATHY	STABLE	10,800	2.46	6.7	1+	11	5	NOT DONE	YES	200	5
34	IMMAMSAB SANAGOLI	66	M	943435	DIABETIC NEPHROPATHY	STABLE	9.8	14.6	2.57	1+	31	25	9.5	NO	300	4
35	SUDHAKAR SHETTI	59	M	851710	DIABETIC NEPHROPATHY	STABLE	3,600	3.94	9.79	2+	7	5	7.8	NO	100	5
36	ESTHER HALAKI	48	F	898117	DIABETIC NEPHROPATHY	STABLE	6,100	5.06	4.36	3+	15	11	NOT DONE	YES	300	5
37	SAMBHAJI PATIL	60	M	851820	DIABETIC NEPHROPATHY	STABLE	9,000	15	9.14	2+	7	6	NOT DONE	NO	250	5
38	PRAKASHRAO GAIKWAD	73	M	848806	DIABETIC NEPHROPATHY	STABLE	5,900	3.3	2.79	2+	20	22	NOT DONE	NO	246	4
39	ABHIJIT GAVANANG	33	M	850605	DIABETIC NEPHROPATHY	STABLE	5,000	18	9.77	1+	9.43	6	NOT DONE	NO	200	5
40	BALASAHEB PATIL	88	M	848570	DIABETIC NEPHROPATHY	STABLE	5,000	22.5	5.76	3+	5.76	8	NOT DONE	YES	150	5
41	MAHESH DASARADDI	42	M	914255	DIABETIC NEPHROPATHY	STABLE	8,000	5.92	9.62	3+	6.61	6	8.3	YES	201	5
42	RUKKUM BIRADER	58	M	914897	DIABETIC NEPHROPATHY	STABLE	8,100	15.33	3.2	2+	20.64	20	NOT DONE	NO	130	4
43	SHIVARAYAPPPA KALBAVI	72	M	916123	DIABETIC NEPHROPATHY	STABLE	10,000	14.83	6.74	2+	13.61	7	NOT DONE	YES	100	5
44	RAJASHEKARRAYYA HIREMATH	63	M	911489	DIABETIC NEPHROPATHY	STABLE	8,000	9.75	3.28	1+	18.26	19	NOT DONE	NO	146	4
45	GURAPPA PUTTI	48	M	916927	DIABETIC NEPHROPATHY	STABLE	9,700	8.88	15.73	2+	4.39	3	9	YES	132	5
46	CHARLES MULLER	59	M	917905	DIABETIC NEPHROPATHY	STABLE	8,100	10.62	13.48	3+	4.67	4	NOT DONE	YES	150	5
47	NINGAPPA KAMBLE	55	M	915514	DIABETIC NEPHROPATHY	STABLE	5,000	11.25	5.45	1+	12.13	11	NOT DONE	NO	340	5
48	PRAKASH SALENKE	58	M	939911	DIABETIC NEPHROPATHY	STABLE	9,800	4.27	1.23	1+	56	64	NOT DONE	NO	149	2
49	SIDDAPPA DHARWAD	72	M	939915	DIABETIC NEPHROPATHY	STABLE	7,200	4.56	2.09	1+	28	31	9	NO	159	3B
50	ANANT PORE	57	M	938704	DIABETIC NEPHROPATHY	STABLE	10,000	5.71	1.29	2+	58	61	10.5	NO	100	2
51	SAROJA BUCHADI	57	F	937423	DIABETIC NEPHROPATHY	STABLE	10,100	4.8	8.91	1+	5	4	NOT DONE	NO	146	5
52	LAXMAN PATIL	63	M	938920	DIABETIC NEPHROPATHY	STABLE	4,900	3.3	6.22	2+	12	9	NOT DONE	NO	110	5
53	ANBARASAN SELVAM	38	M	931827	DIABETIC NEPHROPATHY	STABLE	8,400	2.32	7.47	3+	11	8	NOT DONE	YES	200	5
54	SOMANNA KAMBLE	65	M	937059	DIABETIC NEPHROPATHY	STABLE	10,000	9.3	5.92	3+	11	9	NOT DONE	YES	120	5
55	MANAJI KALAL	70	M	936729	DIABETIC NEPHROPATHY	STABLE	8,000	32	8.43	3+		6	NOT DONE	YES	230	5

