"STUDY OF ASSOCIATION BETWEEN SERUM FERRITIN AND GLYCATED HEMOGLOBIN IN TYPE 2 DIABETES MELLITUS"

By

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Dissertation submitted to BLDE University, Vijayapur

In partial fulfillment of the requirements for the degree of

MD

IN

GENERAL MEDICINE

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Dr. TIMMANNA GIRADDI.

LIST OF ABBREVIATIONS USED

α	-	Alpha
β	-	Beta
μg	-	Micro gram
DM	-	Diabetes Mellitus
NIDDM	-	Non-insulin dependent diabetes mellitus
IDDM	-	Insulin dependent diabetes mellitus
ADA	-	American Diabetes Association
BMI	-	Body mass index
CDC	-	Center for Disease Control
ICMR	-	Indian Council of Medical Research
ESRD	-	End stage renal disease
GLUT	-	Glucose transporter
FPG	-	Fasting plasma glucose
IGT	-	Impaired Glucose Tolerance
HNF	-	Hepatocyte Nuclear Transcription Factor
MODY	-	Maturity Onset Diabetes of Young
DNA	-	Deoxyribonucleic acid
IPF	-	Insulin promoter factor
GDM	-	Gestational diabetes mellitus
PODIS	-	Prevalence of Diabetes in India Study
IDF	-	International Diabetes Federation
DKA	-	Diabetic Ketoacidosis
HHS	-	Hyperglycemic Hyperosmolar State

CAD	-	Coronary Artery disease
PAD	-	Peripheral Arterial disease
AGE	-	Advanced Glycosytion Endproducts
PKC	-	Protein Kinase C
TGF-β	-	Transforming Growth Factor –β
PAI	-	Plasminogen Activator Inhibitor
VEGA	-	Vascular Endothelial Growth Factor
DCCT	-	Diabetes Control and Complications Trial
UKPDS	-	United Kingdom Prospective Diabetes Study
HbA1c	-	Glycated hemoglobin
IL-1	-	Interleukin-1
TNF	-	Tumor Necrosis Factor
TIM	-	T cell Mucin Domain
2M	-	α-2-Macroglobulin
HDL	-	High density lipoprotein
LDL	-	Low density lipoprotein
TG	-	Triglyceride
TC	-	Total Cholesterol
FBS	-	Fasting Blood Sugar
PPBS	-	Post prandial Blood Sugar
IU	-	International units
Kg	-	Kilogram
LDL	-	Low density lipoprotein
m	-	Meter
mg/dl	-	Milligram per deciliter

min	-	Minute
mm Hg	-	Millimeter of mercury
n	-	Total number
NK	-	Natural killer
nmol	-	Nano mole
nmols/L	-	Nano moles per liter
SD	-	standard deviation

ABSTRACT

Background and objectives

Recently, Serum ferritin has sparked widespread interest in the pathogenesis and complications of diabetes. The present study was undertaken to estimate the levels of serum ferritin in patients with Type 2 Diabetes Mellitus and to correlate levels of serum ferritin with Glycemic status of Diabetes Mellitus.

Methodology

The present one and half year year cross sectional study was carried out in the Department of Medicine, BLDE University's Shri B M Patil medical college and research center , Vijayapur from January 2014 to june 2015. A total of 131 patients with type 2 diabetes mellitus were studied.

Results

Maximum number of cases were in the age group of 51 to 60 that is 54 patients (41.2%). The mean age of study population was 53.48 ± 8.56 years. Out of 131 patients, 70 (53.43%) were males and 61 patients (46.56%) were females, with a ratio of male to female 1.15:1. In 15 patients (11.5%), the levels of serum ferritin were high, in 55 patients (41.9%) the levels were upper normal.

Most of the patients had fasting and post prandial glucose abnormality (96% and 95% respectively).

Conclusion and interpretation

There was an positive association between serum ferritin levels and HbA1c in patients with type 2 diabetes mellitus. Higher serum ferritin levels were associated with poor glycemic control. Additionally we observed a significant correlation of serum

ferritin levels with variable factors body mass index, fasting blood sugar, post prandial blood sugar, high density lipoprotein (HDL), triglycerides and low density lipoprotein (LDL). However, we did not find significant correlation with variable factors age, gender, duration of diabetes and total cholesterol.

Keywords

Diabetes mellitus; Glycaemic control; serum ferritin;

CONTENT

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INTRODUCTION

Diabetes Mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.

Diabetes mellitus (DM) is a chronic and potentially disabling disease which is reaching an epidemic proportion in many parts of the world. It is a major and growing threat to global public health. The biggest impact of the disease is on adults of working age; particularly in developing countries. The vast majority of cases of the diabetes fall into two broad categories: those having little or no endogenous insulin secretory capacity (IDDM or type 1 DM) and those who retain endogenous insulin secretory capacity but have a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (NIDDM or Type 2 DM)[1,2].

Centers for Disease Control and Prevention (CDC) report in 2011 estimated that nearly 26 million Americans have diabetes.[3] Type 2 diabetes mellitus (DM) accounts for more than 90% of the diabetic population world wide. Additionally, an estimated 79 million Americans have prediabetes. Rates of diabetes are increasing worldwide. The International Diabetes Federation predicts that the number of people living with diabetes will to rise from 366 million in 2011 to 552 million by 2030.[4]The top 10 countries in number of people with diabetes are currently India, China, the United States, Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh. The prevalence of diabetes and its adverse health effects have risen more rapidly in South Asia than in any other region of the world.[5]

Thirty years ago, the prevalence of diabetes in India based on the Indian Council of Medical Research (ICMR) multicentric survey[6] was around two percent in urban India and one percent in rural India. In just three decades, these prevalence rates

have shot up to nine percent in urban India and three to eight percent in rural India, in adults over 20 years of age. These represents a 600 to 800% increase in prevalence rates of diabetes something which is unparallel in any Western nation. Indeed, India is now referred to as the "Diabetic Capital" of the world.

Further, DM is associated with several complications. The complications of diabetes mellitus include retinopathy, nephropathy and neuropathy (both peripheral and autonomic). The risk for atherosclerotic vascular disease is also increased in persons with DM. The risk for microvascular and neuropathic complications is related to both duration of diabetes and the severity of hyperglycemia; the increased risk for vascular disease actually antedates the onset of hyperglycemia to the degree associated with diabetes mellitus[1].

Ferritin is the major iron storage protein and plays a key role in iron metabolism. Ferritin in the circulation is a secretory form of the protein which is glycosylated and differs in subunit composition from the storage form found in the cells.[7] Serum ferritin concentration is directly related to body iron stores in healthy individuals, and is also an acute phase reactant which increases during inflammation.[8]

Iron is a transition metal that can be easily become oxidized and thus acts as an oxidant. The general effect of catalytic iron is to convert poorly reactive free radicals such as hydrogen peroxide into highly reactive radicals such as the hydroxyl radical. Increased accumulation of iron affects insulin synthesis and secretion from the pancreas,[9] interferes with the insulin extracting capacity of the liver[10] and Iron deposition in muscle decreases glucose uptake because of muscle damage.[11] Conversely, insulin stimulates cellular iron uptake through increased transferrin receptor externalization.[12] Iron deposition in the liver may also cause insulin resistance by interfering with the ability of insulin to suppress hepatic glucose

production.[13] Reactive oxygen species interfere with insulin uptake through direct effect on insulin receptor function[14] and inhibiting the translocation of glucose transporter (GLUT4) in plasma membrane.[15]

Serum ferritin concentration is directly related to body iron stores in healthy individuals. In general population, body ferritin stores are positively associated with the development of glucose intolerance, type 2 diabetes mellitus.[16]

This study intended to perform to find a link between serum ferritin and type 2 diabetes mellitus and also glycated hemoglobin as a blood glucose control marker in diabetic patients.

OBJECTIVE OF THE STUDY:

To determine the relation between serum ferritin and glycated hemoglobin in type 2 diabetes mellitus patients.

REVIEW OF LITERATURE

HISTORICAL REVIEW

Diabetes is perhaps as old as mankind. Cognizance of symptoms related to diabetes and recognition of the disorder was confined to a few geographic and cultural locations in the Ancient Era (up to 600 AD).

The knowledge acquired during this period was lost sight of and progress was tardy and indiscrete during the medieval period (600 to 1500 AD).

With the advent of modern age (1500 to 1758 AD) and its progression to renaissance and industrial revolution (1750 to 1850 AD), certain key features of diabetes were rediscovered and some new information was generated which stand out as landmarks in characterizing diabetes.

During the later decades of the 19th and first half of the 20th century, all round progress was achieved in the knowledge of pathology, predisposing factors, management, course and complications of diabetes mellitus. Growth of knowledge has been very fast in course of the second half of the last century(contemporary period) involving epidemiology, genetics, immunology and molecular biology which has led to accumulation of voluminous information on various aspects of this versatile disorder.[6,17]

Some key developments in scientific and clinical understanding of diabetes may be summarized as follows:

The earliest mention of diabetes like illness characterized by polyuria can be traced to Eqyptian Papyrus dating back to around 1550 B.C.[17]

- The sweet taste of diabetic urine was noted in the 5th and 6th century AD by the Indian physicians and in the 17th century by Thomas Willis. The term ‘Diabetes mellitus’, an allusion to the honeyed taste of urine, was first used in

the late 18th century by John Rollo and others, to distinguish it from other polyuric states in which urine was tasteless[17].

