"IMPAIRED PROTEIN TOLERANCE TEST" AS A MARKER OF EARLY RENAL DYSFUNCTION IN TYPE 2 DIABETES MELLITUS

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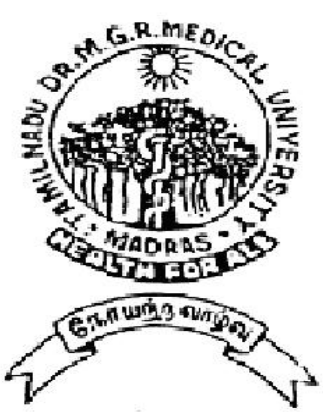

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CERTIFICATE

This is to certify that this dissertation titled "**Impaired Protein tolerance test'' as a marker of early Renal dysfunction in Type 2 Diabetes Mellitus**" submitted by **DR.D.P.PUNITHA** to the faculty of General Medicine, The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of the requirement for the award of MD degree branch I General Medicine, is a bonafide research work carried out by her under our direct supervision and guidance.

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CONTENTS

Abbreviations

AGEs -advanced glycosylation end products

AT- Angiotensin

BG – Blood Glucose

BMI – Body Mass Index

BS-Blood Sugar

CAD- Coronary Artery Disease

CKD-Chronic Kidney Disease

DM- Diabetes Mellitus

DN-Diabetic Nephropathy

IDF-International Diabetes Federation

ESRD-End-Stage Renal Disease

e-GFR-Estimated Glomerular Filtration Rate

GTT-Glucose Tolerance Test

MDRD-Modification of Diet in Renal Disease

PCR-Protein Creatinine Ratio

PTT-Protein Tolerance Test

SC-Serum Creatinine

TGF-β Transforming growth factor β

TScr -Tubular Secretion of creatinine

VEGF -vascular endothelial growth factor

INTRODUCTION

India is frequently referred to as the diabetic capital of the world. Diabetes mellitus is widely prevalent in our country and its incidence is rising in alarming proportions. The worldwide prevalence[1] of diabetes has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 177 million in 2000. Based on current trends, >360 million individuals worldwide will have diabetes by the year 2030. Although the prevalence of both type 1 and type 2 diabetes is increasing worldwide, the prevalence of type 2 diabetes is rising much more rapidly because of increasing obesity and reduced activity levels as countries become more industrialized. Worldwide estimates project that in 2030 the greatest number of individuals with diabetes will be 45–64 years of age .

According to the Diabetes Atlas published by the International Diabetes Federation (IDF), there are an estimated 40 million persons with diabetes in India in 2007, now it has risen to 60 million in 2009 and this number is predicted to rise to almost 120 million people in 2025 by which time every fifth diabetic subject in the world would be an Indian. Diabetes is a major cause of mortality, but several studies indicate that diabetes is likely under reported as a cause of death. A recent estimate suggested that diabetes was the fifth leading cause of death worldwide and was responsible for almost 3 million deaths annually (1.7–5.2% of deaths worldwide).

Diabetes is the most common cause of kidney failure[2], accounting for nearly 44 percent of new cases. More than 100,000 people are diagnosed with kidney failure, a serious condition in which the kidneys fail to rid the body of wastes. Kidney failure is the final stage of chronic kidney disease (CKD). Even when diabetes is controlled, the disease can lead to CKD and kidney failure. Most people with diabetes do not develop CKD that is severe enough to progress to kidney failure. There are interplay of factors leading to kidney disease of diabetes—factors[3] including heredity, diet, and other medical conditions, such as high blood pressure. It has been found that high blood pressure and high levels of blood glucose increase the risk that a person with diabetes will progress to kidney failure. So there is a need to identify subnormal kidney function[4] at an earlier stage in order to initiate treatment in retarding the progression of kidney damage. Glucose tolerance test (GTT) has been used to assess the patient at risk of diabetes mellitus. The stress of glucose load in GTT unravels the patient with marginal pancreatic dysfunction. It has been suggested that analogous to GTT, a protein tolerance test (PTT) may help in identifying individuals with subnormal renal function before they manifest clinically. Increased serum creatinine level is often considered the first sign of a renal problem, but now, it may not be so. Microalbuminuria, another test to detect early renal dysfunction has now been used as a marker of endothelial dysfunction. By the time the serum creatinine levels increase, a good amount of

irreversible kidney damage is done .so, now this stresses the need for the tolerance test on the kidney in patients who have low glomerular filtration rate.

An acute oral protein load causes a transient hyperfiltration that might reveal a loss of glomerular permselectivity properties .So, acute protein load test is of great utility in revealing a silent glomerular filtration disturbance.

The stress of PTT will enable us to determine individuals with impaired functional reserve. The present study utilises this principle to identify those patients with diabetes who are at risk of developing renal failure.

REVIEW OF LITERATURE

DIABETES MELLITUS

Diabetes mellitus (DM) refers to a group of metabolic disorders which have a common denominator namely hyperglycemia. Factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system.[5]

Classification

DM is classified on the basis of the pathogenic process that leads to hyperglycemia. The two broad categories of DM are designated type 1 and type 2.

- Type 1 diabetes is the result of complete or near-total insulin deficiency.
- Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Type 2 DM is preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).

ETIOLOGIC CLASSIFICATION OF DIABETES MELLITUS

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

A. Immune-mediated

B. Idiopathic

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

III. Other specific types of diabetes

A. Genetic defects of beta cell function characterized by mutations in

1. Hepatocyte nuclear transcription factor (HNF) 4 (MODY 1)
2. Glucokinase (MODY 2)
3. HNF-1 (MODY 3)
4. Insulin promoter factor-1 (IPF-1; MODY 4)
5. HNF-1 (MODY 5)
6. NeuroD1 (MODY 6)
7. Mitochondrial DNA
8. Subunits of ATP-sensitive potassium channel
9. Proinsulin or insulin conversion

B. Genetic defects in insulin action

1. Type A insulin resistance

2. Leprechaunism

3. Rabson-Mendenhall syndrome

4. Lipodystrophy syndromes

C. Diseases of the exocrine pancreas—pancreatitis, pancreatectomy, neoplasia, cystic fibrosis, hemochromatosis, fibrocalculous pancreatopathy, mutations in carboxyl ester lipase.

D. Endocrinopathies—acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma

E. Drug- or chemical-induced—Vacor, pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, beta-adrenergic agonists, thiazides, phenytoin, protease inhibitors, clozapine

F. Infections—congenital rubella, cytomegalovirus, coxsackie

G. Uncommon forms of immune-mediated diabetes—"stiff-person" syndrome, anti-insulin receptor antibodies

H. Other genetic syndromes sometimes associated with diabetes—Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram's syndrome, Friedreich's ataxia, Huntington's chorea, Laurence-Moon-Biedl syndrome, myotonic dystrophy, porphyria, Prader-Willi syndrome

IV. Gestational diabetes mellitus (GDM)

Glucose intolerance may develop during pregnancy. Insulin resistance is related to the metabolic changes of late pregnancy and the increased insulin

requirements may lead to IGT. GDM occurs in ~4% of pregnancies in the United States; most women revert to normal glucose tolerance post-partum but have a substantial risk (30–60%) of developing DM later in life. [5]

Diagnosis of diabetes mellitus

The National Diabetes Data Group and World Health Organization (WHO) have issued certain diagnostic criteria:

- Symptoms of diabetes plus random blood glucose concentration 11.1 mmol/L (200 mg/dL)[a] (or)
- Fasting plasma glucose 7.0 mmol/L (126 mg/dL)[b] (or)
- Two-hour plasma glucose 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test[c]

[a]Random is defined as without regard to time since the last meal.

[b]Fasting is defined as no caloric intake for at least 8 h.

[c]The test should be performed using a glucose load containing the equivalent of 75gms anhydrous glucose dissolved in water; not recommended for routine clinical use.

Glucose tolerance is classified into three categories based on the FPG:

(1) FPG < 5.6mmol/L (100 mg/dL) is considered normal

(2) FPG = 5.6–6.9 mmol/L (100–125 mg/dL) is defined as IFG

(3) FPG ≥ 7.0 mmol/L (126 mg/dL) warrants the diagnosis of DM.

Based on the OGTT, IGT is defined as plasma glucose levels between 7.8 and 11.1 mmol/L (140 and 199 mg/dL) and diabetes is defined as a glucose > 11.1

mmol/L (200 mg/dL) 2 h after a 75-g oral glucose load. Some individuals have both IFG and IGT. Individuals with IFG and/or IGT, recently designated 'pre-diabetes' by the American Diabetes Association (ADA), are at substantial risk for developing type 2 DM (25–40% risk over the next 5 years) and have an increased risk of cardiovascular disease. [6]

Risk Factors for Type 2 Diabetes Mellitus

1. Family history of diabetes (i.e., parent or sibling with type 2 diabetes)
2. Obesity (BMI >25 kg/m^2)
3. Habitual physical inactivity
4. Race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
5. Previously identified IFG or IGT
6. History of GDM or delivery of baby >4 kg (>9 lb)
7. Hypertension (blood pressure >140/90 mmHg)
8. HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
9. Polycystic ovary syndrome or acanthosis nigricans
10. History of vascular disease

Pathophysiology

Type 2 DM is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. Obesity, particularly visceral or central (as evidenced by the hip-waist ratio), is

very common in type 2 DM. In the early stages of the disorder, glucose tolerance remains near-normal, despite insulin resistance, because the pancreatic beta cells compensate by increasing insulin output. As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets in certain individuals are unable to sustain the hyperinsulinemic state. IGT, characterized by elevations in postprandial glucose, then develops. A further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia. Ultimately, beta cell failure may ensue.[7]

DIABETIC NEPHROPATHY

The natural history of Diabetic Nephropathy in patients with type 2 DM is less well understood than in patients with type 1 DM. This partly reflects the fact that type 2 DM is largely a disease of an older population with associated obesity ,hypertension ,dyslipidemia and high rates of cardiovascular disease that restrict the manifestation of renal disease. In addition approximately 7% of the patients with type 2 DM already have microalbuminuria at the time of diagnosis.With in 5 years of diagnosis 18% have microalbuminuria especially those with poor metabolic control and high blood pressure levels[8.]

It is commoner to see more patients of type 2 DM with nephropathy than those with type 1 DM (9:1) even though the incidence of nephropathy is high in type 1 DM(30%) when compared to type 2 DM(20%)[9]

Definition

Nephropathy is one of the commonest complications of type 2 diabetes mellitus. Diabetic nephropathy[10] (DN) is typically defined by macroalbuminuria—that is, a urinary albumin excretion of more than 300 mg in a 24-hour collection and abnormal renal function as represented by an abnormality in serum creatinine, calculated creatinine clearance, or glomerular filtration rate (GFR). Clinically, diabetic nephropathy is characterized by a progressive increase in proteinuria and decline in GFR, hypertension, and a high risk of cardiovascular morbidity and mortality[11].

Prevalence and risk factors[12]

Diabetes has become the primary cause of end-stage renal disease (ESRD) in the United States, and the incidence of type 2 diabetes mellitus continues to grow in the United States and worldwide. Approximately 44% of new patients entering dialysis in the United States are diabetics. Approximately 20% to 30% of all diabetics will develop evidence of nephropathy, although a higher percentage of type 1 patients progress to ESRD[13].

Risk factors for diabetic nephropathy[14] include:

-African American, Hispanic, or American Indian origin

-Family history of kidney disease or high blood pressure

-Poor control of blood pressure

-Poor control of blood sugars

-Type 1 diabetes before age 20

-Smoking

Diabetic nephropathy generally coexists along with other diabetes complications including hypertension, retinopathy, and atherosclerosis.

Pathophysiology and natural history

The common progression from microalbuminuria to overt nephropathy has led many to consider microalbuminuria to define early or incipient nephropathy.[15] Renal disease is suspected to be secondary to diabetes in the clinical setting of long-standing diabetes. This is supported by the history of diabetic retinopathy, particularly in type 1 diabetics, in whom there is a strong correlation. The natural history of diabetic nephropathy is a process that progresses gradually over years.

Early diabetes is heralded by glomerular hyperfiltration and an increase in GFR. This is believed to be related to increased cell growth and expansion in the kidneys, possibly mediated by hyperglycemia itself. Microalbuminuria typically occurs after 5 years in type 1 diabetes.[16] Overt nephropathy, with urinary protein excretion higher than 300 mg/day, often develops after 10 to 15 years. ESRD develops in 50% of type 1 diabetics, with overt nephropathy within 10 years.