56	VAMAN JADHAV	58	M	936447	DIABETIC NEPHROPATHY	STABLE	7,600	10.5	5.27	2+	13	11	10.7	YES	90	5
57	SUMANGALA CHIKODI	71	F	932912	DIABETIC NEPHROPATHY	STABLE	5,000	31.66	6.94	1+	9	5	NOT DONE	NO	200	5
58	SHAKUNTALA PATIL	75	F	927769	DIABETIC NEPHROPATHY	STABLE	9,000	23.5	5.29	3+	12	7	NOT DONE	NO	90	5
59	NEELAVATHI PATIL	72	F	932038	DIABETIC NEPHROPATHY	STABLE	8,900	11	5.47	1+	10	7	8	NO	350	5
60	SHOBHA HENADRI	63	F	883965	DIABETIC NEPHROPATHY	STABLE	6,000	32	6.67	3+	6	6	8.4	NO	200	5
61	VIJAY MALI	48	M	926963	DIABETIC NEPHROPATHY	STABLE	3,000	42	10.34	1+	6.2	5	NOT DONE	YES	140	5
62	PRAKASH DESHMUKH	66	M	662574	DIABETIC NEPHROPATHY	STABLE	7,500	8	8.08	3+	10.2	6	6.9	NO	100	5
63	BASAVARAJ SHIVASHINPI	47	M	890525	DIABETIC NEPHROPATHY	STABLE	9,500	3.13	2.21	3+	21.3	34	7.6	NO	120	3B
64	VASAPPA BAME	60	M	918158	DIABETIC NEPHROPATHY	STABLE	9,100	13.1	11.36	2+	6.78	4	NOT DONE	NO	132	5
65	SHOBHA MULLOLI	49	F	812365	DIABETIC NEPHROPATHY	STABLE	5,000	30	9.8	3+	6.21	4	8.1	YES	89	5
66	PARAPPA YALIGOUDA	70	M	930454	DIABETIC NEPHROPATHY	STABLE	8,400	6	5.98	2+	8.94	9	NOT DONE	NO	300	5
67	SUSHILA NANDRE	66	F	928943	DIABETIC NEPHROPATHY	STABLE	4,900	2.7	9.74	2+	7.14	4	7.3	YES	162	5
68	SHRINIVAS MEDIKERI	64	M	905574	DIABETIC NEPHROPATHY	STABLE	7,100	2.32	3.13	1+	22	20	10.1	NO	298	4
69	SAVITA MAVAL	64	F	919026	DIABETIC NEPHROPATHY	STABLE	2,280	9.2	6.11	1+	8.81	7	NOT DONE	NO	195	5
70	RAJANI BORGI	63	F	919896	DIABETIC NEPHROPATHY	STABLE	6,400	2.8	3.35	2+	22	14	NOT DONE	NO	102	5
71	LENKANAGOUDA ARAKERI	70	M	920755	DIABETIC NEPHROPATHY	STABLE	10,000	3	2.91	3+	22.05	21	NOT DONE	NO	265	4
72	VASANT LONDHE	61	M	921157	DIABETIC NEPHROPATHY	STABLE	9,500	4.54	7.91	2+	8.32	7	NOT DONE	NO	170	5
73	SHAMBHULINGAPPA MAHANSHETTI	67	M	922164	DIABETIC NEPHROPATHY	STABLE	8,100	3.21	3.08	2+	26.33	20	6.3	NO	100	4
74	YAMANAVVA HARIGERI	55	F	924293	DIABETIC NEPHROPATHY	STABLE	9,800	3.6	1.82	3+	33.08	31	NOT DONE	NO	310	3B
75	JANARDHAN PATIL	79	M	924076	DIABETIC NEPHROPATHY	STABLE	7,500	14.33	7.88	1+	6.45	6	NOT DONE	NO	230	5
76	RANGAGOUDA PATIL	46	M	891844	DIABETIC NEPHROPATHY	STABLE	6,000	12.7	4.72	3+	15.21	14	12	NO	410	5
77	LAXMIBAI KALYAGOL	46	F	925787	DIABETIC NEPHROPATHY	STABLE	4,300	30	4.9	1+	13.6	10	NOT DONE	NO	210	5
78	DEVIRAMMA ODANAVAR	59	M	899314	DIABETIC NEPHROPATHY	STABLE	8,000	2.031	3.19	3+	26.38	20	6.6	NO	110	4
79	RAMESH KHADED	59	M	925210	DIABETIC NEPHROPATHY	STABLE	4,000	8.3	2.86	3+	34.62	23	NOT DONE	NO	220	4
80	SANJAY HONAWAD	58	M	926721	DIABETIC NEPHROPATHY	STABLE	3,500	9.1	7.54	2+	8.46	7	NOT DONE	YES	150	5
81	SHARANAPPA CHOUDHRI	57	M	940704	DIABETIC NEPHROPATHY	STABLE	4,800	5.57	2.13	2+	44	33	7.8	YES	270	3B
82	SHANKAR SAIBANNAVAR	68	M	943616	DIABETIC NEPHROPATHY	STABLE	7,000	7.03	0.92	1+	83	85	8.8	NO	130	2
83	ASHA SATTIGERI	61	F	943453	DIABETIC NEPHROPATHY	STABLE	3,500	18	0.94	1+	50	66	10.1	no	300	2
84	TULSABAI NAGONI	75	F	940717	DIABETIC NEPHROPATHY	STABLE	5,700	2.7	4.65	1+	4	9	8.6	NO	200	5
85	KUBER SULAKUDE	68	M	940715	DIABETIC NEPHROPATHY	STABLE	9,000	6.6	2.7	2+	20	23	NOT DONE	NO	150	4
86	APPASAHEB PATIL	71	M	980756	DIABETIC NEPHROPATHY	STABLE	6,900	2.17	1.11	1+	69	66	6.4	NO	110	2
87	BASAPPA ULLAGADDI	51	M	926647	DIABETIC NEPHROPATHY	STABLE	8,900	6.75	1.77	1+	45	44	NOT DONE	NO	90	3B
88	KASHIBAI PATIL	58	F	929108	DIABETIC NEPHROPATHY	STABLE	5,000	22.25	3.66	1+	5	13	NOT DONE	NO	230	5
89	HALIMA SAYYED	52	F	925560	DIABETIC NEPHROPATHY	STABLE	6,700	1.9	2.16	4+	31	22	NOT DONE	NO	130	4