- In 1776, Matthew Dobson discovered that diabetic serum as well as urine contained sugar, and concluded that diabetes was a systemic condition rather than a disease of kidneys[17].
- Claude Bernard made numerous discoveries in the field of metabolism and diabetes during the mid to late 19th century, describing the storage of glucose in the liver as glycogen and hyperglycemia in experimental animals[17].
- In 1889, Oskar Minkowski and Josef Von Mering observed that total pancreatectomy produced diabetes in dogs[17].
- In 1893, Edovard Laguesse named that pancreatic islets after Paul Langerhans, who had described them and suggested that they produced a glucose lowering substance. This then hypothetical hormone was named ‘insulin’ by Jean de Meyer in 1909, over a decade before its discovery[17].
- Various workers, including George Zueler (Germany) and Nicolas Paulesco (Romania), isolated active but impure hypoglycemic extracts from the pancreas during the first two decades of the 20th century; but toxic side effects precluded their formal testing in diabetic patients[17].
- Insulin was discovered at the University of Toronto in 1921, through collaboration between Frederick G Banting, Charles H Best, James B Collip and J J R Macleod Insulin was extracted from chilled pancreas in an acid / ethanol mixture; the extracts were found to lower blood glucose levels in pancreatectomized dogs and were first tested in a human diabetic in January 1922.[17]

Major advances in the understanding of diabetes and metabolism have included:

- The sequencing of insulin in 1955 by Frederick Sanger and elucidation of its three dimensional structure in 1969 by Dorothy Hodgkin.
- The measurement of insulin concentration using the first radioimmunoassay by Solomon Berson and Rosalyn Yalow in 1959.
- The isolation of proinsulin in 1967 by Donal Steiner's group.
- Identification of specific insulin receptors by Pierre Freychet and colleagues in 1971 and
- The sequencing of the insulin receptor in 1985.

Landmarks in insulin discovery and development[6]

Year	Contribution	Discovery, Development
1869	Paul Langerhans	Identified Islet cells
1889	Joseph Von Mehring and Oskar Minkowski	Identified pancreas as the origin of fatal diabetes mellitus
1908	George Ludwig Zeuler	Injected acomatrol pancreatic extract into dying patient
1921	Paulesco	Pancreatin(Insulin)
1921	Banting and Best	Work started at the University of Toronto in the month of April
1922	Banting and Best	Insulin Isolation
1923	Nordisk Insulin Laboratory	Started production of Insulin
1926	Abel	Prepared the first crystalline Insulin
1934	Svedberg	Molecular weight of insulin was determined
1936	Hagedorn(Novo Nordisk)	Development of the first protamine Insulin(PZI)
1946	Hagedorn(Novo Nordisk)	Development of first prolonged acting Insulin-Neutral Protamine Hagedorn(NPH) or Isophane Insulin
1952	Hallas-Moller and Schlichtkrull	Development of the Lente series of Insulin

1955	Frederik Sanger	Elucidation on the structure of insulin and awarded with Nobel prize
1964	Novo Nordisk	Premixed insulin preparation were made available
1981	Jan Markussen and associates	First commercially available human insulin preparation using DNA technology
1996	Eli Lilly and company	First commercially introduced insulin analog, Lispro
2000	Novo Nordisk	Rapid acting insuliln analog – Insulin aspart made availabe
2000	Aventis Pharmaceuticals	Marketing of long lasting form of insulin-Insulin Glargine
2003	Novo Nordisk	Detemir another long acting insulin analogue introduced

Diabetes mellitus refer to a group of common metabolic disorder that shares the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduce insulin secretion, decreased glucose utilization and increased glucose production.[1,18]

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. In the United States, DM is the leading cause of end-stage renal disease (ESRD), non traumatic lower extremity amputations, and adult blindness. It also predisposes to cardiovascular diseases. With an increasing incidence worldwide, DM will be leading cause of morbidity and mortality for the foreseeable future[1,6,18,19].

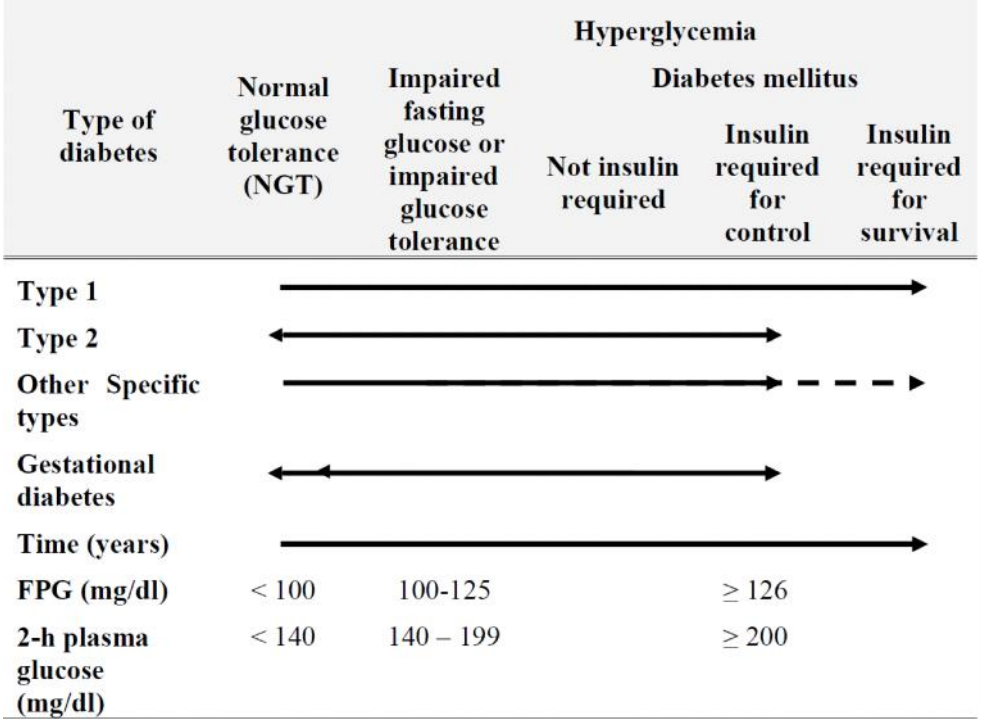
Classification of Diabetes Mellitus

DM is classified on the basis of the pathogenic process that leads to hyperglycemia, as opposed to earlier criteria such as age of onset or type of therapy. The two broad categories of DM are designated as[6]

- Type 1
- Type 2

Both types of diabetes are preceded by a phase of abnormal glucose homeostasis as the pathogenic processes progresses. 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. Distinct genetic and metabolic defects in insulin action and/or secretion give rise to the common phenotype of hyperglycemia in type 2 DM and have important potential therapeutic implications now that pharmacologic agents are available to target specific metabolic derangements. Type 2 DM is preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).

Spectrum of glucose homeostasis and diabetes mellitus[20]



Etiologic classification of diabetes mellitus[6]

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 β - cell function characterized by mutations in :

1. Hepatocyte nuclear transcription factor (HNF) 4α maturity onset diabetes of young (MODY) 1
2. Glucokinase (MODY 2)

3. HNF –1α (MODY 3)
4. Insulin promoter factor (IPF) 1 (MODY 4)
5. HNF – 1β (MODY 5)
6. Neuro D1 (MODY 6)
7. Mitochondrial deoxyribo nucleic acid (DNA)
8. Sub units of adenosine triphosphate (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, fibrocalculouspancreatopathy.

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, α-interferon, protease inhibitors, clozapine, beta blockers.

F. Infections – congenital rubella, cytomegalovirus, coxsackie.

G. Uncommon forms of immune - mediated diabetes "stiff-man" 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)

EPIDEMIOLOGY

Epidemiology

Diabetes is fast becoming the epidemic of the 21st century. Type 2 diabetes, which is more prevalent (more than 90% of all diabetes cases) and the main driver of the diabetes epidemic, now affects 5.9% of the world's adult population with almost 80% of the total in developing countries.[21]

Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000.[17] World Health Organization reported that, 346 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from consequences of high blood sugar. More than 80% of diabetes deaths occur in low and middle income countries. WHO projects that, diabetes deaths will double between 2005 and 2030.[22]

Race

The prevalence of type 2 diabetes mellitus varies widely among various racial and ethnic groups. Type 2 diabetes mellitus is becoming virtually pandemic in some groups of Native Americans and Hispanic people. The risk of retinopathy and nephropathy appears to be greater in blacks, Native Americans, and Hispanics.[23]

Sex

Type 2 DM is slightly more common in older women than men.[23]

Age

While type 2 diabetes mellitus traditionally has been thought to affect individuals older than 40 years, it is being recognized increasingly in younger persons, particularly in highly susceptible racial and ethnic groups and the obese. In some areas, more type 2 than type 1 diabetes mellitus is being diagnosed in pre pubertal children, teenagers and young adults. Virtually all cases of diabetes mellitus in older individuals are type 2.[23]

Indian scenario

India is in the midst of an ever - increasing epidemic of diabetes mellitus Data on type 1 diabetes mellitus from our country is scant. Clinic based data from the mid sixties to the eighties reported the prevalence of childhood diabetes with onset below 15 years of age as being one to four percent of all the diabetic subjects attending clinics in different parts of the country.[6,18]

According to recent study also, almost 95% of childhood diabetes reportedly belongs to Type 1 DM. Early onset type 2 diabetes, MODY, fibrocalculous pancreatic diabetes and diabetes associated with genetic syndromes accounted for the remaining cases.[6]

Type 2 DM accounts for more than 90% of all patients with diabetes in India. According to WHO there were an estimated 19.4 million diabetes individuals in 1995, and this number is projected to increase in 80 million by 2030. The ICMR study (1972 to 1975) was the first systematic nationwide collaborative study on the prevalence of diabetes mellitus.[6,18]

The prevalence of diabetes was found to be 2.8% in rural and five percent in the urban population above the age of 40 years. The prevalence of Diabetes in India

Study (PODIS) carried out in 77 centres recently reported a standardized prevalence rate for DM, in the total urban and rural population of 4.3, 5.9 and 2.7% respectively.[6]

Several epidemiological studies in migrant Indians and India itself show that, the population has a high genetic predisposition for diabetes, which is precipitated by environmental factors such as urbanization.[21] The prevalence of diabetes is four to six fold lower in rural areas, which is probably attributed to a conventional lifestyle which has beneficial effect on glucose tolerance (IGT).National Urban Diabetes Survey done in six cities, found age standardized prevalence rates of 12% for diabetes; with a slight male preponderance and 14% for impaired glucose tolerance. Subjects under the age of 40 years, had a prevalence of five percent for DM and 13% prevalence of impaired glucose tolerance.