Type 2 diabetes has a more variable course. Patients often present at diagnosis with microalbuminuria because of delays in diagnosis and other factors affecting protein excretion. Fewer patients with microalbuminuria progress to advanced renal disease. Without intervention, approximately 30% progress to overt nephropathy and, after 20 years of nephropathy, approximately 20% develop ESRD. Because of the high prevalence of type 2

compared with type 1 diabetes; however, most diabetics on dialysis are type 2 diabetics.

Long-standing hyperglycemia is known to be a significant risk factor for the development of diabetic nephropathy.[17] Hyperglycemia may directly result in mesangial expansion and injury by an increase in the mesangial cell glucose concentration. The glomerular mesangium expands initially by cell proliferation and then by cell hypertrophy. Increased mesangial stretch and pressure can stimulate this expansion, as can high glucose levels.[18] Transforming growth factor β (TGF-β) is particularly important in the mediation of expansion and later fibrosis via the stimulation of collagen and fibronectin. Glucose can also bind reversibly and eventually irreversibly to proteins in the kidneys and circulation to form so-called advanced glycosylation end products (AGEs) [19]. AGEs can form complex cross-links over years of hyperglycemia and can contribute to renal damage by stimulation of growth and fibrotic factors via receptors for AGEs. In addition, mediators of proliferation and expansion, including platelet-derived growth factor, TGF-β, and vascular endothelial growth factor (VEGF) that are elevated in diabetic nephropathy can contribute to further renal and microvascular complications.

Proteinuria, a marker and potential contributor to renal injury, accompanies diabetic nephropathy. Increased glomerular permeability will allow plasma proteins to escape into the urine. Some of these proteins will be taken up by the proximal tubular cells, which can initiate an inflammatory response that contributes to interstitial scarring eventually leading to fibrosis.

Tubulointerstitial fibrosis is seen in advanced stages of diabetic nephropathy and is a better predictor of renal failure than glomerular sclerosis. Hyperglycemia, angiotensin II, TGF-β, and likely proteinuria itself all play roles in stimulating this fibrosis.[20] There is an epithelial-mesenchymal transition that takes place in the tubules, with proximal tubular cell conversion to fibroblast-like cells. These cells can then migrate into the interstitium and produce collagen and fibronectin[21]. In diabetic nephropathy, the activation of the local renin-angiotensin system occurs in the proximal tubular epithelial cells, mesangial cells, and podocytes. Angiotensin II (ATII) itself contributes to the progression of diabetic nephropathy. ATII is stimulated in diabetes despite the high-volume state typically seen with the disease, and the intrarenal level of ATII is typically high, even in the face of lower systemic concentrations. ATII preferentially constricts the efferent arteriole in the glomerulus, leading to higher glomerular capillary pressures. In addition to its hemodynamic effects, ATII also stimulates renal growth and fibrosis through ATII type 1 receptors, which secondarily upregulate TGF-β and other growth factors.

Control of hypertension has clearly shown to be an important and powerful intervention in decreasing the progression of diabetic nephropathy.[22] In diabetics who have disordered autoregulation at the level of the kidney, systemic hypertension can contribute to endothelial injury.[23] Human studies of type 2 diabetics have shown that blood pressure lowering, regardless of the agent used, retards the onset and progression of diabetic nephropathy. In animal studies, the degree and severity of the diabetic nephropathy were

strongly linked to systemic blood pressure. The fact that most types 1 and 2 diabetics do not develop diabetic nephropathy (DN) suggests that other factors may be involved. Genetic factors clearly play a role in the predisposition to diabetic nephropathy in family members who have DN, and linkage to specific areas on the human genome is evolving.[24] The theory of a reduction in nephron number at birth indicates that individuals born with a reduced number of glomeruli may be predisposed to subsequent renal injury and progressive nephropathy. This has been shown in animal studies in which the mother was exposed to hyperglycemia at the time of pregnancy. If this linkage is true in humans, that would have important implications concerning the role of maternal factors in the eventual development of kidney disease.

The Course of Kidney Disease in Diabetes

Diabetic kidney disease takes many years to develop. In some people, the filtering function of the kidneys is actually higher than normal in the first few years of their diabetes. Over several years, people who are developing kidney disease will have small amounts of the blood protein albumin begin to leak into their urine. This first stage of CKD is called microalbuminuria. The kidney's filtration function usually remains normal during this period. As the disease progresses, more albumin leaks into the urine.[25] This stage may be called macroalbuminuria or proteinuria.

As the amount of albumin in the urine increases, the kidneys' filtering function usually begins to drop. The body retains various wastes as filtration falls. As kidney damage develops, blood pressure often rises as well. Overall,

kidney damage rarely occurs in the first 10 years of diabetes, and usually 15 to 25 years will pass before kidney failure occurs. For people who live with diabetes for more than 25 years without any signs of kidney failure, the risk of ever developing it decreases.

Diagnosis of CKD

People with diabetes should be screened regularly for kidney disease.[26] Some of the abnormalities detected in renal dysfunction are as follows.

Marker	Findings Indicating Kidney Damage
Proteinuria	Increased excretion of albumin or total urine protein
Urine Sediment Abnormalities	Red blood cells*, white blood cells*, cellular casts, coarse granular casts, fat
Imaging Tests	Abnormalities in kidney size Asymmetry in kidney size or function Irregularities in shape (cysts, scars, mass lesions) Stones Hydronephrosis and other abnormalities of the urinary tract Arterial stenosis and other vascular lesions
Abnormalities in Blood or Urine Composition	Nephrotic syndrome Tubular syndromes (renal tubular acidosis, potassium secretory defects, renal glycosuria, renal phosphaturia, Fanconi's syndrome)

*Red blood cells (hematuria) or white blood cells (pyuria) may originate at any site in the urinary tract, and do not necessarily indicate kidney damage. Patients with hematuria and pyuria should be evaluated for CKD

The two key markers for kidney disease are eGFR and urine albumin.eGFR- eGFR stands for estimated glomerular filtration rate[27]. The calculation of eGFR is based on the amount of creatinine, a waste product, found in a blood sample. As the level of creatinine goes up, the eGFR goes down. Glomerular filtration rate (GFR) measures the amount of glomerular filtrate (a substance similar to the plasma part of blood but without the proteins) formed in the kidneys per minute. The results help indicate the kidney's ability

to filter and remove waste products from the body. The following table shows the creatinine values corresponding to GFR.[28]

Serum Creatinine Corresponding to an Estimated GFR of 60 mL/min/1.73 m2 by the Abbreviated MDRD Study and Cockcroft-Gault Equations

	MDRD Study Equation					
	Non-African American		African-American		Cockcroft-Gault Equation	
Age (Years)	Men	Women	Men	Women	Men	Women
30	1.47	1.13	1.73	1.34	1.83	1.56
40	1.39	1.08	1.65	1.27	1.67	1.42
50	1.34	1.03	1.58	1.22	1.50	1.28
60	1.30	1.00	1.53	1.18	1.33	1.13
70	1.26	0.97	1.49	1.15	1.17	0.99
80	1.23	0.95	1.46	1.12	1.00	0.85

Calculations in this table use serum creatinine values obtained in the MDRD Study Central Laboratory, which were a mean of 0.23 mg/dL lower than duplicate samples analyzed at the NHANES III Central Laboratory. Calculations in this table assume a weight of 72 kg and body surface area (BSA) of 1.73 m². Units for serum creatinine are mg/dL (multiply by 88.4 µmol/L = 1 mg/dL). Reprinted with permission.[1]

GFR is used to screen for early signs of kidney damage. In people already diagnosed with kidney disease (nephropathy), GFR is used to monitor the patient for signs of deterioration in kidney function. In general, values are interpreted based on the following table[29]:

Stage of disease	Description of condition	GFR (mL/min/ 1.73 mÂ²)
At an increased risk	Risk factors for kidney disease (e.g., diabetes, high blood pressure, family history of kidney disease, older age, ethnic group)	More than 90
1	Kidney damage (proteinuria) and normal GFR	More than 90
2	Kidney damage and slight decrease in GFR	60-89
3	Moderate decrease in GFR	30-59
4	Severe decrease in GFR	15-29
5	Kidney failure	Less than 15

The benefits of GFR include:

-Measuring the filtering capacity of the kidneys.

-Outlining the progression of kidney disease.

-Predicting the time to onset of kidney failure.

-Predicting the risk of complications of chronic kidney disease.

-Providing physicians with information that may be needed for determining medication dosage.

Types and differences of GFR

The glomerular filtration rate (GFR) of the kidneys cannot be directly measured. However, various methods have been developed to provide indirect measurements and estimates.[30]

One such method is an inulin clearance test[31]. Inulin, a complex fructose sugar (a sugar found in fruit), is considered an ideal filtration marker for the measurement of GFR in humans. Inulin is injected into the patient and the amount of inulin filtered at the glomeruli (blood vessels in the kidney) normally equals the amount of excreted inulin. However, this method is not often used because it is costly, inconvenient and better suited for research studies. It is also difficult to perform on infants.

The use of radioactive markers (radioactive marker clearance test) also provides an accurate measurement of GFR.[32] However, they are not readily

available in many centers. The most commonly used measurements of GFR are serum creatinine tests and 24-hour creatinine clearance tests.

Creatinine is a waste product that comes from two sources: meat products in the diet and muscle use. Almost all creatinine eventually ends up in a person's urine. Creatinine measurements from blood and urine samples are used to calculate GFR because the chemical is normally present in the bodyand very little of it is reabsorbed. A serum creatinine test measures the level of creatinine in the blood.[33]

A creatinine clearance test compares the levels of creatinine in the urine and the blood, along with urine volume. A 24-hour urine sample is usually collected and a blood sample is taken from a vein, and the estimated GFR is calculated.

There are certain drawbacks to using a creatinine clearance test to calculate GFR.[34] These include:

- Collecting a 24-hour urine sample may be inconvenient for the patient.
- The level of creatinine excreted in urine varies. The levels differ from day to day, and thus even a 24-hour sample can yield inaccurate GFR estimations.
- Errors are common during the collection of a urine sample, and these errors can influence test results.
- Creatinine is secreted by the kidneys. In addition to being filtered by the kidneys, a small amount of creatinine is also secreted by the kidneys. As a result, the amount of creatinine excreted in the urine is the

combination of both the filtered and secreted creatinine and is not exactly equivalent to the GFR. This usually results in overestimating the GFR of the kidneys.

Another method of estimating a patient's GFR is by using a serum creatinine based prediction equation. These equations are more accurate in estimating GFR than serum creatinine measurements alone. Several equations have been developed. The equations are useful because they take into consideration that creatinine production varies according to age, gender, race or ethnicity, and muscle mass. All or some of these factors are then used along with the serum creatinine concentration to estimate a patient's GFR.

Equations to Estimate GFR from Serum Creatinine Concentration

Adults	
Cockcroft-Gault equation [123]	$C_{Cr}(\text{ml/min}) = \frac{(140 - Age) \times Weight}{72 \times S_{Cr}} \times (0.85 \text{ if female})$
Abbreviated MDRD Study equation [124, 125]	$GFR(\text{ml/min/1.73m}^2) = 186 \times (S_{Cr})^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African - American})$
Children	
Schwartz formula [126*]	$C_{Cr}(\text{ml/min}) = \frac{0.55 \times Length}{S_{Cr}}$
Counahan-Barratt equation [127]	$GFR(\text{ml/min/1.73m}^2) = \frac{0.43 \times Length}{S_{Cr}}$

GFR, glomerular filtration rate; C_{Cr}, creatinine clearance; S_{Cr}, serum creatinine in mg/dL; age, in years; weight, in kg; length, in cm.
*Formula for children >1 year. Coefficients vary for low birth-weight infants to 1 year (0.33), term infants up to 1 year (0.45), adolescent girls (0.55), and adolescent boys (0.70)

The Cockcroft-Gault and Jelliffe were originally developed for estimating creatinine clearance. However, they have been widely tested as predictors of GFR in adults.

A newly developed equation, the MDRD Study equation, also provides an estimate of GFR in adults. The abbreviated version of the equation is based

on serum creatinine concentration, age, gender and race and is standardized for body surface area. The National Institute of Diabetes and Digestive and Kidney Diseases, the National Kidney Foundation and the American Society of Nephrology recommend estimating GFR from serum creatinine concentration using the MDRD Study equation.[35]

Equations to estimate GFR in children have also been developed.[36] Although imprecise, these equations are considered practical and convenient. They both estimate GFR based on a constant multiplied by the patient's height (height is proportional to muscle mass) divided by the serum creatinine. The MDRD Study equation has not been widely tested in children and its' reliability is unknown.