90	BHUTAPPA WADER	72	M	927321	DIABETIC NEPHROPATHY	STABLE	4,400	5.06	7.4	2+	5	7	NOT DONE	YES	200	5
91	ASHOK AVATI	48	M	888946	DIABETIC NEPHROPATHY	STABLE	9,800	12.28	5.42	2+	10	11	NOT DONE	NO	134	5
92	CHANDRASHEKHAR	59	M	926933	DIABETIC NEPHROPATHY	STABLE	10,000	5.84	5.52	3+	15	10	NOT DONE	NO	280	5
93	MALLIKARJUN INCHALMATH	42	M	924420	DIABETIC NEPHROPATHY	STABLE	7,500	17.8	8.93	1+	10	7	NOT DONE	YES	170	5
94	KHAITAN CRUZ	75	M	889751	DIABETIC NEPHROPATHY	STABLE	10,300	3.6	1.72	2+	42	38	NOT DONE	NO	280	3B
95	BASAPPA JULPI	46	M	927428	DIABETIC NEPHROPATHY	STABLE	9,000	9.77	4.3	2+	19	15	6.3	NO	130	4
96	RAVINDRA SHETTI	84	M	941981	DIABETIC NEPHROPATHY	STABLE	9,500	1.61	1.18	3+	37	56	9.7	NO	300	3A
97	BASAVARAJ SUTAR	70	M	941955	DIABETIC NEPHROPATHY	STABLE	8,600	2.28	1.24	1+	47	59	8.8	NO	140	3A
98	SHUSHILA RAJANATH	65	F	596529	DIABETIC NEPHROPATHY	STABLE	5,000	3	9.7	2+	7.14	4	7.3	YES	200	5
99	NEELAKANTHA NAGAPPA	43	M	894439	DIABETIC NEPHROPATHY	STABLE	10,000	32.6	15.53	3+	5	3	NOT DONE	YES	340	5
100	PRAMOD SHIVAPPA	35	M	857559	DIABETIC NEPHROPATHY	STABLE	3,800	28.27	7.02	3+	12	10	NOT DONE	YES	138	5

ANNEXURE-V

KEY TO MASTER CHART

SI.NO	:	SERIAL NUMBER
I.P NO	:	IN- PATIENT NUMBER
TLC	:	TOTAL LEUCOCYTE COUNT
N : L RATIO	:	NEUTROPHIL LYMPHOCYTE RATIO
CREAT CLEARANCE	:	CREATININE CLEARANCE
EGFR	:	ESTIMATED GLOMERULAR FILTRATION RATE
HbA1C	:	HEMOGLOBIN A1C
HD	:	HEMODIALYSIS
RBS	:	RANDOM BLOOD SUGARS
CKD STAGE	:	CHRONIC KIDNEY DISEASE STAGE

Introduction

Objectives

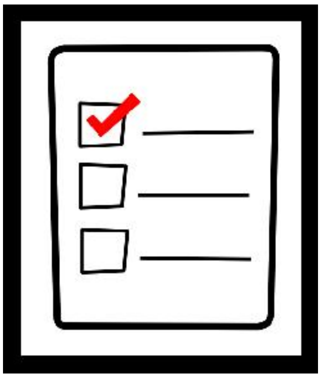
Review of Literature

Methodology

Results

Discussion

Conclusion

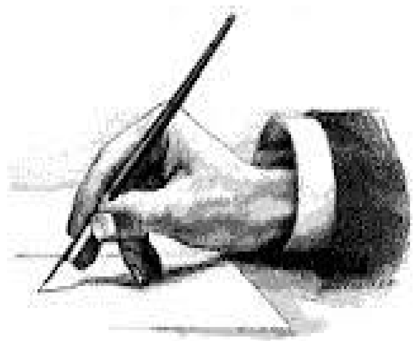


Limitations

Recommendations

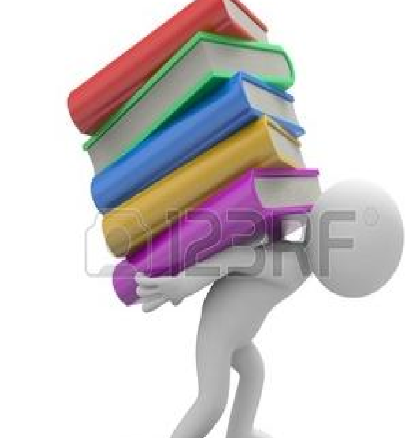
Summary

Bibliography



Annexure-I

Annexure-II



Annexure-III

Annexure-IV

Annexure-V