The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025.[24] It is clear that in the last two decades, there has been a marked increase in the prevalence of diabetes among both urban as well as the rural Indians, with a suggestion that Southern India has seen the sharpest increase. Subsequent studies confirmed this high prevalence of diabetes in urban south India. Although in rural India the prevalence of diabetes is much lower than in the urban population, even here the prevalence rates are rapidly rising, though clearly more studies are needed. Variations in the prevalence rates of diabetes in different urban populations of India are expected because of the large variation in the prevalence of cardiovascular risk factors in different regions and states. It is evident that there is a shift in age of onset to younger age groups, which is alarming and this could have adverse effects on the nation's economy. Hence, the early identification of at - risk individuals and appropriate intervention to increase physical activity, bring about

changes in dietary habits could to a great extent help to prevent/ delay, the onset of diabetes and thus reduce the burden due to its associated complications in India.[21]

The world wide prevalence of DM 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, more than 360 million individuals will have diabetes by the year 2030. Although the prevalence of both type 1 and type 2 DM is increasing world wide, the prevalence of type 2 DM is rising much more rapidly because of increasing obesity and reduced activity levels as countries become more industrialized. This is true in most countries and 6 of the top 10 countries with the highest rates are in Asia. In the United States, the centre for Disease control and prevention (CDC) estimated that 20.8 million persons, or seven percent of the population, had diabetes in 2005 (30% of individuals with diabetes were undiagnosed).[6,18]

The prevalence is similar in men and women throughout most age ranges but is slightly greater in men more than 60 years. World wide estimates project that in 2030 the greatest number of individuals with diabetes will be 45 to 64 years of age.[18]

Causes for diabetic pandemic

The type 2 DM epidemic is tightly and consistently linked to that of obesity, both geographically and chronologically. Many factors like, urbanization and mechanization, together with globalized pattern of western pattern of lifestyle, together with poverty, lack of education and low socio-economic status and inner city deprivation are emerging as significant risk factors for DM. Lack of breast feeding, low birth weight is associated with insulin resistance and type 2 DM in adult life (especially in subjects who become obese) due to long term metabolic response during poor fetal nutrition.[25]

Obesity

Prevention of obesity, in women of child bearing age, is another primary goal because exposure to environment of a diabetic pregnancy places the fetus at increased risk for future onset diabetes. About 80% of patients are obviously obese at the time of diagnosis, usually with a central fat distribution in and around the abdominal cavity. In addition, many of those who are not traditionally obese, by weight criteria have increased percentage of fat predominantly distributed in the abdominal region. It is the most obvious target to prevent DM.

Body mass index (BMI)

Three key anthropometric measurements are important to evaluate the degree of obesity – weight, height and waist circumference. The BMI, calculated as weight (kg)/height(m)^2 or as weight (lbs)/height(inches)^2 x 703, is used to classify weight status and risk of disease. Body mass index, is used since it provides an estimate of body fat and is related to risk of disease. Lower BMI thresholds for overweight and obesity have been proposed for the Asia–Pacific region since this population appears to be at risk at lower body weights for glucose and lipid abnormalities.[1]

Classification of weight status and risk of disease[26]

	BMI (Kg/m^2)	Obesity Class	Risk of Disease
Underweight	<18.5		
Healthy weight	18.5 – 24.9		
Overweight	25.0 – 29.9		Increased
Obesity	30.0 – 34.9	I	High
Obesity	35.0 – 39.9	II	IIigh
Extreme Obesity	≥ 40	III	Extremely high

CRITERIA FOR THE DIAGNOSIS OF DIABETES MELLITUS[17,18]

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

Note:

In the absence of unequivocal hyperglycemia and acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day.

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

75 g anhydrous glucose dissolved in water; not recommended for routine clinical use.

SCREENING[1]

Widespread use of the fasting plasma glucose (FPG) as a screening test for type 2 DM is recommended because:

1. A large number of individuals who meet the current criteria for DM are asymptomatic and unaware that they have the disorder.
2. Epidemiologic studies suggest that type 2 DM may be present for up to a decade before diagnosis.
3. As many as 50% of individuals with type 2 DM have one or more diabetes - specific complications at the time of their diagnosis.
4. Treatment of type 2 DM may favorably alter the natural history of DM.

The ADA recommends screening all individuals more than 45 years every three years and screening individuals at an earlier age if they are overweight [body mass index (BMI) more than 25 kg/m^2] and have one additional risk factor for diabetes. In contrast to type 2 DM, a long asymptomatic period of hyperglycemia is rare prior to the diagnosis of type 1 DM.[1,6]

PATHOGENESIS

Type 2 DM

Insulin resistance and abnormal insulin secretion are central to the development of type 2 DM. Although the primary defect is controversial, most studies support the view that insulin resistance precedes an insulin secretory defect but that diabetes develops only when insulin secretion becomes inadequate.

Genetic Considerations

Type 2 DM has a strong genetic component. The concordance of type 2 DM in identical twins is between 70 and 90%. Individuals with a parent with type 2 DM have an increased risk of diabetes; if both parents have type 2 DM, the risk approaches 40%

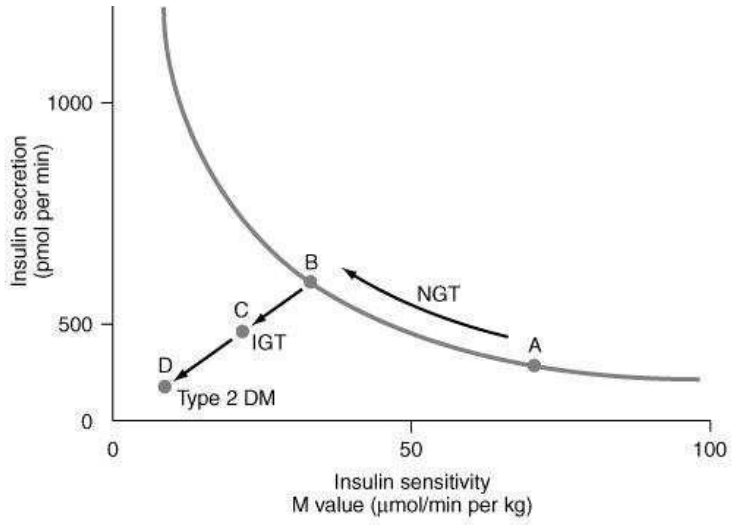
Insulin resistance, as demonstrated by reduced glucose utilization in skeletal muscle, is present in many non diabetic, first-degree relatives of individuals with type 2 DM. The disease is polygenic and multi factorial since in addition to genetic susceptibility, environmental factors (such as obesity, nutrition and physical activity) modulate the phenotype. The genes that predispose to type 2 DM are incompletely identified but recent genome–wide association studies have identified several genes that convey a relatively small risk for type 2 DM (relative risk of 1.1 to 1.5). Most prominent is a variant of the transcription factor 7 like 2 gene that has been associated with type 2 diabetes in several populations and with impaired glucose tolerance in one population at high risk for diabetes. Genetic polymorphisms associated with type 2 diabetes have also been found in the genes encoding the peroxisome proliferators – activated receptor -r, inward rectifying potassium channel expressed in beta cells, zinc transporter expressed in beta cells, IRS and calpain 10. The mechanisms by which these genetic alterations increase the susceptibility to type 2 diabetes are not clear but several are predicted to alter insulin secretion. Investigation using
genome -wide scanning for polymorphisms associated with type 2 DM is ongoing.[1]

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.[1]



Metabolic changes during the development of type 2 diabetes mellitus[1]

COMPLICATIONS OF DIABETES MELLITUS[6,18]

Acute Complications of DM

Diabetic ketoacidosis (DKA) and hyperglycemic hyperosmolar state (HHS) are acute complications of diabetes. DKA was formerly considered a hallmark of type 1 DM but it also occurs in individuals who lack immunologic features of type 1 DM and who can subsequently be treated with oral glucose-lowering agents (these obese

individuals with type 2 DM are often of Hispanic or African-American descent). HHS is primarily seen in individuals with type 2 DM. Both disorders are associated with absolute or relative insulin deficiency, volume depletion and acid-base abnormalities. DKA and HHS exist along a continuum of hyperglycemia with or without ketosis. Both disorders are associated with potentially serious complications if not promptly diagnosed and treated.[6,18]

Chronic Complications of DM

The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and nonvascular complications. The vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy, nephropathy) and macrovascular complications [coronary artery disease (CAD), peripheral arterial disease (PAD), cerebrovascular disease]. Nonvascular complications include problems such as gastroparesis, infections, and skin changes. Long-standing diabetes may be associated with hearing loss. Whether type 2 DM in elderly individuals is associated with impaired mental function is not clear.[18]

Chronic complications of diabetes mellitus

1) Microvascular
    a. Eye disease
        i. Retinopathy (nonproliferative/proliferative)
        ii. Macular edema
    b. Neuropathy
        i. Sensory and motor (mono - and polyneuropathy)

ii. Autonomic

c. Nephropathy

2) Macrovascular

a. Coronary artery disease

b. Peripheral arterial disease

c. Cerebrovascular disease

3) Other

a. Gastrointestinal (gastroparesis, diarrhea)

b. Genitourinary (uropathy/sexual dysfunction)

c. Dermatologic

d. Infectious

e. Cataracts

f. Glaucoma

g. Periodontal disease

The risk of chronic complications increases as a function of the duration of hyperglycemia; they usually become apparent in the second decade of hyperglycemia. Since type 2 DM often has a long asymptomatic period of hyperglycemia, many individuals with type 2 DM have complications at the time of diagnosis.[19]

The Microvascular complications of both type 1 and type 2 DM result from chronic hyperglycemia. Large, randomized clinical trials of individuals with type 1 or type 2 DM have conclusively demonstrated that a reduction in chronic hyperglycemia prevents or delays retinopathy, neuropathy and nephropathy. Other incompletely defined factors may modulate the development of complications.[27,28]

Mechanisms of Complications

Although chronic hyperglycemia is an important etiologic factor leading to complications of DM, the mechanism(s) by which it leads to such diverse cellular and organ dysfunction is unknown.[21]

Four prominent theories, which are not mutually exclusive, have been proposed to explain how hyperglycemia might lead to the chronic complications of DM.[1,27,29]

One theory is that increased intracellular glucose leads to the formation of advanced glycosylation end products (AGEs) via the nonenzymatic glycosylation of intra and extracellular proteins. Nonenzymatic glycosylation results from the interaction of glucose with amino groups on proteins. AGEs have been shown to cross-link proteins (for example collagen, extracellular matrix proteins), accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction and alter extracellular matrix composition and structure. The serum level of AGEs correlates with the level of glycemia and these products accumulate as glomerular filtration rate declines.