There are some drawbacks to using prediction equations. One is that the equations are much less accurate at measuring a higher range of GFR, such as occurs in a healthy person or in the early stages of chronic kidney disease. As a result, other indications of early kidney disease, such as proteinuria (abnormally high levels of protein in the urine) are needed to detect early deterioration in kidney function. In newly diagnosed type 2 diabetic patients, particularly those with a GFR >/=90 ml/min per 1.73 m2, both CG and MDRD equations significantly underestimate eGFR. This highlights a limitation in the use of eGFR in the majority of diabetic subjects outside the setting of chronic kidney disease.

There are also some situations in which the GFR estimate provided by a creatinine clearance test is more desirable than that based on a prediction

equation. This is because certain individual variations (e.g., diet and muscle mass) are not taken into consideration in prediction equations. These situations include:

-Extremes of age

-Extremes of body size

-Disease of the skeletal muscles

-Paraplegia or quadriplegia

-Vegetarian diet

-Use of creatine (dietary) supplements

-Rapid changes in kidney function

-Amputation

-Malnutrition

-Muscle wasting

-Pregnancy

Cystatin C test is another method of detecting and monitoring kidney malfunction.[37] Cystatin C is found in most cells, is filtered out of the blood by the glomeruli and forms a fluid filtrate. The cystatin C left in the filtrate is then reabsorbed by the body and not returned to the blood. When the kidneys malfunction, the blood levels of cystatin C increase, and the test can reflect the reduction in the formation of fluid filtrate. The increased levels of cystatin C may be detected before there is a decrease in the GFR. In addition, gender, muscle mass and race or ethnicity does not influence the test. The cystatin C

test may become a diagnostic standard in detecting kidney malfunction in the future.

Once calculated, people's GFR can be used to measure their level of kidney function and/or determine their stage of kidney disease.

In general, an estimated GFR greater than or equal to 90 milliliters per minute per 1.73 square meters (mL/min/1.73 mÂ²) is normal. The 1.73 mÂ² value represents the average adult body surface area in square meters.

An estimated GFR less than 90 mL/min/1.73 mÂ² is abnormal.

However, normal value ranges may vary among laboratories. In addition to aging and kidney disease, there are several other factors that may affect GFR.

Factors that can decrease GFR include:

-Vascular diseases

-Congestive heart failure

-Sodium and water depletion (dehydration)

-Hemorrhage

-Vigorous exercise

-Shock

Factors that can increase GFR include:

-Dietary protein intake

-Ketoacidosis

-Hyperglycemia (high blood sugar)

-Pregnancy

PROTEIN TOLERANCE TEST[38]

In this test, a patient is exposed to high level of protein and his/her GFR is calculated in a span of two-three days, the GFR should increase by 20% without protein leaking into the urine. Glomerular Filtration Rate (GFR) is the most widely used indicator of kidney function in patients with renal disease. The severity and prognosis of the renal disease is often predicted on basis of this parameter alone. The recent K-DOQI[39] guidelines also recommend the stratification of renal disease according to the GFR and other risk factors. Generally, it is a well accepted notion, that GFR is remarkably stable from day-to-day over a period of years. However, in their pioneering report, Bosch et al described their findings in a group of studies performed to examine the influence of protein intake on GFR.

A direct relationship was also found between the protein intake and GFR, i.e., with an increase in protein intake there was an increase in GFR in both short term and long term studies. The possibility of a variation in GFR and the capacity of kidney to augment its level of function suggest a renal functional reserve[40]. The renal functional reserve represents the capacity of the kidney to increase its level of operation under certain demands. This reserve may be considered analogous to the cardiac functional reserve. When increased physiological demands are placed on the heart, it responds with an increase in cardiac output. Similarly, when the kidneys are subjected to greater physiological demands, they also respond with an increase in GFR.

Conceptionally, the renal capacity to increase GFR from the baseline to a maximal one can alter the results of GFR studies.

In renal diseases, this functional reserve increases GFR of the residual nephrons, replacing the lost function and maintaining the whole organ GFR.[41] Only after the residual nephrons can no longer compensate for the functional loss, will the changes in resting GFR and rise in serum creatinine occur. On the other hand, the patient with a renal disease on a low protein diet may have a reduction in GFR unrelated to the progression of renal disease. Resting GFR therefore is not only an insensitive index for early detection of renal disease but is also inappropriate for renal disease follow up. Glucose tolerance test (GTT) has been used to assess the patient at risk of diabetes mellitus. The stress of glucose load in GTT unravels the patient with marginal pancreatic dysfunction. It has been suggested that analogous to GTT, a protein tolerance test (PTT) may help in identifying individuals with subnormal renal function before they manifest clinically. The stress of PTT will enable us to determine individuals with impaired functional reserve.[42] Thus PTT is a better test than resting GFR or serum creatinine

Mechanisms of renal haemodynamic response to protein feeding[43].

An acute oral protein load causes a transient hyperfiltration that might reveal a loss of glomerular permeable selectivity properties. A protein meal, on digestion, which acutely raises the plasma amino acid concentration; this increase can also be mimicked by an intravenous amino acid infusion. These amino acids are filtered at the glomerulus and act directly on the kidney to

stimulate proximal tubular absorption in a healthy metabolic state. Amino acid may also change sensitivity of the macula densa sensing mechanisms by altering cell permeability. Sensing a reduced tubular sodium chloride concentration, the macula densa cells release EDRF and prostaglandins locally, which cause afferent arteriolar vasodilatation. This afferent vasodilatation results in increased blood flow and GFR. On the other hand, the patient with a renal disease on a low protein diet may have a reduction in GFR unrelated to the progression of renal disease. Resting GFR therefore is not only an insensitive index for early detection of renal disease but is also inappropriate for renal disease follow up.

Utility of the protein tolerance test in clinical Nephrology

The protein tolerance test can be utilised in:

a. Assessing the baseline and progression of renal disease in certain high risk groups – especially in disorders known to have a subsequent decline in renal function – like diabetics, hypertensives, polycystic kidney disease patients, and patients with a solitary kidney. These patients can be accurately prognosticated and planned for more aggressive intervention if required, by testing with stress GFR as compared to resting GFR.

b. Assessment of borderline donors. Due to shortage of live related donors, elderly and hypertensive individuals are now being taken up as potential renal donors. Stress GFR in atleast these high risk donors will be desirable to reject those who are likely to have renal compromise subsequently, though they might be having a normal resting GFR.

Procedure for conducting PTT[44]

The protein tolerance test has two components:

1. Stress GFR

2. Tubular stress test

Stress GFR

Patients should be fasting and should receive oral hydration with 20 ml/kg of water. Once hydration is complete, urine volume is replaced by an equal quantity of water. Endogenous creatinine clearance is used for assessing the test and baseline GFR. Baseline GFR– Two blood samples are collected for serum creatinine measurement at the start and 30 minutes apart for calculation of creatinine clearance by Cockroft and Gault equation (CG formula) and the mean is taken as baseline creatinine clearance.

Tubular stress test

Purpose: To assess the functional reserve of the kidney by performing tubular function.

Increase in tubular secretion of creatinine (TScr) after a test meat meal. They demonstrated that in normal individual TScr was three times the baseline, while patients with moderate renal failure were unable to raise their TScr. However, it requires standardisation and further studies to prove its utility.

Interpretation

Any individual with normal protein tolerance test will show an increase in GFR from baseline in absence of urinary protein. In contrast, those with abnormal test will have proteinuria and no increase in GFR. The maximal

filtration capacity attained after the protein load in various western studies is reported to be around 140 -160 ml/min/1.73 m2 with a percentage increase in GFR of 20 - 40% from basal state2, 3, 5. Increase in GFR without any proteinuria suggests normal response, while increase in GFR with proteinuria would suggest renal injury and no increase in GFR would suggest incipient renal failure. Hence, protein tolerance test can be used to ascertain an individual's renal reserve, with incipient renal failure and Normal GFR and serum creatinine. Thus, appropriate measures can be initiated at the earliest in such cases. Not much literature is available at the moment in relation to this test. Only two studies have evaluated the protein tolerance test, one was way back in 1950 and one was in 2005 in india. Both the studies proved that protein tolerance test was a useful tool in identifying at risk patients.

AIMS AND OBJECTIVES

The aims of the study were-

1. To identify individuals with impaired protein tolerance test as a marker of early renal dysfunction in type 2 diabetes mellitus.

2. To compare individuals with impaired protein tolerance with normal population.

MATERIALS AND METHODS

The study was conducted on patients attending the out patient department of Government Rajaji Hospital, Madurai. Approval from the hospital ethical committee was obtained.

STUDY DESIGN

The study was a case control study conducted for a period of one year between October 2008- September 2009.

Inclusion criteria

- Patients with type 2 diabetes mellitus were included in the study.
- Fifty healthy, age and sex matched controls without diabetes or its complications were also included in the study for comparison.

Exclusion criteria

- Patients with type 2 diabetes mellitus with proteinuria
- Systemic hypertension
- Renal Failure

Diagnosis of Type 2 diabetes mellitus was made by clinical details and routine blood investigations including fasting and postprandial blood sugar values. The WHO criteria were employed for the diagnosis of diabetes mellitus.[2]

The presence of absence of renal dysfunction was made on the basis of the following:

1. Clinical details
2. Routine Blood investigations

3. Measurment of baseline creatinine clearance and. creatinine clearance after a protein meal(100 gm of protein as cottage cheese)
4. Spot urinary protein estimation at baseline and after a protein meal.

METHODS

After the diagnosis of Type 2 diabetes mellitus,

All the selected patients were subjected to a high protein meal. To detect renal dysfunction in type 2 diabetes mellitus, blood samples were collected after 8 hours of fasting for fasting blood sugar and after two hours of postprandial state. Blood samples were collected at 0, 30, 60 and 120 minutes for serum creatinine after giving high protein meal. Serum was separated and stored in the refrigerator. Serum creatinine was measured from this serum.

e GFR was calculated by using **Cockcroft-Gault equation**

Estimated creatinine clearance (mL/min)

= (140-age x body weight in kg)/72 x Plasma creatinine (mg/dL)

Urine samples were collected at 0, and 120 minutes for Urine PCR .

The information collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done with the help of computer using **Epidemiological Information Package (EPI 2008).** Using this software, range, frequencies, percentages, means, standard deviations, chi square and ‘p’ values were calculated. Kruskul Wallis chi-square test was used to test the significance of difference between quantitative variables. A 'p' value less than 0.05 is taken to denote significant relationship.

RESULTS AND STATISTICAL ANALYSIS

EPIDEMIOLOGY

Majority of the patients were from in and around Madurai city. The total number of patients included in the study was 52. Fifty controls were also included in the study for comparative analysis.

Among the total of 52 Type 2 diabetes mellitus patients **[Female (F)-24; Male (M)-28]** , **32 diabetic patients [Female (F)-16; Male (M)-16]** had no evidence of Renal Dysfunction (**Group-I**), whereas **9 diabetic patients (F-4; M-5)** had evidence of Renal injury(**Group-II**) and **11 diabetic patients (F-4; M-7)** had evidence of incipient Renal Failure (**Group-III**)

Out of the 50 controls, 24 were female and 26 were male, 2 **diabetic patients (F-1; M-1)** had evidence of renal injury (**Group-II**)

The age of the controls ranged from 32 to 67 years with a mean age of 52.1 years. The age of the patients in the study group ranged from 32-67 years with a mean of 54.11 years. In the study group, 9 patients were in the age group of upto 40 years (18%) , 15 patients in 41-50 age group (30%), 22 patients in 51-60 age group (44%),4 patients (8%) were in the age group of >60years.

The age groups of the cases and controls were comparable and there was no statistical difference (p=0.3594).

The age distribution of the patients are shown in tables 1

TABLE 1: Age distribution

Age group	**cases**		**controls**	
	No.	**%**	**No.**	**%**
Upto 40 years	2	3.8	9	18
41-50	13	25	15	30
51-60	31	59.6	22	44
Above 60 years	6	11.5	4	8
Total	52	100	44	100
Mean S.D.	54.11 7.27		52.1 9.0	

The gender of the patients and controls were comparable. The sex distribution of the patients are shown in tables 2 .

TABLE 2: Sex distribution

Sex	**Cases**		**Controls**	
	No.	**%**	**No.**	**%**
Males	28	53.8	26	52
Females	24	46.2	24	48
Total	52	100	50	100

It was observed that the number of female patients was equal in both control and study groups , but a slightly higher number of Male patients in control group (1:1.08).

Patients and controls were classified as overweight, normal weight and underweight according to the body mass index. Out of 50 controls, 13 were in the underweight patients (26%), 25 were in the normal weight patients (50%) and12 were (24 %) in the overweight patients. In study group, 15 out of 52 (28.8%) were in the underweight patients, 25 out of 52 (48.1%) were in the normal weight patients and 12 out of 52 (23'1%) were in the ov erweight patients.