A second theory is based on the observation that hyperglycemia increases glucose metabolism via the sorbitol pathway. Intracellular glucose is predominantly metabolized by phosphorylation and subsequent glycolysis but when increased, some glucose is converted to sorbitol by the enzyme aldose reductase. Increased sorbitol concentration alters redox potential, increases cellular osmolality, generates reactive oxygen species and likely leads to other types of cellular dysfunction. However, testing of this theory in humans, using aldose reductase inhibitors, has not demonstrated significant beneficial effects on clinical endpoints of retinopathy, neuropathy or nephropathy.

A third hypothesis proposes that hyperglycemia increases the formation of diacylglycerol leading to activation of protein kinase C (PKC). Among other actions, PKC alters the transcription of genes for fibronectin, type IV collagen, contractile proteins and extracellular matrix proteins in endothelial cells and neurons. Inhibitors of PKC are being studied in clinical trials.

A fourth theory proposes that hyperglycemia increases the flux through the hexosamine pathway, which generates fructose-6-phosphate, a substrate for O-linked glycosylation and proteoglycan production. The hexosamine pathway may alter function by glycosylation of proteins such as endothelial nitric oxide synthase or by changes in gene expression of transforming growth factor B (TGF-B) or plasminogen activator inhibitor-1 (PAI-1).

Growth factors appear to play an important role in DM–related complications and their production is increased by most of these proposed pathways. Vascular endothelial growth factor A (VEGF-A) is increased locally in diabetic proliferative retinopathy and decreases after laser photocoagulation. TGF-B is increased in diabetic nephropathy and stimulates basement membrane production of collagen and fibronectin by mesangial cells. Other growth factors, such as platelet-derived growth factor, epidermal growth factor, insulin-like growth factor I, growth hormone, basic fibroblast growth factor and even insulin, have been suggested to play a role in DM-related complications. A possible unifying mechanism is that hyperglycemia leads to increased production of reactive oxygen species or superoxide in the mitochondria; these compounds may activate all four of the pathways described above. Although hyperglycemia serves as the initial trigger for complications of diabetes, it is still unknown whether the same pathophysiologic processes are

operative in all complications or whether some path ways predominate in certain organs.[6]

Glycemic Control and Complications

The Diabetes Control and Complications Trial (DCCT) provided definitive proof that reduction in chronic hyperglycemia can prevent many of the early complications of type 1 DM. This large multicenter clinical trial randomized over 1400 individuals with type 1 DM to either intensive or conventional diabetes management and prospectively evaluated the development of retinopathy, nephropathy and neuropathy. Individuals in the intensive diabetes management group received multiple administrations of insulin each day along with extensive educational, psychological and medical support.[30]

Individuals in the conventional diabetes management group received twice-daily insulin injections and quarterly nutritional, educational and clinical evaluation. The goal in the former group was normoglycemia; the goal in the latter group was prevention of symptoms of diabetes. Individuals in the intensive diabetes management group achieved a substantially lower hemoglobin A1C (7.3%) than individuals in the conventional diabetes management group (9.1%).[30]

The DCCT demonstrated that improvement of glycemic control reduced nonproliferative and proliferative retinopathy (47% reduction), microalbuminuria (39%reduction), clinical nephropathy (54% reduction) and neuropathy (60%reduction). Improved glycemic control also slowed the progression of early diabetic complications. There was a nonsignificant trend in reduction of macrovascular events during the trial (most individuals were young and had a low risk of cardiovascular disease). The results of the DCCT predicted that individuals in the intensive diabetes management group would gain 7.7 additional years of vision, 5.8

additional years free from ESRD and 5.6 years free from lower extremity amputations. If all complications of DM were combined, individuals in the intensive diabetes management group would experience 15.3 more years of life without significant microvascular or neurologic complications of DM, compared to individuals who received standard therapy. This translates into an additional 5.1 years of life expectancy for individuals in the intensive diabetes management group.[30]

The benefit of the improved glycemic control during the DCCT persisted even after the study concluded and glycemic control worsened. For example, individuals in the intensive diabetes management group for a mean of 6.5 years had a 42–57% reduction in cardiovascular events [nonfatal myocardial infarction (MI), stroke or death from a cardiovascular event] at a mean follow-up of 17 years, even though their subsequent glycemic control was the same as those in the conventional diabetes management group.[30]

The benefits of an improvement in glycemic control occurred over the entire range of A1C values, suggesting that at any A1C level, an improvement in glycemic control is beneficial. The goal of therapy is to achieve an A1C level as close to normal as possible, without subjecting the patient to excessive risk of hypoglycemia.[30]

The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that each percentage point reduction in A1C was associated with a 35% reduction in microvascular complications. As in the DCCT, there was a continuous relationship between glycemic control and development of complications. Improved glycemic control did not conclusively reduce (nor worsen) cardiovascular mortality but was associated with improvement with lipoprotein risk profiles, such as reduced triglycerides and increased HDL.[31]

One of the major findings of the UKPDS was that strict blood pressure control significantly reduced both macro- and microvascular complications. In fact, the beneficial effects of blood pressure control were greater than the beneficial effects of glycemic control. Lowering blood pressure to moderate goals(144/82 mmHg) reduced the risk of DM-related death, stroke, microvascular end points, retinopathy, and heart failure (risk reductions between 32 and 56%).[31]

Similar reductions in the risks of retinopathy and nephropathy were also seen in a small trial of lean Japanese individuals with type 2 DM randomized to either intensive glycemic control or standard therapy with insulin (Kumamoto study). These results demonstrate the effectiveness of improved glycemic control in individuals of different ethnicity and presumably, a different etiology of DM (that is phenotypically different from those in the DCCT and UKPDS).[31]

The findings of the DCCT, UKPDS and Kumamoto study support the idea that chronic hyperglycemia plays a causative role in the pathogenesis of diabetic microvascular complications. These landmark studies prove the value of metabolic control and emphasize the importance of (1) intensive glycemic control in all forms of DM and (2) early diagnosis and strict blood pressure control in type 2 DM.[31]

Renal complications of diabetes mellitus

Diabetic nephropathy, a relatively common microvascular complication of both type 1 and type 2 DM contributes maximally to the pool of patients with chronic renal failure. It is defined clinically as the presence of persistent proteinuria in a diabetic patient usually with retinopathy, elevated blood pressure and declining glomerular function, in the absence of UTI, other renal disease and/or heart failure.[29,32]

SERUM FERRITIN

Ferritin was discovered in 1937 by the French scientist Laufberger, who isolated a new protein from horse spleen that contained up to 23% by dry weight of iron. The appearance of ferritin in human serum was documented several years thereafter. However , quantification of serum ferritin awaited the purification of ferritin and anti-ferritin antibodies and the development of sensitive immunoassay techniques.[33]

In 1972, using an immunoradiometric assay, Addison et al.. convincingly demonstrated that ferritin could be reliably detected in human serum.[34] To determine the relationship between serum ferritin level and total body iron stores, the authors measured serum ferritin in normal population, patients with iron deficiency and individuals with iron overload. They demonstrated that serum ferritin was elevated in patients with iron overload and decreased in patients with iron deficiency diseases.[33]

In 1975 Jacobs and Worwood suggested that the assay of serum ferritin might provide a "useful and convenient method of assessing the status of iron storage." [35] Serum ferritin continues to be measured to this day, although it is now known that many additional factors, including inflammation, infection and malignancy—all of which may elevate serum ferritin—complicate the interpretation of this value. What is most surprising is that despite this long history of clinical use, fundamental aspects of the biology of serum ferritin are still unclear. For example its tissue of origin, secretory pathway, receptor interactions and cellular effects remain topics of active debate. In this chapter, we will discuss recent studies on serum ferritin and its roles in iron delivery, immunity, inflammation, angiogenesis and cancer as well as its current use as a clinical tool.[33]

BASIC BIOLOGY

Ferritin is present in most tissues as a cytosolic protein, although a mitochondrial form has recently been described and nuclear localization and functions have been proposed. Ferritin plays an important role in the storage of intracellular iron, and has been the subjective of extensive recent reviews. Ferritin is a 24-subunit protein that is composed of two types of subunits, termed H and L. H refers to the original isolation of isoforms of ferritin from human heart, which are rich in the H subunit, or to its electrophoretic migration as the heavier of the two subunits. L refers to ferritin isolated from human liver, which is rich in a lighter subunit. The ratio of H to L subunits within the assembled ferritin protein varies depending of tissue type and developmental stage. Genes encoding the H and L subunits of human ferritin are located on chromosomes 11q and 19q respectively. Both H and L ferritin also have multiple pseudogenes. Amino acid sequence similarity between ferritin H and L subunits in mammals is bout 50%; sequence conservation between subunit types is even greater (among mammalian H subunits, there is approximately 90% homology; among L subunits approximately 80% homology).[33]