The mean height, weight and BMI were comparable in both the groups. The results are shown in table 3.

TABLE 3: BMI

BMI	Cases		Controls	
	No.	%	No.	%
Underweight< (18.5)	15	28.8	13	26
Normal(18.5-24.9)	25	48.1	25	50
Overweight (>25)	12	23.1	12	24
'p'	0.9016 Not significant			

The BMI of the above groups was comparable and there was no statistical difference (p=0.9016).

Urine PCR was analyzed in the two groups. The results are summarised in table 4.

TABLE 4: Urine PCR

Urine PCR	**Cases**		**Controls**	
	No.	**%**	**No.**	**%**
O min				
Normal	52	100	50	100
Abnormal	-	-	-	-
'p'	-			
120 min				
Normal	32	61.5	48	96
Abnormal	20	38.5	2	4
'p'	0.0001 Significant			

. Urine PCR was more in the test group then the control group and both the values (0 and 120 min) were statistically significant **(p=0.0001).**

Fasting (0 hr) and postprandial (2 hour) blood sugar values were analyzed in the two groups. The mean FPG in control group was 90.3mg/dl compared to 154.5 mg/dl in study group. On the other hand mean 2 hour PPG values in control group were 129mg/dl as opposed to 254.8mg/dl in study group.

Serum creatinine-1 (0, 30 min & mean) and post protein load Serum creatinine-2 levels (60, 120 min & mean) were analysed. The mean Serum creatinine-1 values were 0.86 mg/dl in control group, 0.91mg/dl in study group. **The difference between the two groups was statistically not significant (p=0.0544).**

The mean post protein load Serum creatinine-2 values were 0.72mg/dl in control group, 0.81mg/dl in study group. **The difference between the two groups was statistically significant (p=0.0025).**

The e GFR-1 mean value was 77.46 in the control group compared to 72.28 in the study group .**The difference between the two groups was not statistically significant (p=0.1016).**

The e GFR-2 values remained persistently decreased with a mean of 92.42 in the control group compared to 82.33 in the study group .**The difference between the two groups was statistically significant (p=0.0008).**
The results are summarised in table 5.

TABLE 5: Quantitative parameters

Parameter	**Cases**		**Controls**		**'p'**
	Mean	**SD**	**Mean**	**SD**	
Weight(kgs)	58.9	7.9	57.5	9.2	0.2837 Not significant
Height(cm)	157.9	6.9	156	6.5	0.0946 Not significant
BMI	23.7	3.3	23.6	3.1	0.7914 Not significant
Blood sugar(0 min)	154.5	27.6	90.3	13.3	**0.0001 significant**
Blood sugar(120min)	254.8	47	129	8.5	**0.0001 significant**
Duration	6.35	2.68	0	-	**0.0001 significant**
Sr.creatinine(0min)	0.94	0.18	0.88	0.15	0.0611 Not significant
Sr.creatinine(30min)	0.88	0.15	0.83	0.17	0.1076 Not significant
Sr.creatinine1(mean)	0.91	0.11	0.86	0.13	0.0544 Not significant
Sr.creatinine(60min)	0.88	0.15	0.76	0.15	**0.0001 significant**
Sr.creatinine(120min)	0.73	0.16	0.64	0.11	**0.0025 significant**
Sr.creatinine2(mean)	0.81	0.14	0.70	0.15	**0.0001significant**
e GFR-1	72.28	8.12	76.36	11.5	0.1016 Not significant
e GFR-2	82.33	13.94	95.36	13.8	**0.0008 significant**

RENAL FUNCTION ANALYSIS

Renal function was analyzed in the two groups. Normal Renal Function was observed in 48 patients (96%) in the control group compared to 32 patients (61.5%) in the test group. Renal injury was observed in 2 patients (4%) in the control group compared to 9 patients (17.3%) in the test group**.** Renal Failure was observed in none of the patients in the control group compared to 11 patients (21.2%) in the test group**. The prevalence of Renal dysfunction was more in the study group (38.5%) compared to 4% in controls and the difference was statistically significant (p=0.0018).** The results are summarised in table 6.

TABLE 6: Renal Function

Renal Function	**Cases**		**Controls**	
	No.	**%**	**No.**	**%**
Normal	32	61.5	48	96
Renal Injury	9	17.3	2	4
Renal Failure	11	21.2	-	-
'p'	**0.0018 significant**			

The relation between age and renal function was analyzed. It was found that renal failure was more in patients over 60 years (83.3%) as compared to 16.1% in the 51-60 years group and 7.7% in the 41-50 years group.

TABLE 7: Age and Renal Function

Age group	**Renal Function**					
	Normal		**Injury**		**Failure**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Upto 40 years	2	100	-	-	-	-
41-50	11	84.6	1	7.7	1	7.7
51-60	19	61.3	7	22.6	5	16.1
Above 60 years	-	-	1	16.7	5	83.3
Mean S.D.	51.3 6.8		57.9 5.3		59.3 5.9	
'p'	**0.0005 significant**					

The difference was found to be statistically significant (p=0.0005).

TABLE 8: Sex and Renal Function

Sex	Renal Function					
	Normal		Injury		Failure	
	No.	%	No	%	No	%
Males (28)	16	57.1	5	17.9	7	25
Females (24)	16	66.7	4	16.7	4	16.7
'p'	0.6761 Not significant					

The difference was not statistically significant (p=0.6761).

TABLE 9: BMI and Renal Function

BMI	Renal Function					
	Normal		Injury		Failure	
	No.	%	No.	%	No.	%
BMI(Total)						
Underweight(15)	10	66.7	3	20	2	13.3
Normal (25)	16	64	3	12	6	24
Overweight (12)	6	50	3	25	3	25
Mean	23.5		23.7		24.2	
S.D.	2.9		3.9		3.4	
'p'	**0.7303 Not significant**					
BMI(Male)						
Underweight(9)	6	66.7	2	22.2	1	11.1
Normal (12)	7	58.3	1	8.3	4	33.3
Overweight (7)	3	42.9	2	28.6	2	28.6
Mean	23.06		23.91		23.9	
S.D.	2.65		4.27		2.91	
'p'	0.5309 Not Significant					
BMI(Female)						
Underweight(6)	4	66.7	1	16.7	1	16.7
Normal (13)	9	69.2	2	15.4	2	15.4
Overweight (5)	3	60	1	20	1	20
Mean	23.87		23.5		24.59	
S.D.	3.2		3.96		4.54	
'p'	0.995 Not Significant					

The relation between duration of disease and renal dysfunction was analyzed. It was seen that 10 out of 12 patients (83.3%) with a longer duration of disease (>8 years) had renal failure compared to one out of 13 patients (7.7%) with duration of disease between 6-8 years. None of the patients with duration of disease <6 years had renal failure**.** The values are summarized in table 10.

TABLE 10: Duration of Disease and Renal Function

Duration of Diseases	**Renal Function**					
	Normal		**Injury**		**Failure**	
	No.	**%**	**No.**	**%**	**No.**	**%**
<6 years(27)	26	96.3	1	3.7	-	-
6-8 years(13)	6	46.2	6	46.2	1	7.7
>8 years(12)	-	-	2	16.7	10	83.3
Mean	4.62		7.33		10.55	
S.D.	1.01		1.32		1.51	
'p'	**0.0001 Significant**					

. **The difference was found to be statistically significant (p=0.0001).**

Analysis was done in relation to urine PCR and renal dysfunction. It was found that abnormal urine PCR was seen in 20 patients at 120 minutes compared to none at 0 minutes. Eleven out of these 20 patients (55%) had renal failure and 9 out of 20 patients (45%) had renal injury. The results are presented in the following table 11.

TABLE 11: Urine PCR and Renal Function

Urine PCR	**Renal Function**					
	Normal		**Injury**		**Failure**	
	No.	**%**	**No**	**%**	**No**	**%**
O min						
Normal(52)	32	61.5	9	17.3	11	21.2
Abnormal(0)	-	-	-	-	-	-
'p'	-					
120 min						
Normal(32)	**32**	**100**	**-**	**-**	**-**	**-**
Abnormal(20)	**-**	**-**	**9**	**45**	**11**	**55**
'p'	**0.0001 Significant**					

. **The difference was found to be statistically significant (p=0.0001).**

The results of Quantative parameters and Renal Function are summarised in the following table 12.

TABLE 12: Quantitative parameters and Renal Function

Parameter	Renal Function						'p'
	Normal		Injury		Failure		
	Mean	SD	Mean	SD	Mean	SD	
Blood sugar(0 min)	136.2	11.0	173.3	20.8	192.4	14.2	**0.0001**(S)
Blood sugar(120min)	227.5	31.3	296.9	36.4	299.5	31.5	**0.0001**(S)
Sr.creatinine(0min)	0.93	0.18	0.91	0.22	0.95	0.16	0.8519(NS)
Sr.creatinine(30min)	0.88	0.15	0.84	0.17	0.89	0.14	0.802(NS)
Sr.creatinine1(mean)	0.91	0.1	0.87	0.17	0.94	0.11	0.3995(NS)
Sr.creatinine(60min)	0.85	0.12	0.83	0.18	1.03	0.12	**0.0019**(S)
S.creatinine(120min)	0.65	0.1	0.76	0.17	0.94	0.12	**0.0001**(S)
Sr.creatinine2(mean)	0.75	0.09	0.8	0.16	0.98	0.11	**0.0001**(S)
e GFR-1	73.1	8.2	71.0	7.5	70.9	8.9	0.4887(NS)
e GFR-2	88.9	11.2	77.6	9.6	67.1	10.7	**0.0001**(S)

Fasting serum creatinine levels (0 min), and post load serum creatinine levels (30min, 60min and 120min) were analysed in relation to renal function.

The mean serum creatinine-1(0 and 30 min) values were 0.91mg/dl in group with Normal Renal Function, 0.87mg/dl in group with Renal Injury, 0.94mg/dl in group with Renal Failure.

The mean post protein load serum creatinine-2 values (60 and 120 min) were 0.75mg/dl in group with Normal Renal Function , 0.8 mg/dl in group with Renal Injury , 0.98mg/dl in group with Renal Failure.

Serum creatinine-2 values remained persistently elevated in patients with Renal Injury and patients in group with Renal Failure compared to patients in group with Normal Renal Function. The relationship was statistically significant. **(p=0.0001).**

DISCUSSION

Diabetes remains a common menace in the developing population and the number has been increasing at an alarming rate in the recent years. The key to successful prevention of complications lies in early diagnosis and control. The United Kingdom Prospective Diabetes Study, conducted from 1976 to 1997, showed conclusively that, in people with improved blood glucose control, the risk of early kidney disease was reduced by a third.

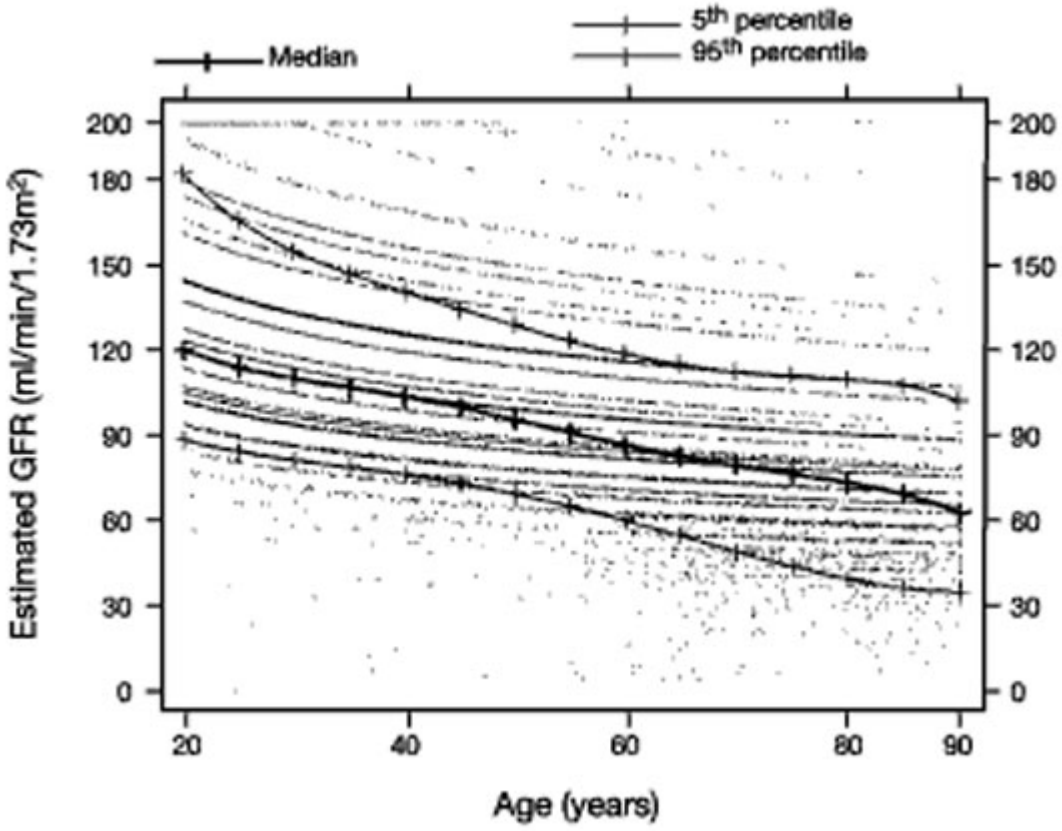
Additional studies conducted over the past decades have clearly established that any program resulting in sustained lowering of blood glucose levels will be beneficial to patients in the early stages of CKD.

Despite adequate control of diabetes, patients may land up with one or more of the macro vascular or micro vascular complications. Diabetic nephropathy is a common problem and it remains one of the challenges for us to diagnose the presence of renal dysfunction at an early stage. Early diagnosis of renal dysfunction in our patients would allow us to protect our patients from treatments that may damage further nephrons and tip them into the clinical stages of renal failure and to initiate renoprotective management regimens at an early stage

The early diagnosis is a challenge because the kidney has considerable functional reserve so that standard laboratory tests for renal function can only detect abnormalities once more than 66% of functioning renal tissue has been lost. Precise evaluation of renal function would also allow more effective

monitoring of the rate of decline of renal function over time and help determine the efficacy of a therapeutic intervention.

Many of the results in this study correlated well with literature. There was an age related decline in GFR similar to that seen in most studies in the past. Around 85% of patients in this study above 60 years had renal failure compared to only 13% in age group <60 years. The following graph shows the age related decline in GFR as studied in the MDRD study. This was as a result of natural history of renal disease as well as increase in conditions like diabetes and hypertension.



It was found in our study that increase in duration of diabetes was strongly related to renal failure. This is well in concurrence with the study done by coulhon et al where it was seen that increasing age and duration of diabetes were associated with renal failure in Type 2 and Type 1 diabetes. In Type 2 diabetes duration of diabetes was a more important risk factor than age.

In the same study, both Type I and Type II diabetic retinopathy and proteinuria were strongly associated with renal failure. This was also seen in our study where 55% of patients with abnormal urine PCR had renal failure.

The protein tolerance test is an upcoming investigation and has not been extensively evaluated in the past. Very few studies have utilised this test to identify patients at risk of renal dysfunction.

Protein tolerance test was developed way back in 1950 by Horn et al where they proved that PTT could identify early onset diabetic nephropathy based on the fact that protein loading could exert a stress on both the glomeruli and tubules. Subsequently no major study has been done with regard to PTT. In this study it was seen that the mean creatinine- 2 values (post protein challenge) were more in patients with renal failure compared to those with renal dysfunction and normal renal function. The decline in eGFR-2 (post protein challenge) was also statistically significant (67 in renal failure versus 88 in normal renal function) implying that this could be a useful marker of renal reserve. Though there are no major studies for comparison, the results of the study were quite significant statistically. This can be done on a larger scale to prove the utility of the protein tolerance test.

Protein tolerance test can be very useful to detect incipient renal failure in a person with normal GFR and serum creatinine value, thus identifying the patients who are most likely to be benefited by an aggressive intervention. This is especially important in evaluating high risk persons like diabetics, post-renal transplants, and polycystic kidney disease patients. PTT can also be used to check the borderline renal donor, and to give accurate prognostication in a progressive renal disease. Tubular stress test still requires standardisation and further studies to prove its utility.

Summary

The study “impaired protein tolerance test as a marker of early renal dysfunction in type 2 diabetes mellitus” was undertaken to find out the usefulness of protein tolerance test in detecting patients with type 2 diabetes mellitus who were at risk of developing renal dysfunction.

The present study was a case control study done at Govt. Rajaji Hospital Madurai. After institutional Ethical Committee clearance, 52 patients with type 2 diabetes mellitus and 50 healthy controls were selected according to the inclusion criteria. There were almost equal males and females in the study. A baseline fasting and post prandial blood sugar, serum creatine and baseline GFR was calculated. This was followed by a protein challenge with 100 grams of protein food. Serum creatinine and GFR were measured at 30, 60 and 120 minutes after protein challenge. Using statistical data, correlation was analyzed between pre/post protein challenge serum creatinine in cases and controls in relation to GFR and renal function. It was found that patients with renal failure had more persistent elevation of serum creatinine and sustained decrease in GFR as compared to patients with normal renal function or those with mild renal dysfunction. There was also an age related decline in renal function. Proteinuria was found to be an independent risk factor for renal failure.

It was also found that patients with long duration of diabetes and poor glycemic control have more chance of early renal injury and dysfunction than those with short duration of diabetes and good glycemic control .

Conclusions

1. Kidney damage starts in Diabetic patients even before microalbuminuria and clinical nephropathy starts.

2. It was found that longer the Duration of diabetes ,more the chance of early renal injury and dysfunction.

3. Renal injury and dysfunction directly correlates with poor metabolic control.

4. Protein tolerance test can be a very useful test to detect such incipient renal failure in patients with normal GFR and normal serum creatinine values.

5. Identifying those patients with subnormal renal function may enable us to initiate an early aggressive intervention.

6. This Protein Tolerance Test may be very much useful in high risk patients like Diabetics,Hypertensive patients.

7. Patients with diseases like solitary kidney, polycystic kidney disease, post renal transplants can also be subjected to this test to identify incipient renal failure.

8. Protein Tolerance Test can also be used to check the borderline renal donor in order to give accurate prognostication in a progressive renal disease.

APPENDIX

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Vol. 6, No. 3 July-September, 2005

PROFORMA

Name: Age: Sex:

Occupation: Address:

Height: ID:
Weight:
BMI:

Duration of Diabetes: yrs
Hypertension: yes / no
Renal Disease: yes /no
Diabetic Nephropathy: yes / no

Diet History:
Personal History:
Family History:

General examination:
Pulse: Blood pressure: Temp: RR:
CVS:
RS:
ABDOMEN:
CNS:

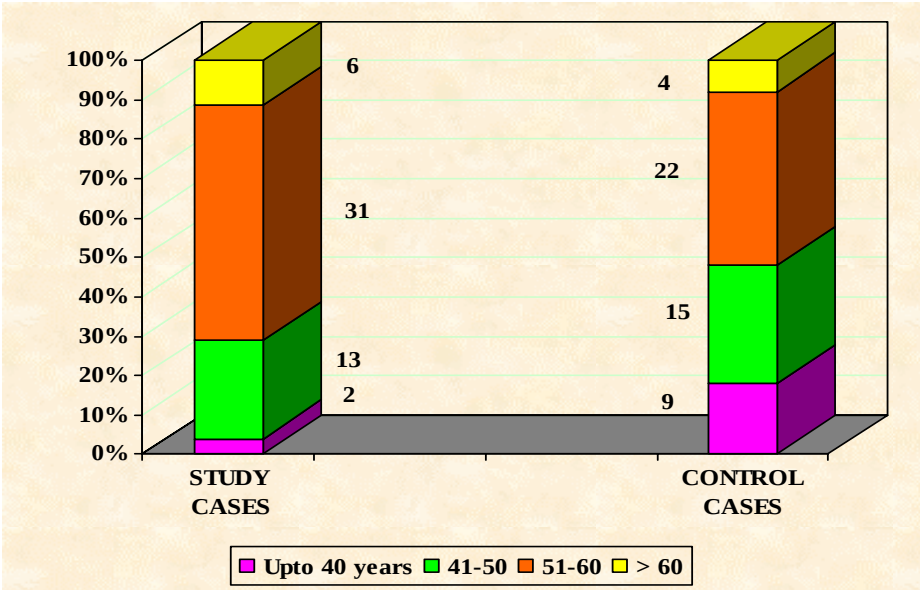
Investigations:
Blood TC: DC: HB: ESR:
Blood Urea: Urine Alb: Sugar: Dep:

Blood Sugar: 0 hour: 2hour:

Serum Creatinine: 0 min: 30min: mean1: e GFR1:
60min 120min: mean2: e GFR2:
Urine PCR: 0min: 120min:

Renal Function Result:

AGE DISTRIBUTION



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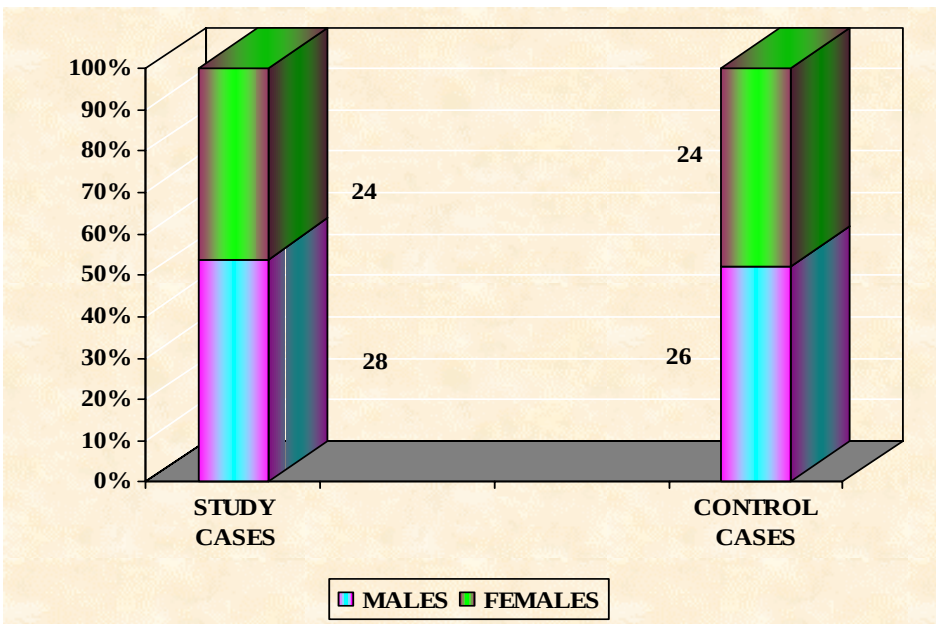
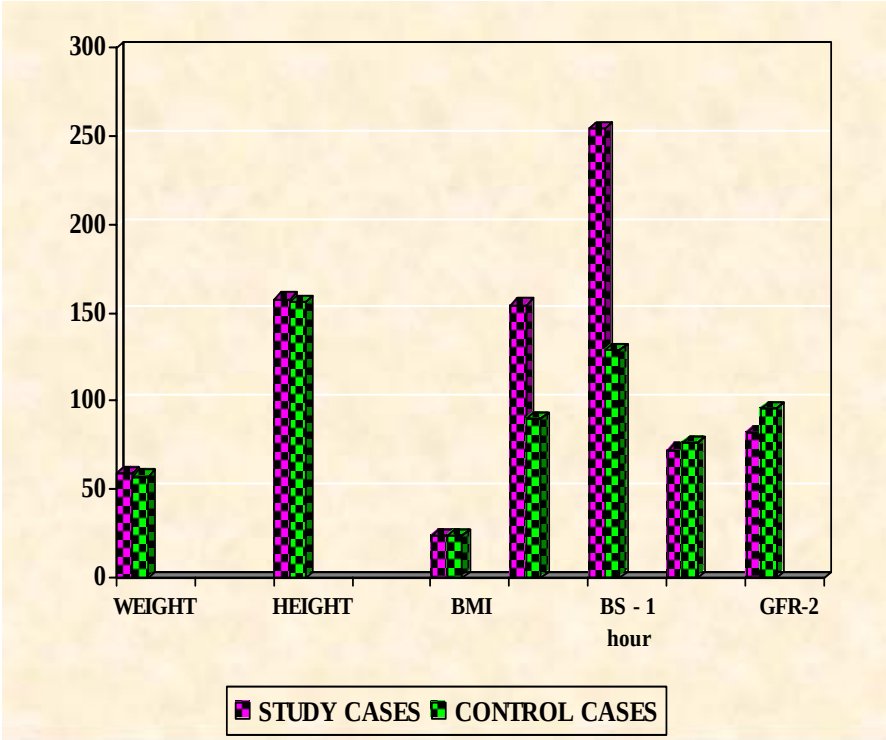
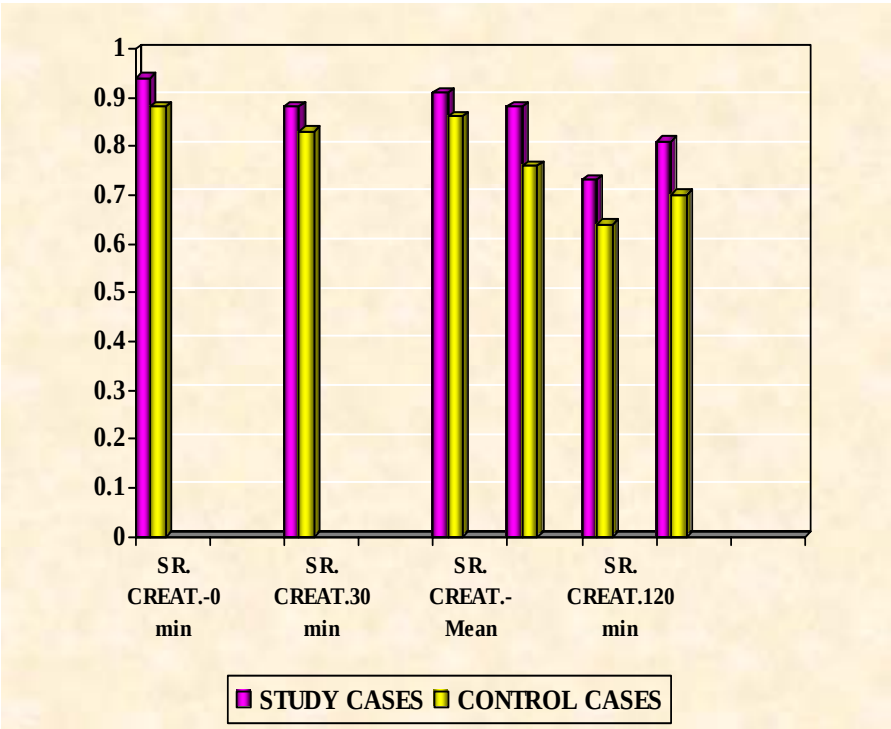