Serum ferritin is relatively iron-poor. Based on its ability to bind concanavilin A, serum ferritin is believed to be glycosylated. It is composed primarily of the L subunit type, as measured by immunological cross reactivity with anti-ferritin L antibodies. The source and detailed secretory pathway of serum ferritin are not completely understood. Hepatocytes, macrophages and Kupffer cells have been shown to secrete ferritin. Despite the absence of a conventional secretary signal on ferritin L, it appears and serum ferritin L and tissue ferritin L are encoded by the same gene. Thus, ferritin L was secreted from hepatocytes transfected with ferritin L cDNA via a classic secretory pathway. Nevertheless, due to lack of a signal peptide sequence

that mediates ferritin secretion, the mechanisms of how ferritin enters the secretory pathway require further characterization. Ferritin secretion into the medium of cultured cells is increased by iron and the cytokines interleukin-1(IL-1) and tumor necrosis factor- α(TNF-α). This enhanced secretion was blocked by co-treatment with dichlorofuranosylbenzimidazole (DRB), a specific transcriptional inhibitor, suggesting that these cytokines transcriptionally upregulate ferritin and its secretion.[33]

In rare cases, hyperferritinemia arises from hereditary disorders that do not cause iron overload. This includes mutations in the gene for tissue ferritin L, and this has been used as evidence that serum ferritin is encoded by this gene. For example, individuals with hyperferritinemia- cataract syndrome who have mutations that increase production of tissue ferritin L also show increased levels of serum ferritin. Recently a new missense mutation in the ferritin L coding sequence was identified and shown to be associated with hyperferritinemia without overall iron overload. The mutation, which mapped to the amino terminus of the L ferritin subunit and did not cause clinical symptoms, was proposed to cause hyperferritinemia by increasing ferritin secretion. Hereditary hyperferritinemia also results from mutations in the ferroportin and ceruloplasmin genes, as well as genes causing genetic hemochromatosis such as HAMP, HFE, TFR2 and HJV. Although the extent of iron overload differs among these patients, in these cases, the increase in serum ferritin is secondary to an increase in systemic iron.[33]

The decreased serum iron, increased macrophage iron and decreased dietary iron absorption of anaemia of inflammation are explained by increase in hepcidin expression induced by inflammatory cytokines; the increased serum iron, depleted macrophage iron and accelerated dietary iron absorption in hereditary

hemochromatosis result from aberrant regulation of hepcidin expression from genetic defects.[33]

Extracellular ferritin in physiological and pathological processes[33]

Due to difficulties in isolating serum ferritin in quantity, few if any experiments have directly assessed effects of exogenous administration of serum ferritin. However investigators have studied the effects of exogenous tissue ferritin on cells. It is uncertain whether this accurately models serum ferritin or whether it instead models paracrine effects of ferritin released from adjacent cells. Despite this uncertainty, several interesting observations have been made using tissue ferritin as a model, including the identification of ferritin receptors and the discovery of proliferative and signalling responses to ferritin.

Iron delivery system

Studies have shown that extracellular ferritin can function as an iron carrier to provide iron to cells. Compared to transferring, which carries a maximum of 2 iron atoms, a single ferritin molecule can sequester up to 4500 iron atoms, thus making it potentially a very effective iron delivery system. Serum ferritin, which is believed to be iron poor, carries much less iron than this, but could nevertheless make a significant impact on iron delivery.

In a study[36] they studied ferritin release by Kupffer cells loaded with iron. Their results showed that about 50% of the iron content of these cells was released to the culture medium within 24 hours in the form of ferritin. When this conditioned medium was used to culture isolated hepatocytes, released ferritin was quickly taken up by the cells. The authors calculated that one hepatocyte could accumulate over 1,60,000 iron molecules per minute via this efficient mechanism. This study

demonstrates that exogenous ferritin can function as a highly efficient iron delivery mechanism.

Although erythroid cells take up iron primarily via the transferrin-transferrin receptor pathway, it has also been shown that ferritin secreted by macrophages can function as an iron source for erythroid precursor cells. Using a two-phase culture protocol, the authors of this study showed that in the absence of transferrin, monocyte- derived macrophages provided enough iron for the proliferation of erythroid precursor cells. Although the exact pathway that mediates ferritn uptake by erythroid cells has not been not characterized, receptor-mediated endocytosis might be involved in this process. However, since a primary effect in the development of TfR knockout mice is a failure of erythropoiesis, it is likely that the transferring-mediated pathways plays the primary role in iron delivery to the developing erythrocyte[33]

In order for extra cellular ferritin to carry out a physiological role, a cell surface receptor must be envisioned. Indeed, saturable binding of ferritin to a variety of a different cell types has been observed for many years. Fargion et al. identified a saturable binding site for ferritin on the surface of human lymphocytes. Binding was specific to H ferritin, not L ferritin. Further studies showed that most B cells and about 30% of CD4 and CD8 T- lymphocytes possessed this binding ability. The binding of ferritin to lymphocytes was shown to decrease cell proliferation. Specific and saturable binding of ferritin has also been observed in liver cells, brain oligodendrocytes, enterocytes, and erythroid precursor cells. Studies using recombinant human ferritin indicated that at least two different types of ferritin receptors are present on liver cells. The first type of ferritin receptor had similar binding affinities for ferritin H and L, while the second type of receptor showed a

specific binding of H ferritin. When H ferritin was added to the culture medium, cells expressing H receptors showed decreased proliferation and colony formation. Interestingly, a specific H ferritin receptor is also present on activated, but not quiescent, liver lipocytes. The activated lipocytes can internalize ferritin via this receptor. As activated lipocytes are responsible for increased collagen production and liver cirrhosis in many iron overload diseases, the authors speculate that the H ferritin receptor on the surface of activated lipocytes may mediate the transfer of iron from outside to lipocytes and thus activate them.[33]

Binding of exogenous ferritin to cell surface receptors has also been implicated as an important iron delivery pathway in the brain. Although the transferrin-transferrin receptor pathway is the main iron import system in most cells, TfR mRNA is not detectable in white matter tracts, even in rats that are fed iron deficient diets. As iron is required for oligodendrocytes to produce myelin and these cells contain more iron than any other cells in the central nervous system, other iron uptake systems that are independent of transferring must be present.

A study[37,38] identified an H ferritin receptor on the cell surface of oligodendrocytes that could take up ferritin via receptor-mediated endocytosis. Study proposed that iron delivered by ferritin is the major source of iron for oligodendrocytes. Other study[39] demonstrated binding of ferritin to other cell types, although specificity for H- or L- ferritin was not explicitly examined.

Thus experiments using intestinal Caco-2 cells indicated that enterocytes possess a ferritin receptor and absorb ferritin via receptor-mediated. A ferritin receptor is also present on placental membranes. Interestingly, in pregnant women with mild or moderate iron deficiency, ferritin receptor binding sites are much more abundant than in pregnant women with normal iron status.[33]

Although many studies[40,41] have identified ferritin binding sites on cells, the first cell surface receptor for ferritin to be cloned was mouse T cell immunoglobulin-domain and mucin-domain 2(TIM-2). TIM-2 is a transmembrane protein expressed in liver, kidney, T cells and B cells.

There is no known human ortholog of TIM-2, although TIM-1 shares sequence homology with TIM-2. TIM-2 has been shown to inhibit T cell activation. TIM-2 was identified as a ferritn H receptor in a screen for TIM-2 ligands. The authors demonstrated that TIM-2 specifically bound ferritin H and not ferritin L. The interaction between ferritin H and

TIM-2 on the cell surface cause internalization of ferritin H into endosomes. This study is consistent with a role for TIM-2 in delivering iron- containing ferritin into cells.[33]

A study[42] demonstrated that TIM-2 is expressed on oligodendrocytes and that its expression level is responsive to iron challenge, as iron repletion decreased its expression while iron chelation increased its expression. Since there no detectable Tf-TfR pathway for delivery in oligodendrocytes, ferritin TIM-2 was suggested to be the primary mechanism for iron uptake by these cells.

Recently a study[43] identified another cell surface receptor for ferritin, Scara-5. Scara-5 is a scavenger receptor that can bind various ligands. In contrast to TIM-2 ,which is a ferritin H receptor, Scara-5 preferentially binds ferritin L.

Scara-5 plays an important role in kidney organogenesis, presumably by delivering iron to cells. Identification of Scara-5 grew out of the observation that despite the embryonic lethality of a TfR1 knockout, some organogenesis still occurs in early embryos. In addition, hypotransferrinemic mice that produce less than 1% of serum transferrin of normal mice show normal organogenesis, and patients with

familial hypotransferrinemia also have normal organ development. These studies led the authors to speculate that there must be other mechanisms responsible for cellular iron uptake besides Tf-TFR. Using murine chimeric embryos composed of unlabelled TfR1 wild type cells and TfR cells tagged with green fluorescent proein, they demonstrated two independent iron delivery systems during kidney organogenesis. They showed that the ureteric bud takes up iron via the classic TfR1 pathway, while capsular cells take up iron via a TfR1- independent pathway, which was identified as a ferritin L receptor, Scara-5. The authors further showed that iron-containing ferritin bound to Scara-5 and underwent endocytosis, releasing iron into the cytoplasm. It will be interesting to determine mechanisms that dictate cell type specificity for transferring-dependent and ferritin- dependent iron delivery, and to explore the role of Scara-5 in the adult animal.[33]

A human ferritin receptor was recently identified. Using expression cloning, a study[43] identified human TfR1 as a cell surface receptor for H ferritin. No binding to L ferritin was observed. The binding of H ferritin to TfR1 was independent of HFE and was only partially inhibited by diferric transferrin, suggesting that binding sites for transferrin and ferritin on the receptor do not entirely overlap. The binding of H ferritin to TfR1 induces H ferritin to enter endosomes and lysosomes and accounts for most of the binding of H ferritin to cell surface.