QUANTITATIVE PARAMETERS: comparison between cases and controls



QUANTITATIVE PARAMETERS: Serum creatinine



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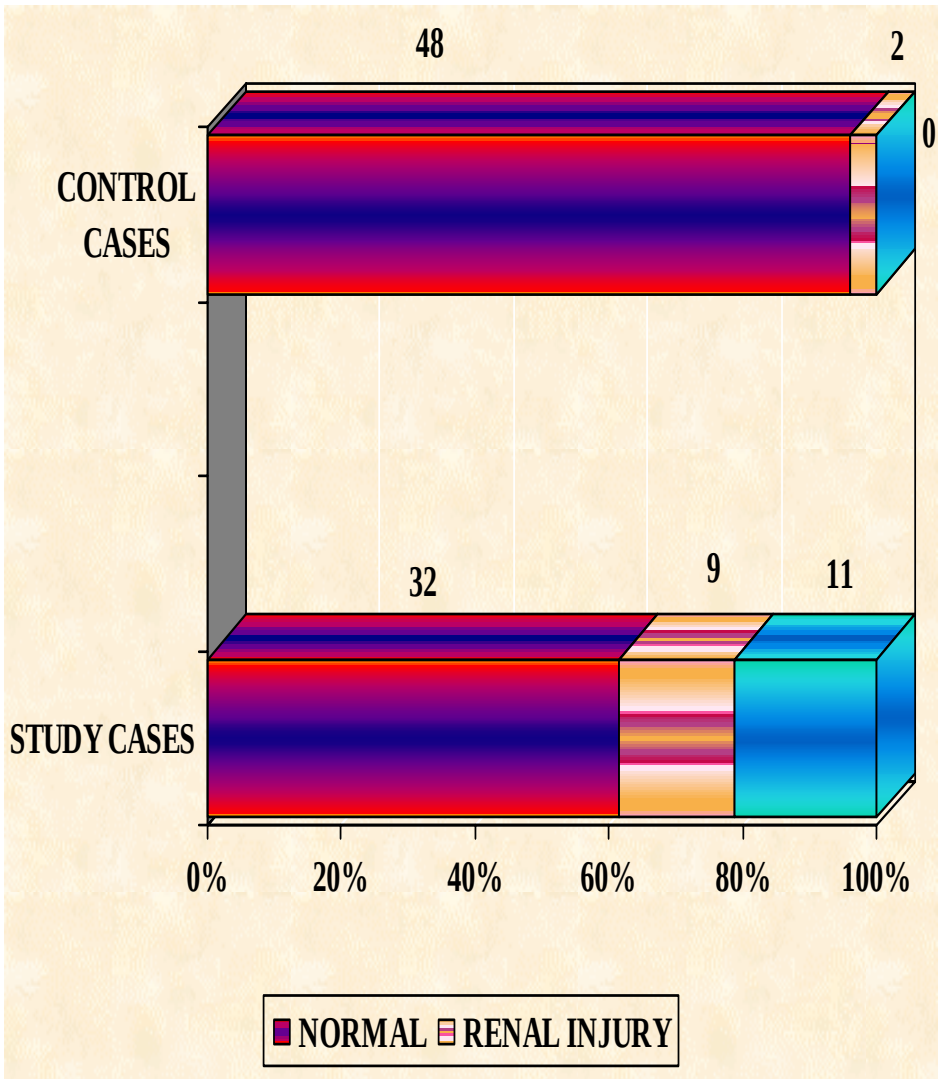

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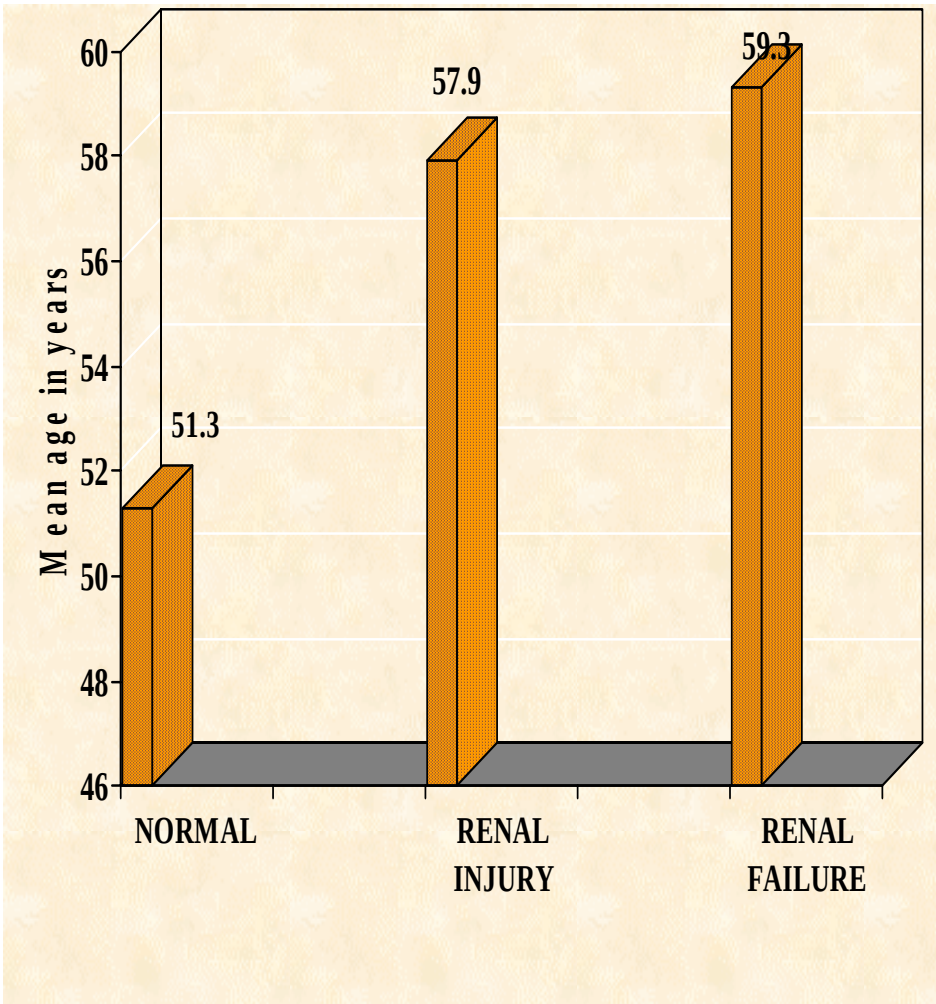