Mechanisms by which iron is released from ferritin for intracellular use are currently being investigated. It has been suggested that iron may exit the protein through gated pores. Using deferoxamine(DFO) as a iron chelator, a study demonstrated that, lysosome- dependent ferritin degradation is required for iron release.[33]

A study[44] confirmed this result and described an additional route for iron release following treatment with the more permeant iron chelators deferriprone and desferasirox, which were found to induce ferritin degradation. It will be interesting to identify pathways of iron trafficking following its release from ferritin.

Signalling molecule

Very recently, a study[45] proposed a new role for extracellular ferritin as a pro-inflammatory signalling molecule in hepatic stellate cells. They observed that cells treated with ferritin activated a pathway comprising PI3 kinase phosphorylation, protein kinase C zeta activation and MAP kinase activation, ultimately culminating in activation of NFkB. Activation of NFkB in turn enhanced the expression of pro-inflammatory mediators, including lnterleukin1 beta, iNOS and others. Interestingly, this function was independent of the iron content of ferritin, suggesting that exogenous ferritin may subsume roles entirely independent of its classic role as an iron binding protein.

Immunity

For many years, it has been known that patients with hematologic malignancies, such as Hodgkin's disease and acute leukemia, have impaired cell-mediated immunity. These patients also exhibit elevated levels of serum ferritin. This suggested a possible relation between serum ferritin and immunity. Early in vitro studies indicated that ferritin modulated body immune function by inhibiting lymphocyte function. When human lymphocytes were treated with splenic ferritin, lymphocyte cell activation by phytohaemagglutinin(PHA) and concanavalin A(Con A) was inhibited. Later in vivo studies also suggested that ferritin inhibits immunity[33]

Chemokines are a family of proteins with chemotactic and activating effects on various leukocyte lineages that play important roles in T helper cell responses, hematopoiesis, hemostasis and angiogenesis. A study[43] observed that the chemokine CSCL12 induced binding of ferritin heavy chain to the CXC chemokine receptor 4 (CXCR4) both in vitro and in vivo. Ferritin H overexpression repressed CXCR4-mediated ERK 1/2 activation, while ferritin H knockdown enhanced ERK 1/2 activation.

The signalling pathways that mediate the anti-immune function of ferritin H are not completely understood. However, the identification of TIM-2 as a specific cell surface receptor for ferritin H makes it tempting to speculate that there may be a link between the immune suppressive function of ferritin H and TIM-2. TIM-2 is a member of the T cell immunoglobulin and mucin-domain (TIM) gene family, which is involved in the regulation of immune responses. The TIM gene family is found within the TAPR locus(T cell and airway phenotype regulator) on mouse chromosome 11 and human chromosome 5. Genetic variations in the TAPR locus are associated with various immune-related diseases. A number of polymorphisms are associated with asthma and other allergic diseases. The mouse TIM gene family consists of eight members(TIM-1 to TIM-8), in human, however, the TIM family seems to include only three members(TIM-1, TIM-2 and TIM-4). There is no human orthologue of mouse TIM-2. However, given to its close sequence homology, human TIM-1 may share the same or atleast some of the functions of murine TIM-2. In contrast to TIM-1, which is expressed on Th1 cell surface and regulates Th1 immune responses, TIM-2 is mainly expressed in differentiated Th2 cells and negatively regulates Th2 cell responses. Knockout TIM-2 mice were generated recently. TIM-2 deficient mice display increased inflammation and Th2 cytokine production in a

mouse atopic model. These results indicate that TIM-2 is a negative regulator of Th2 immune responses. However, despite these suggestive relationships, whether ferritin H plays its immunosuppression function via the activation of TIM-2 receptor has not been studied.[33]

Inflammation

Serum ferritin is widely recognized as an acute phase reactant and marker of acute and chronic inflammation, and is nonspecifically elevated in a wide range of inflammatory conditions, including chronic kidney disease, rheumatoid arthritis and other autoimmune disorders, acute infection, and malignancy. The elevated ferritin in these states reflects increases total body iron storage, but paradoxically, these stores are sequestered and not available for hematopoiesis, a process which contributes to the widely recognized anemia of inflammation. This relative iron deficiency in inflammation and malignancy is presumed to have developed as a defense mechanism to restrict serum iron from utilization by pathogens and tumours. Still's disease and hemophagocytic syndrome represent two clinical entities in which serum ferritin elevations are particularly remarkable.[33]

Angiogenesis

The research for binding partners of ferritin in human serum led to the identification of high molecular weight kininogen (HK) as a ferritin interacting protein. HK is a 120 kDa abundant plasma protein which was initially described as a co-factor in the intrinsic coagulation cascade. HK is cleaved by the serine protease kallikrein to produce two independently active proteins: bradykinin(BK) and two-chain high molecular weight kininogen (HKa). BK is a 9 amino acid rapid acting

peptide which induces NO release, pain and vasodilation. BK is also a pro-angiogenic peptide. In contrast, the other by product of HK cleavage, HKa is anti-angiogenic.[33]

Angiogenesis, the process of creating new blood vessels from pre-existing vessels, is a key step in multiple physiologic and pathologic processes ranging from wound healing to the menstruation cycle to tumour growth and metastasis. The process of angiogenesis is regulated by a balance of multiple pro and anti-angiogenic factors. Interestingly, the two HK cleavage products have opposing roles in angiogenesis: BK promotes vessel formation while HKa inhibits this process. Ferritin, through a direct interaction with both HK and HKa, is a newly defined angiogenic regulator.[33]

Deletion mapping and solid phase binding assays revealed that ferritin directly interacts with the light chain of HK with a Kd of 140 nM. Ferritin decreases the cleavage of HK by kallikrein and by two inflammatory proteases, neutrophil elastase and mast cell tryptase. Though decreasing the cleavage of HK, ferritin decreases the production of BK and HKa, thus reducing the levels of both of these angiogenic regulators.[33]

Additionally, ferritin directly binds HKa. In fact, ferritin has a 10-fold higher affinity for HKa than HK. Ferritin binds within domain 5 of HKa. This domain is responsible for the anti-angiogenic properties of HKa and is exposed when HK is cleaved to release BK and form HKa. Though binding to the anti-angiogenic domain of HKa, ferritin antagonizes HKa's effects, leading to increased blood vessel growth. Indeed, in a mouse tumour model where HKa reduces tumour blood vessel growth, the addition of ferritin counteracts the effects of HKa, leading to significantly increased intratumour blood vessel density.[33]

As described below, serum ferritin levels rise significantly during inflammation and certain malignancies times when angiogenesis, both physiologic and pathologic, occurs. The pro-angiogenic activity of ferritin exerted through its ability to bind HK/HKa may provide a rationale for this increase: serum ferritin levels may rise in order to function as an angiogenic modulator, working to increase new blood vessel growth. This may represent a physiologic response in the setting of inflammation and wound healing, and may also represent a pathologic response in the setting of tumour growth.[33]

Interaction between ferritin and other plasma proteins

In addition to HK, several other ferritin binding partners in serum and/or plasma have been identified including apolipoprotein B, α-2-macroglobulin(α2M), anti-ferritin autoantibody and fibrinogen. By binding to apolipoprotein B, ferritin post translationally inhibits its secretion. α2M is a large plasma protein that can bind many ligands and remove them from blood circulation by α2M receptor mediated endocytosis. The identification of α2M as a ferritin binding protein indicated a potential pathway for cellular uptake and/or clearance of ferritin from circulation. In addition, ferritin autoantibodies and a ferritin immune complex were identified in canine serum, which may contribute to the clearance of circulating ferritin.[33]

Ferritin as a clinical tool

Ferritin is a valuable tool for the clinician, both for the evaluation of common disease states, such as iron-deficiency anemia, and for evaluation of hereditary and acquired iron-overload conditions, such as hereditary hemochromatosis and chronic transfusion therapy. Serum ferritin is usually part of panel of several

blood tests routinely ordered to diagnose and manage these conditions, and is arguably the single most useful marker in most populations, though some caveats apply, as discussed below. Elevated serum ferritin levels can also be a diagnostic clue to very rare but devastating autoimmune or inflammatory disorders, such as hemophagocytic syndrome and Still's disease.[33]

Despite its clear utility as a clinical tool to assess body iron stores, much of the biology of serum ferritin remains as elusive today as when it was first discovered. For example, cellular mechanisms involved in the secretion of ferritin, which does not contain a canonical leader sequence, remains unknown. This will be important to unravel, particularly as it is becoming clear that extracellular ferritin can subsume many functions unrelated to its classic role as an intracellular iron storage protein. The delineation of precise relationships between ferritin secretion and immunomodulation, iron delivery, and triggering of signalling pathways all will require further investigation. The study of ferritin isolated from the serum of normal, non-hemochromatotic individuals may shed additional light on the biochemistry of this protein. Finally, identification of cell types responsible for the secretion of human ferritin, and further studies on the cells and receptors targeted by ferritin action may bring us closer to an understanding of this multifunctional protein.[33]

The Role of Ferritin in Diabetes and Its Complications

Non-insulin dependent diabetes mellitus is a common complication of diseases of iron overload such as haemochromatosis; 53-80% of patients with haemochromatosis develop diabetes. The development of diabetes in haemochromatosis is related to the magnitude of the excess iron[46]. In a cross sectional, population based study of over 1000 middle aged men in eastern Finland we found that fasting concentrations of serum insulin and blood glucose were raised in

men with high serum concentrations of ferritin (an indication of raised stores of iron)[47]. Iron is a catalyst of free radical stress, and it has been suggested that free radicals and lipid peroxidation play a part in the etiology of diabetes. Formation of hydroxyl radicals catalysed by iron may play an important part in the development of diabetes since the cells that produce insulin are extraordinarily sensitive to damage from oxidation[48]. In another cohort study low plasma concentrations of vitamin E were associated with an increased incidence of diabetes, which supports this theory[49]. There are, however, no other studies of the association between iron stores and the incidence of diabetes in a healthy population.

The central importance of iron in the pathophysiology of disease is derived from the ease with which iron is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical[50]. Oxygen normally accepts four electrons and is converted directly to water. However, partial reduction of oxygen can and does occur in biological systems. Thus, the sequential reduction of oxygen along the univalent pathway leads to the generation of superoxide anion, hydrogen peroxide, hydroxyl radical, and water[50,51]. Superoxide and hydrogen peroxide appear to be the primary generated species. These species may then play a role in the generation of additional and more reactive oxidants, including the highly reactive hydroxyl radical (or a related highly oxidizing species) in which iron salts play a catalytic role in a reaction. This reaction is commonly referred to as the metal catalyzed Haber-Weiss reaction[50]: Because iron participates in the formation of reactive oxygen species, organisms take great care in the handling of iron. Indeed, iron sequestration in transport and storage proteins may contribute to antioxidant defenses. It is now well established that oxidants can cause

the release of catalytic iron[50]; thus, a vicious cycle is initiated that leads to the formation of more reactive oxygen species.

Iron overload is not a prerequisite for iron to mediate either diabetes or its complications. Important in its pathophysiology is the availability of so-called catalytic iron or iron that is available to participate in free radical reactions.

The role of Ferritin in the induction of diabetes

Systemic iron overload could contribute to abnormal glucose metabolism was first demonstrated by the observation that the frequency of diabetes is increased in classic hereditary hemochromatosis (HH). However, with the discovery of novel genetic disorders of iron metabolism, it is obvious that iron overload, irrespective of the cause or the gene involved, results in an increased incidence of type 2 diabetes. The role of iron in the pathogenesis of diabetes is suggested by 1) an increased incidence of type 2 diabetes in diverse causes of iron overload and 2) reversal or improvement in diabetes (glycemic control) with a reduction in iron load achieved using either phlebotomy or iron chelation therapy. Recently, a link has been established between increased dietary iron intake, particularly eating red meat and increased body iron stores, and the development of diabetes. A causative link with iron overload is suggested by of the improvement in insulin sensitivity and insulin secretion with frequent blood donation and decreased iron stores[52,53].

Although the exact mechanism of iron-induced diabetes is uncertain, it is likely, as discussed below, to be mediated by three key mechanisms:

1) Insulin deficiency

2) Insulin resistance and

3) Hepatic dysfunction

An understanding of the pathogenic pathways of iron-induced diabetes is

derived mainly from studies on animal models of hemochromatosis.

In a mouse model of hemochromatosis, iron excess and oxidative stress mediate apoptosis of pancreatic islets with a resultant decrease in insulin secretory capacity[54]. Pancreatic islets have an extreme susceptibility to oxidative damage, perhaps because of the nearly exclusive reliance on mitochondrial metabolism of glucose for glucose-induced insulin secretion and low expression of the antioxidant defense system[55]. A high expression of divalent metal transporter additionally predisposes them for more accumulation of iron than other cells[56] and potentiates the danger from iron-catalyzed oxidative stress.

In studies on thalassemic patients, insulin resistance is significantly increased[57,58]. It was recently demonstrated in human studies,that a high prevalence of abnormal glucose homeostasis in individuals with hemochromatosis[59]. Using glucose tolerance tests, they demonstrated not only that insulin secretion is impaired but also that there is insulin resistance. The mechanisms for insulin resistance include the possibility of iron overload causing resistance directly or through hepatic dysfunction[60]. In a study of patients with unexplained hepatic iron overload, most were found to be insulin resistant, which suggests a common etiologic link among hepatic iron, hepatic dysfunction, and insulin resistance[61].

Several prospective studies have demonstrated an positive relationship between serum ferritin level and incident Type 2 diabetes

1) Study done by Sumesh R et al[62] (2013)found that there was a positive correlation between serum ferritin and FBS, HbA1c. There was no correlation between serum ferritin and age, sex, metabolic syndrome, coexistent hypertension, total cholesterol, LDL and serum triglycerides.
2) Mukesh G et al[63] (2013) conducted cross sectional study consists of 150 patients out of them 50 patients having type 2 DM with good control (Group II), 50 patients with type 2 DM with poor control (Group III) and 50 normal healthy control (Group-I) were selected, found statistically significant increase in free iron concentration in group III cases compare to Group I and Group II, and there was a statically significant positive correlation between free iron concentration and FBS, PP2BS and Glycated Hemoglobin. They concluded that serum free iron concentration was higher in patients with type 2 diabetes mellitus with poor control and also there was a positive correlation with serum free iron concentration and glycemic control, which suggest important role of iron in metabolic derangement in diabetic patients and its complications.
3) Pankaj B et al[64] (2011) conducted study which involved 200 Indian male type II diabetes mellitus patients, aged 35–65 years and age & sex matched 200 healthy controls found Serum ferritin values were significantly increased in type II diabetic patients compared to the control group and concluded that significant association between serum ferritin levels and type II diabetes mellitus in a representative population. And suggested further studies involving a larger sample size are required to investigate the

pathophysiological mechanism and consequences of increased serum ferritin levels in these patients.

4) Study done by Sultan S et al[16] (2009) found that serum ferritin was significantly higher in patients with type 2 DM compared with the control group and in patients with type 2 DM ferritin level was positively correlated with fasting blood glucose, glycated hemoglobin, fasting insulin level and insulin resistance.
5) Study done by Liang S et al[65] (2008) found that Elevated circulating ferritin concentrations were associated with higher risk of type2 diabetes and metabolic syndrome in middle-aged and elderly Chinese independent of obesity, inflammation, adipokines, and other risk factors. Our data support the crucial role of iron overload for metabolic diseases, even in a country with relatively high prevalence of iron deficiency.
6) Study done by Sumeet S et al[66] (2008)found significant association between serum ferritin and serum insulin(SI), Diabetics with increased level of serum ferritin(SF) had significantly poor glycemic control reflected by higher levels of HbA1c. They found significant relationship between increased serum ferritin and increased TC, TG levels in diabetic patients and with decreased serum HDL levels. A similar relationship was evident between increased SF and nephropathy, retinopathy neuropathy and hypertension, whereas, there was nonsignificant relationship found between increased SF and peripheral vascular disease and ischemic heart disease. They concluded that increased SF levels are associated with increased SI levels reflecting insulin resistance, poor glycemic control and complications of type-2 DM.

7) Study done by Forouhi N G et al[67](2007) found that baseline serum ferritin was higher among cases than control participants, adjusted for known risk factors (age, BMI, sex, family history, physical activity, smoking habit) and dietary factors measured by 7-day food diary, the risk of diabetes was markedly elevated in participants with clinically raised ferritin compared with the lowest quartile. Further adjustment for potential confounding by inflammation C-reactive protein, IL-6 and fibrinogen) had no material impact on the observed association, while adjustment for hepatic enzymes (alanine aminotransferase and γ glutamyl transferase) and adiponectin attenuated the magnitude of association, but it remained statistically significant.

8) Gorokhova S G et al[68] (2007) conducted study on two hundred and twentyfour patients were divided into two groups: group 1 consisted of 170 patients without concomitant diabetes mellitus and group 2 consisted of 54 patients with diabetes mellitus, found that ferritin level was significantly higher in patients with DM and ST depression.

9) Campenhout A et al[69] (2006) conducted case control study with 100 diabetes patients and 100 controls found that serum ferritin was elevated in type 2 diabetes mellitus compared to controls and this increase was not related to inflammation but inversely correlated with soluble transferring receptors.

10) Hernandez C et al[70] (2005) conducted case control study in 84 type 2 diabetic patients and 60 healthy controls found that diabetic patients had higher serum ferritin levels than control groups and there was no difference in circulating transferin receptor levels between both groups.

11) Bozzini C et al[71] (2005) conducted case control study found that mean ferritin levels were higher in metabolic syndrome subjects than control subjects and increased linearly with the increasing number of metabolic syndrome features.

METHODOLOGY

The present study was conducted in the Department of Medicine, BLDE University's Shri B M Patil medical college and research center, Vijayapur from January 2014 to June 2015 on newly detected and known patients with type 2 diabetes mellitus during the study period from January 2014 to June 2015.

Study design

The study design was one and half year cross sectional study.

Study period and duration

The present one year study was conducted from January 2014 to June 2015.

Place

The present study was conducted in Department of Medicine, BLDE University's Shri B M Patil medical college and research center , Vijayapur.

Source of Data

Patients with type 2 diabetes mellitus of BLDE University's Shri B M Patil medical college and research center, Vijayapur were studied.

Sample size

A total of 125 patients with type 2 diabetes mellitus were selected for the study.

Sampling procedure

- With prevalence of Type 2 Diabetes Mellitus – 9%[72]
- At confidence interval of 95% allowing $\pm$ 5 margin of error
- The calculated sample size was 125 using the below statistical formula.
    - $n = \frac{(1.96)^2 (p) (1-p)}{d^2}$

Hence a sample size of 125 patients with type 2 diabetes mellitus was considered.

Selection criteria

Inclusion criteria

- Outpatients and inpatients of Shri B M Patil Medical College Hospital who are diagnosed with type 2 Diabetes Mellitus

Exclusion criteria

- Chronic kidney disease.
- Chronic liver disease.
- Hypothyroidism.
- On corticosteroid therapy.
- Severe anemia (Hb < 7 g %).
- Blood transfusion in last 3 months.