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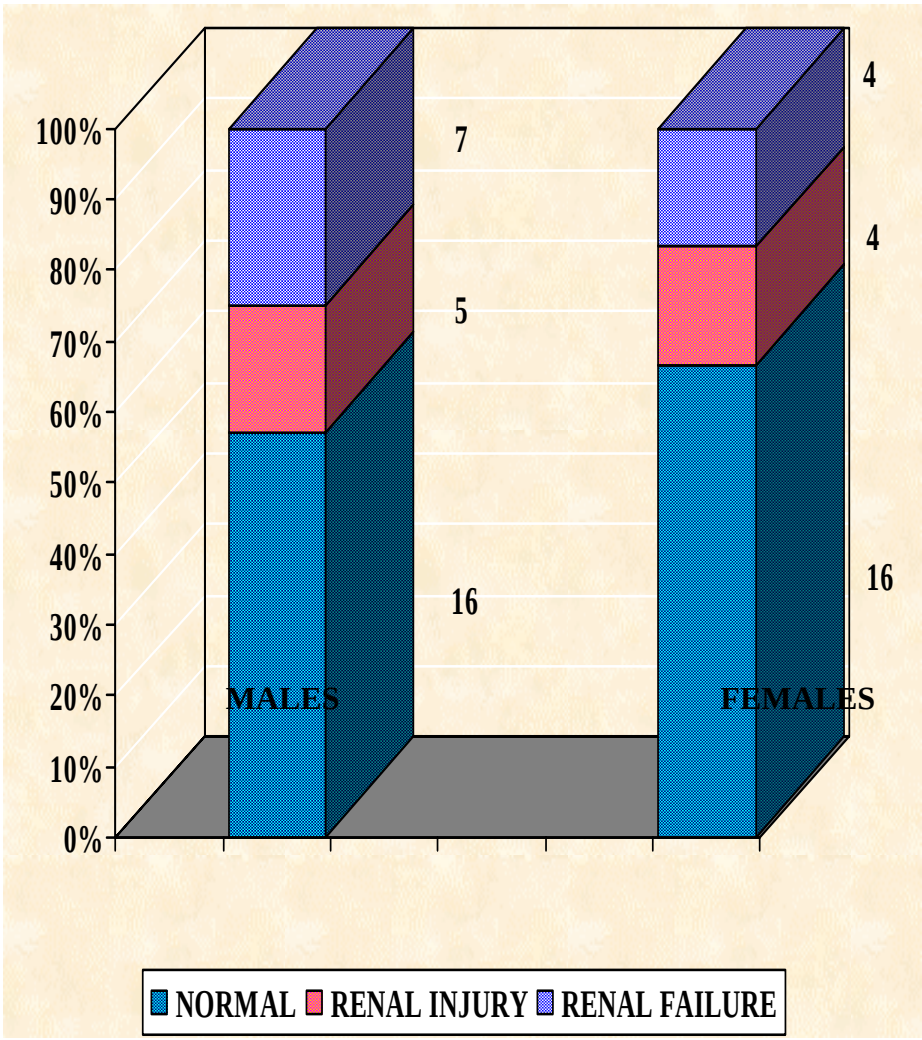
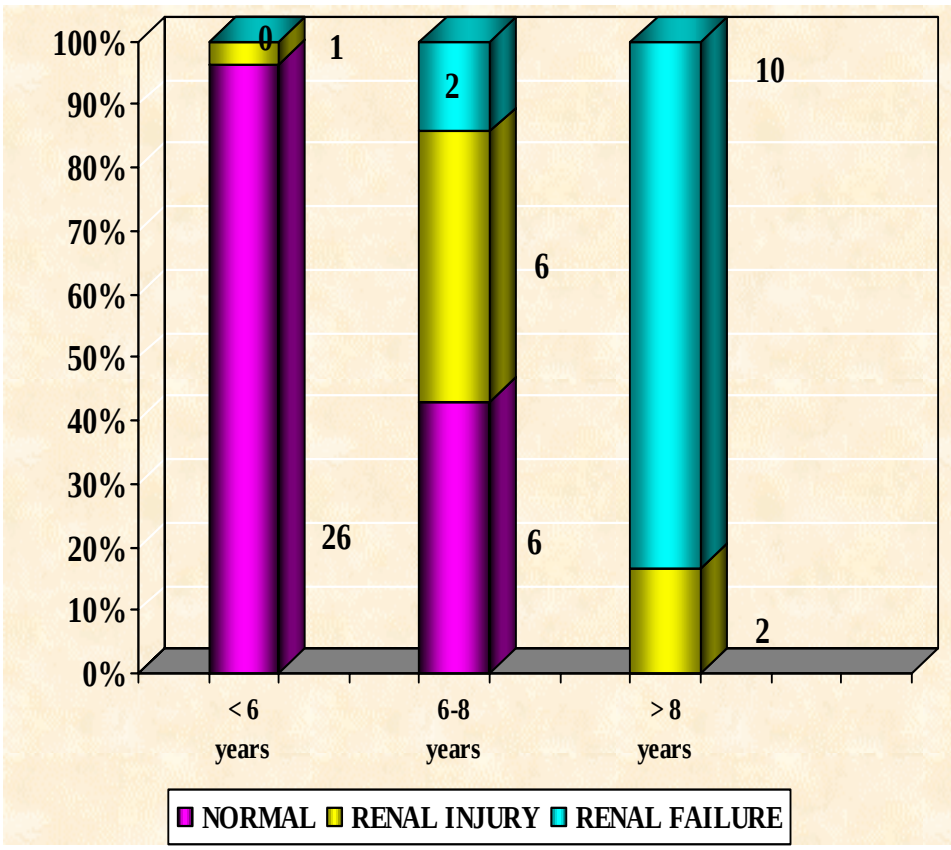
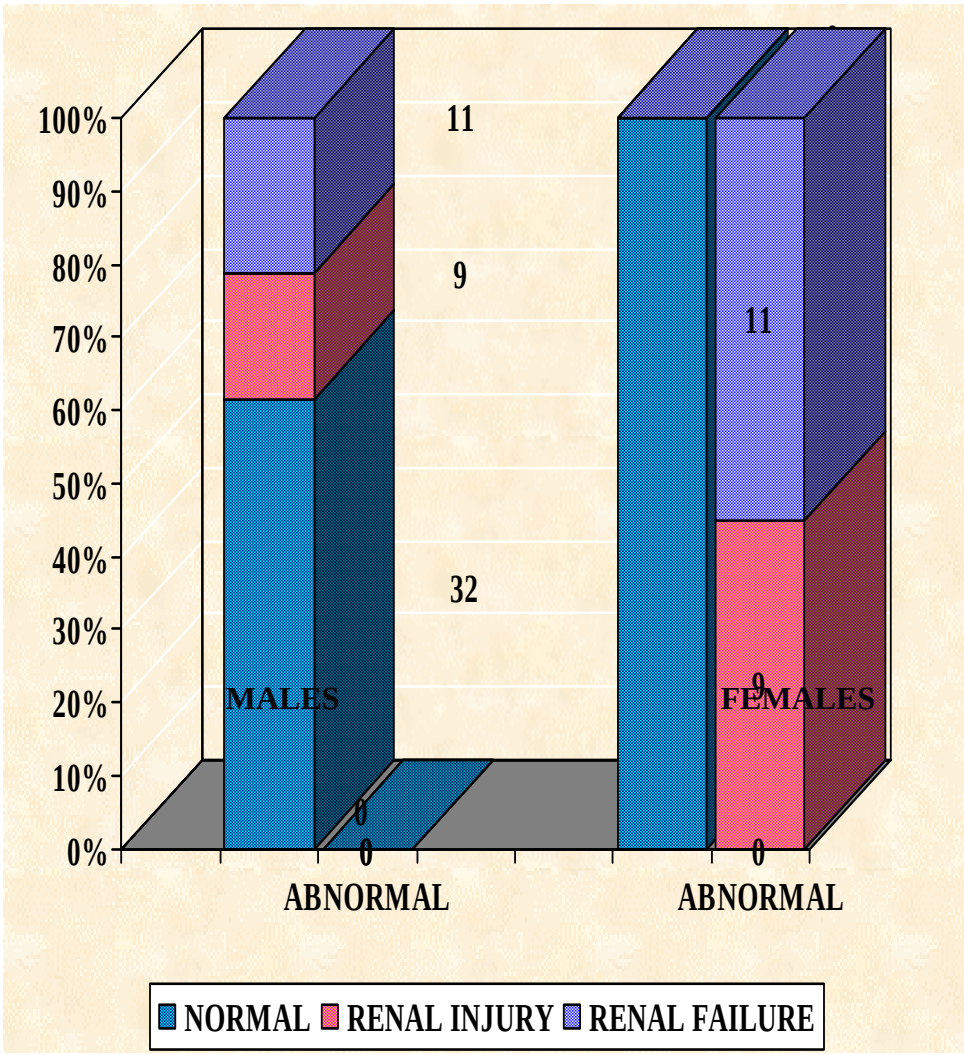


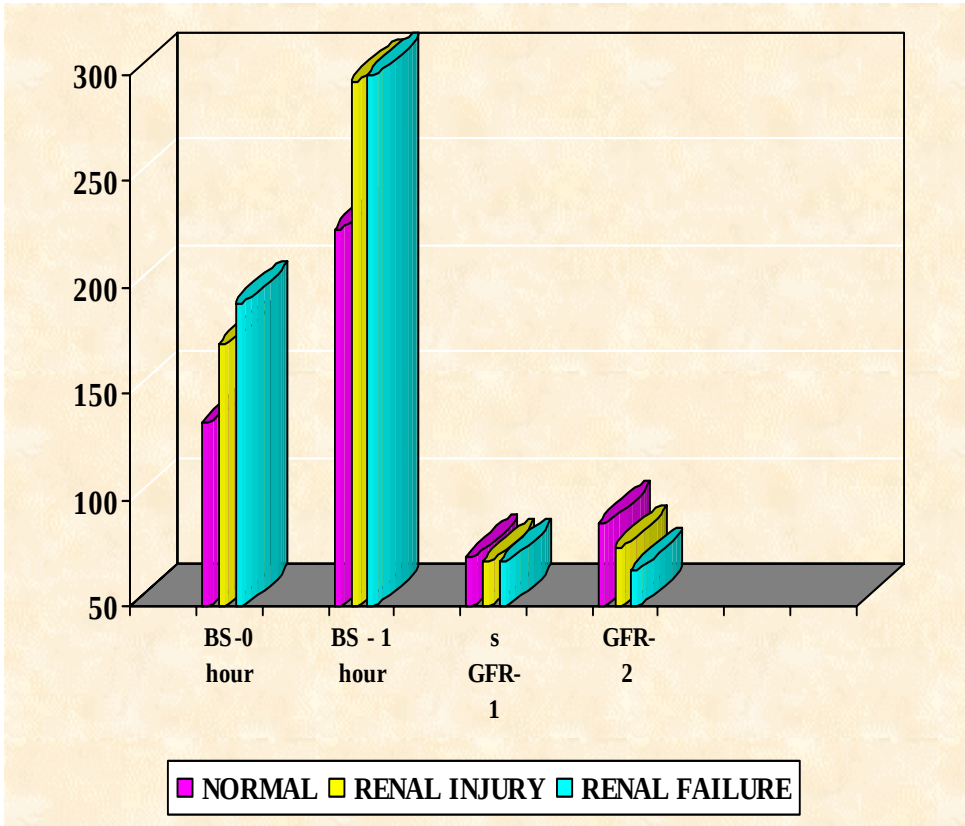
RENAL FUNCTION & DURATION OF DISEASE

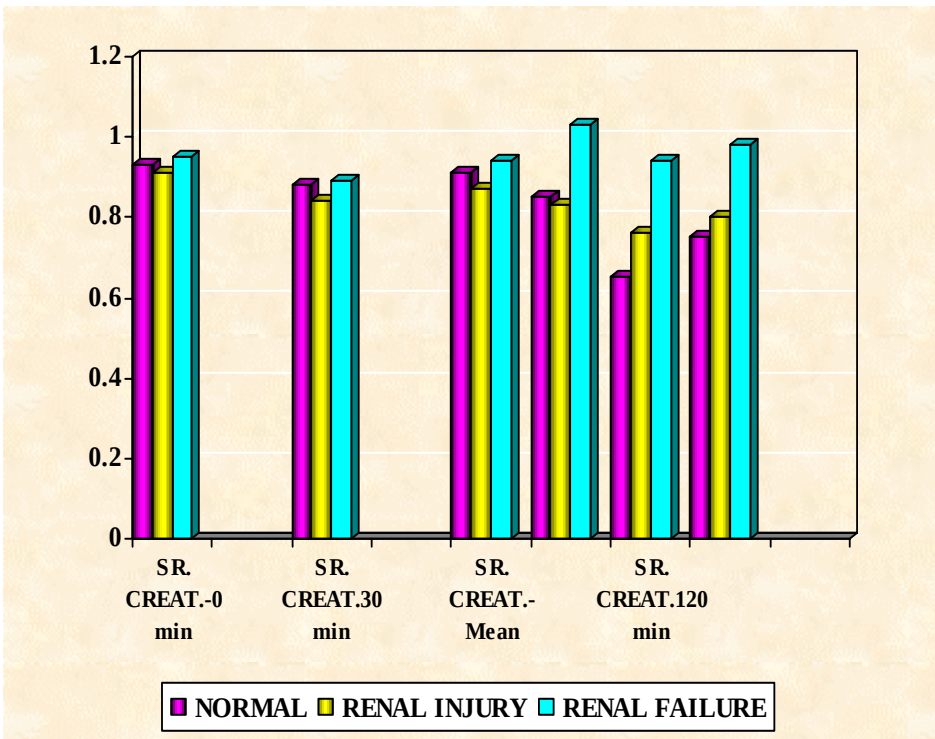


URINE PCR & RENAL FUNCTION



RENAL FUNCTION & QUANTITATIVE PARAMETERS: PTT





MASTER CHART

S.NO	ID	Age	sex	weight	height	BMI	BS-0hr	BS-2hr	Duration	sc-0	sc30	sc1mean	e GFR1	sc60	sc120	sc2mean	e GFR2	PCR(0)	PCR(120)	Renal Function	GROUP
1	T36	52	M	76	165	Overweight	216	324	7	1.2	1.1	1.15	80.77	1.1	1.1	1.1	84.12	N	A	I	II
2	T52	45	M	70	160	Overweight	146	224	5	1.2	1	1.1	89.62	1	0.8	0.9	102.62	N	N	N	I
3	T3	60	M	68	156	Overweight	196	344	9	1.1	1	1.05	71.95	1.1	1.1	1.1	68.42	N	A	F	III
4	T7	37	M	66	154	Overweight	132	248	3	1.2	1.1	1.15	82.1	1	0.8	0.9	104.9	N	N	N	I
5	T5	46	M	68	158	Overweight	128	246	4	1.1	1.1	1.1	80.4	1	0.8	0.9	98.64	N	N	N	I
6	T10	55	M	74	160	Overweight	164	302	5	1.2	1	1.1	79.11	1	0.9	0.95	91.95	N	A	I	II
7	T20	57	M	69	159	Overweight	208	278	11	1.1	1	1.05	75.55	1.2	1.1	1.15	69.16	N	A	F	III
8	T22	50	M	53	154	Normal	134	240	6	0.8	1	0.9	73.61	1	0.6	0.8	82.81	N	N	N	I
9	T23	57	M	61	164	Normal	186	272	10	1.1	1	1.05	66.97	1.2	1	1.1	63.68	N	A	F	III
10	T24	51	M	62	165	Normal	128	240	5	1.1	0.9	1	76.64	1	0.6	0.8	95.8	N	N	N	I
11	T25	65	M	60	162	Normal	174	298	12	1	0.8	0.9	69.44	1	1	1	62.5	N	A	F	III
12	T26	58	M	61	164	Normal	136	202	6	1.2	0.8	1	69.47	0.8	0.8	0.8	86.84	N	N	N	I
13	T27	54	M	56	158	Normal	128	198	5	1.1	1	0.9	74.32	0.8	0.6	0.7	95.55	N	N	N	I
14	T28	58	M	58	160	Normal	168	302	8	0.8	0.9	0.85	77.71	0.8	0.8	0.8	82.56	N	A	I	II
15	T17	55	M	61	162	Normal	194	320	10	1	1	1	70.01	1.1	1	1.05	68.58	N	A	F	III
16	T32	43	M	63	160	Normal	130	210	5	0.8	1.2	1	84.8	1	0.6	0.8	106.09	N	N	N	I
17	T19	56	M	62	158	Normal	132	244	5	1	1	1	72.33	0.8	0.9	0.85	85.09	N	N	N	I
18	T21	55	M	48	146	Normal	138	216	4	0.8	1	0.9	62.96	1	0.5	0.75	75.55	N	N	N	I
19	T11	45	M	65	165	Normal	178	320	9	0.8	1	0.9	95.29	1	0.8	0.9	95.29	N	A	F	III
20	T18	62	M	51	167	Underweight	180	316	8	0.6	1	O.8	69.06	0.9	0.8	0.85	65	N	A	F	III
21	T35	60	M	50	165	Underweight	152	240	6	O.8	0.8	0.8	69.44	0.7	0.8	0.75	74.07	N	A	I	II
22	T20	42	M	54	170	Underweight	136	280	3	1	0.6	0.8	91.88	0.8	0.6	0.7	105	N	N	N	I
23	T15	46	M	48	163	Underweight	131	215	4	0.9	0.7	0.8	78.33	0.6	0.6	0.6	104.44	N	N	N	I
24	T29	56	M	57	176	Underweight	126	184	5	1.2	0,8	1	66.5	0.8	0.8	0.8	83.12	N	N	N	I
25	T30	57	M	55	173	Underweight	122	168	2	1	0.6	0.8	79.45	0.6	0.6	0.6	105.67	N	N	N	I
26	T48	57	M	48	163	Underweight	145	214	5	0.6	1	0.8	69.16	0.7	0.5	0.6	92.22	N	N	N	I
27	T33	60	M	45	162	Underweight	166	348	9	0.6	1	0.8	62.5	0.8	0.7	0.75	66.66	N	A	I	II
28	T39	53	M	52	168	Underweight	130	242	4	1.1	0.8	0.95	66.14	0..9	0.7	0.8	78.54	N	N	N	I
29	T31	60	F	68	155	Overweight	178	298	7	1	0.8	0.9	71.35	1	0.6	0.8	80.28	N	A	I	II
30	T1	55	F	75	157	Overweight	137	206	4	0.9	0.9	0.9	83.62	1	0.6	0.8	94.07	N	N	N	I
31	T2	65	F	68	148	Overweight	212	234	13	1	0.9	0.95	63.37	1	1	1	60.2	N	A	F	III
32	T4	56	F	65	160	Overweight	138	202	6	1	0.8	0.9	71.62	0.8	0.6	0.75	85.94	N	N	N	I
33	T34	50	F	67	145	Overweight	135	268	4	1	0.8	0.9	79.09	0.8	0.6	0.7	101.69	N	N	N	I
34	T3	59	F	62	166	Normal	198	302	12	1	0.8	0.9	65.87	1	0.9	0.95	62.4	N	A	F	III
35	T6	55	F	44	143	Normal	122	196	4	0.8	0.8	0.8	63.63	0.8	0.6	0.7	72.72	N	N	N	I
36	T8	49	F	49	142	Normal	152	243	8	0.8	0.6	0.7	73.54	0.6	0.6	0.6	85.8	N	A	I	II
37	T9	45	F	55	151	Normal	147	220	5	0.6	1	0.8	77.1	0.8	0.6	0.7	88.06	N	N	N	I
38	T12	65	F	53	148	Normal	178	280	11	0.8	0.7	0.75	62.57	1	0.8	0.9	52.14	N	A	F	III
39	T13	48	F	55	150	Normal	138	238	3	1	0.8	0.9	68.53	0.8	0.6	0.7	85.33	N	N	N	I
40	T38	56	F	57	153	Normal	126	240	6	0.9	0.7	0.8	70.65	0.8	0.6	0.7	80.75	N	N	N	I
41	T40	32	F	52	153	Normal	138	256	4	0.8	1	0.9	73.66	0.8	0.6	0.7	94.69	N	N	N	I
42	T41	55	F	61	164	Normal	146	248	5	1	1	1	61.21	1	0.8	0.9	68.01	N	N	N	I

43	T43	60	F	58	160	Normal	196	328	9	1	0.8	0.9	60.86	0.9	0.7	0.85	63.82	N	A	I	II
44	T44	60	F	51	152	Normal	170	268	6	0.6	1	0.8	60.18	0.8	0.6	0.7	68.81	N	N	N	I
45	T45	55	F	60	164	Normal	146	278	4	0.8	0.9	0.85	70.83	0.8	0.7	0.75	80.27	N	N	N	I
46	T46	60	F	62	162	Normal	138	226	5	1	0.8	0.9	65.06	1	0.6	0.8	86.11	N	N	N	I
47	T47	50	F	47	160	Underweight	112	138	5	0.6	0.8	0.7	71.33	0.7	0.6	0.65	76.83	N	N	N	I
48	T49	62	F	45	158	Underweight	212	330	11	0.7	0.6	0.65	63.75	0.7	0.6	0.65	63.75	N	A	F	III
49	T50	60	F	43	154	Underweight	158	246	6	0.7	0.6	0.65	62.47	0.6	0.6	0.6	67.68	N	N	N	I
50	T51	44	F	50	165	Underweight	144	232	5	0.9	0.8	0.85	66.66	0.8	0.6	0.7	80.95	N	N	N	I
51	T14	67	F	48	162	Underweight	168	287	7	0.7	0.6	0.65	63.64	0.6	0.6	0.6	68.94	N	A	I	II
52	T16	54	F	49	163	Underweight	142	248	5	0.6	0.7	0.75	66.33	0.7	0.6	0.65	76.54	N	N	N	I
53	C1	60	M	68	164	Overweight	90	134	0	1.1	0.9	1	75.55	0.8	0.6	0.7	107.93	N	N	N	C
54	C2	47	M	74	162	Overweight	98	136	0	0.8	1	0.9	106.2	0.9	0.8	0.85	114.86	N	N	N	C
55	C3	60	M	78	164	Overweight	102	140	0	1.2	0.8	1	86.66	1	0.7	0.85	104.5	N	N	N	C
56	C4	56	M	72	164	Overweight	98	138	0	1	1	1	94	1	0.7	0.85	101.17	N	N	N	C
57	C5	50	M	70	168	Overweight	108	132	0	1.2	1.2	1.2	72.91	1.2	0.7	0.95	92.1	N	N	N	C
58	C6	39	M	62	159	Normal	106	128	0	1.2	0.8	1	86.97	1	0.6	0.8	108.71	N	N	N	C
59	C7	36	M	70	168	Normal	86	126	0	0.9	1.2	1.05	96.29	0.9	0.8	0.85	118.95	N	N	N	C
60	C8	55	M	60	158	Normal	94	132	0	0.8	1.2	1	70.83	0.7	0.8	0.75	98.88	N	N	N	C
61	C9	35	M	45	144	Normal	82	120	0	0.8	0.6	0.7	93.75	0.6	0.6	0.6	111.45	N	N	N	C
62	C10	59	M	60	158	Normal	94	138	0	1	1	1	67.5	0.7	0.8	0.75	92.22	N	N	N	C
63	C11	58	M	54	152	Normal	68	116	0	0.8	0.7	0.75	82	0.6	0.6	0.6	105	N	N	N	C
64	C12	57	M	56	158	Normal	96	136	0	0.7	0.9	0.8	80.6	0.7	0.7	0.7	94.44	N	N	N	C
65	C13	40	M	64	162	Normal	84	128	0	1	0.9	0.95	93.56	0.8	0.8	0.8	111.11	N	N	N	C
66	C14	59	M	58	155	Normal	64	116	0	0.8	0.9	0.85	76.76	0.9	0.6	0.75	89.14	N	N	N	C
67	C15	60	M	60	158	Normal	104	140	0	1.1	0.9	1	66.66	0.9	0.9	0.9	70.17	N	A	I	C
68	C16	49	M	54	153	Normal	74	128	0	0.9	0.7	0.8	85.31	0.7	0.6	0.65	109.61	N	N	N	C
69	C17	45	M	62	158	Normal	96	124	0	1.1	1.1	1.1	74.36	0.9	0.8	0.85	98.26	N	N	N	C
70	C18	56	M	58	156	Normal	98	126	0	1	1	1	67.66	0.6	0.8	0.7	101.26	N	N	N	C
71	C19	66	M	60	158	Normal	85	124	0	0.8	1	0.9	68.51	0.7	0.6	0.65	108.33	N	N	N	C
72	C20	36	M	48	164	Underweight	102	136	0	0.8	0.6	0.7	99.04	0.7	0.5	0.6	117.77	N	N	N	C
73	C21	36	M	56	174	Underweight	76	140	0	1	0.8	0.9	89.87	0.8	0.8	0.8	105	N	N	N	C
74	C22	37	M	52	170	Underweight	110	137	0	0.9	0.8	0.85	87.51	0.6	0.8	0.7	108.33	N	N	N	C
75	C23	38	M	45	156	Underweight	69	118	0	0.7	0.8	0.75	85	0.7	0.6	0.65	100	N	N	N	C
76	C24	50	M	46	158	Underweight	98	125	0	0.8	0.8	0.8	71.87	0.8	0.6	0.7	82.14	N	N	N	C
77	C25	60	M	54	172	Underweight	90	134	0	0.8	1	0.9	66.66	0.9	0.6	0.75	82	N	N	N	C
78	C26	47	M	53	170	Underweight	92	136	0	0.9	0.6	0.75	91.27	0.6	0.7	0.65	107.58	N	N	N	C
79	C27	56	F	70	147	Overweight	86	132	0	1	1	1	69.41	0.8	0.7	0.75	96.96	N	N	N	C
80	C28	69	F	67	154	Overweight	104	136	0	0.8	1	0.9	62.39	1	0.6	0.8	72.17	N	N	N	C
81	C29	60	F	60	152	Overweight	98	108	0	1	0.8	0.9	62.96	0.8	0.7	0.75	75.55	N	N	N	C
82	C30	48	F	74	165	Overweight	104	138	0	1	1	1	80.37	1	0.6	0.8	102.64	N	N	N	C
83	C31	50	F	53	145	Overweight	106	126	0	0.8	0.6	0.7	80.44	0.6	0.5	0.55	104.66	N	N	N	C
84	C32	49	F	50	142	Overweight	94	132	0	0.9	0.6	0.75	71.62	0.6	0.5	0.55	101.95	N	N	N	C
85	C33	56	F	78	164	Overweight	64	110	0	1	0.9	0.95	81.42	0.9	0.8	0.8	101.29	N	N	N	C
86	C34	60	F	56	165	Normal	94	132	0	0.8	0.8	0.8	66.04	0.7	0.5	0.6	88.14	N	N	N	C
87	C35	43	F	66	164	Normal	96	126	0	0.9	0.8	0.85	88.91	0.8	0.6	0.7	110.19	N	N	N	C

88	C36	59	F	62	160	Normal	72	132	0	1	0.9	0.95	62.4	0.8	0.7	0.75	81	N	N	N	C
89	C37	50	F	50	148	Normal	82	116	0	0.8	0.7	0.75	70.83	0.6	0.5	0.55	96.59	N	N	N	C
90	C38	50	F	50	150	Normal	98	138	0	0.9	0.7	0.8	66.4	0.8	0.6	0.7	75.89	N	N	N	C
91	C39	67	F	51	148	Normal	76	124	0	0.8	0.6	0.7	62.78	0.6	0.5	0.55	82.1	N	N	N	C
92	C40	48	F	55	154	Normal	82	130	0	0.8	0.8	0.9	66.37	0.8	0.5	0.65	95.89	N	N	N	C
93	C41	40	F	54	152	Normal	66	108	0	0.9	0.8	0.85	74.99	0.7	0.6	0.65	98.07	N	N	N	C
94	C42	57	F	50	150	Normal	88	126	0	0.6	0.8	0.7	69.99	0.6	0.6	0.6	85.51	N	N	N	C
95	C43	64	F	56	158	Normal	102	128	0	0.8	0.6	0.7	73.67	0.8	0.5	0.65	79.33	N	N	N	C
96	C44	60	F	50	152	Normal	92	136	0	0.7	0.8	0.75	62.96	0.7	0.5	0.6	78.7	N	N	N	C
97	C45	58	F	52	168	Underweight	98	126	0	0.8	0.8	0.8	62.92	0.8	0.5	0.65	77.44	N	N	N	C
98	C46	60	F	43	154	Underweight	108	136	0	0.7	0.6	0.65	62.48	0.6	0.6	0.6	67.68	N	A	I	C
99	C47	59	F	44	158	Underweight	76	134	0	0.6	0.8	0.7	60.1	0.6	0.6	0.6	90.31	N	N	N	C
100	C48	47	F	48	161	Underweight	62	118	0	0.8	0.6	0.7	75.28	0.6	0.6	0.6	91.61	N	N	N	C
101	C49	54	F	46	162	Underweight	98	132	0	0.6	0.8	0.7	66.71	0.6	0.6	0.6	79.65	N	N	N	C
102	C50	50	F	47	160	Underweight	106	138	0	0.8	0.6	0.7	74.5	0.6	0.6	0.6	86	N	N	N	C

N	Normal
A	Abnormal
I	Injury
F	Failure