Ethical clearance

The ethical clearance was obtained from Institutional Ethics Committee, BLDE University's Shri B M Patil medical college and research center , Vijayapur.

Informed Consent

The patients fulfilling selection criteria were explained about the nature of the study. Those willing to participate were enrolled in the study after obtaining a written informed consent .

Method of collection of data

Demographic data such as age, sex and occupation were recorded. Patients were interviewed and history regarding type 2 diabetes mellitus such as duration of disease, medication, personal history and history pertaining to the other co-morbid conditions was obtained. Further these patients were subjected to a thorough

physical examination such as anthropometry (including height and weight), vitals (pulse rate, blood pressure and respiratory rate) and systemic examination. These findings were recorded on a predesigned and pretested proforma .

Investigations

The selected patients underwent the following investigations.

- Complete blood count.
- Renal function test.
- Liver function test.
- Urine – Routine and microscopy.
- Blood sugar levels – Fasting and post prandial.
- Glycated haemoglobin (HbA1c)
- Fasting lipid profile
- Serum ferritin.

Outcome variables

Body mass index

A thorough clinical examination was conducted. Height and weight was recorded and body mass index was calculated based on formula;

$$\text{Body Mass Index} = \frac{\text{Weight (Kg)}}{\text{Height}^2 \text{ (m)}}$$

Body mass index was classified according to Overweight and obesity by BMI in adult Asians as below.[73]

Classification	BMI (Kg/m^2)	Risk of co-morbidities
Underweight	< 18.5	Low (But increased risk of other clinical problems)
Normal range	18.5 to 22.9	Average
Overweight	$\geq$ 23	
At risk	23.0 to 24.9	Increased
Obese I	25.0 to 29.9	Moderate
Obese II	$\geq$ 30.0	Severe

Lipid profile

Total cholesterol, triglycerides, HDL, LDL levels were noted and the findings were recorded. Normal values of lipid parameters were interpreted as;

- Low density lipoprotein < 100 mg/dL.
- High density lipoprotein < 40 mg/dL
- Total Cholesterol < 200 mg/dL.
- Triglycerides < 150 mg/dL.

Statistical analysis

Cross sectional study design

2) Mean = $\frac{\text{sum of value}}{\text{number of value}} = \frac{\Sigma X}{n}$

3) Standard deviation = $\frac{\Sigma (X - x)^2}{(n - 1)}$

4) Anova study(Analysis of variance)

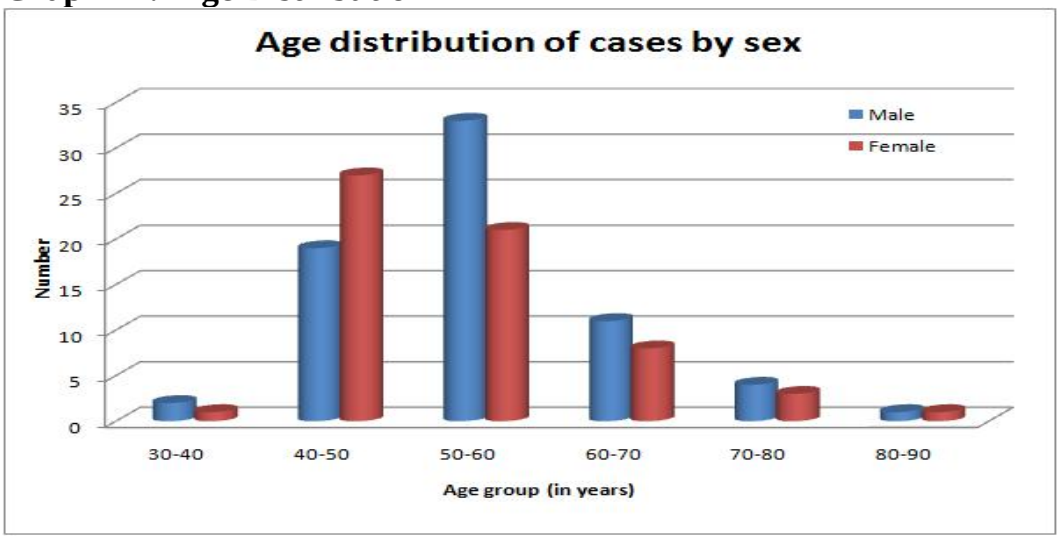
5) F - value (Anova test value)

6) p-value (probability)

Table - 1. Age Distribution

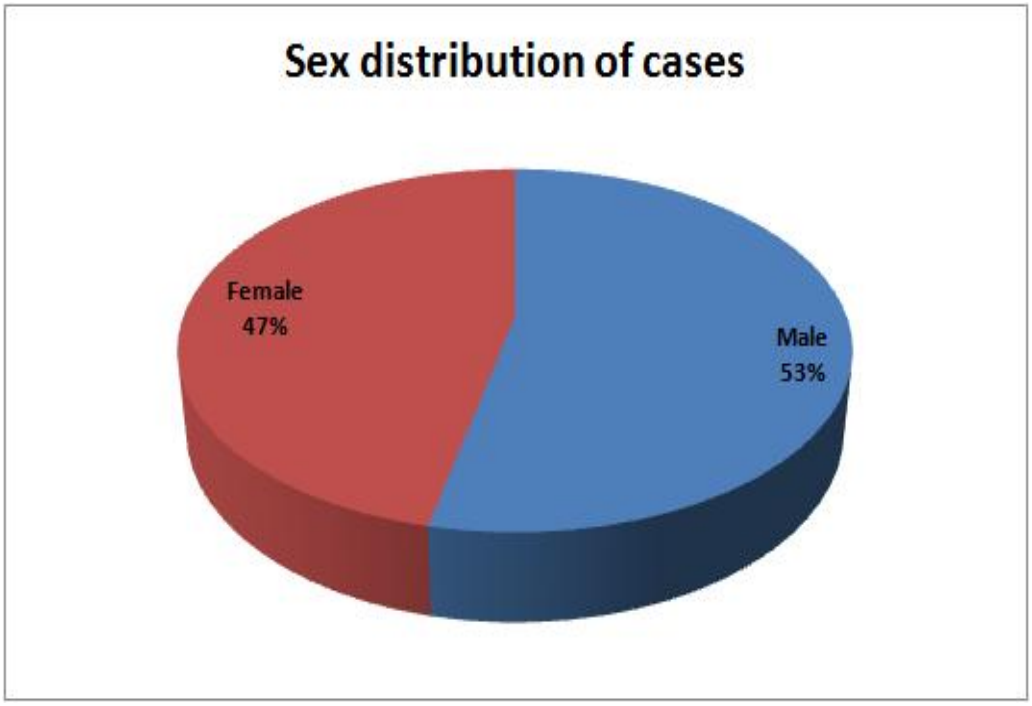
Age group (Years)	Distribution (n=131)			
	Male	**Female**	**Total**	**Percentage**
30 to 40	02	01	3	2.29%
40 to 50	19	27	46	35.1%
50 to 60	33	21	54	41.2%
60 to 70	11	08	19	14.5%
70 to 80	04	03	07	5.34%
80 to 90	01	01	02	1.52%
Total	**70**	**61**	**131**	**100%**

Graph - 1. Age Distribution



Patients age ranged from 30 to 90 years, maximum number of cases were in the age group of 50 to 60 that is 54 patients (41.2%), between 40 to 50 years 46 patients (35.1%) and between 60 to 70 years 19(14.5%) and 70 to 80 years 7 patients (5.34%) in each group. The mean age of study population was 53.46 ± 10.74 years.

Graph - 2. Sex Distribution



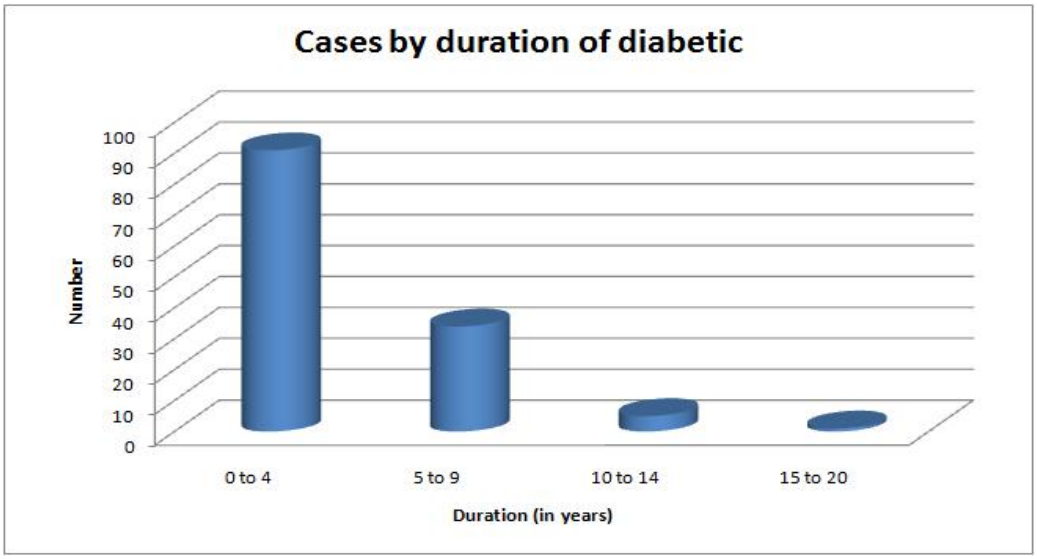
Out of 131 patients 70 (53.43%) were males and 61 patients (46.56%) were females, accounting a ratio of male to female 1.15:1.

Inference *:* Male preponderance was observed.

Table - 2. Duration of diabetes

Duration of diabetes (years)	Distribution (n=131)	
	Number	Percentage
0 to 4	91	69.5%
5 to 9	34	25.9%
10 to 14	05	3.8%
15 to 20	01	0.8%
Total	**131**	**100%**

Graph - 3. Duration of diabetes

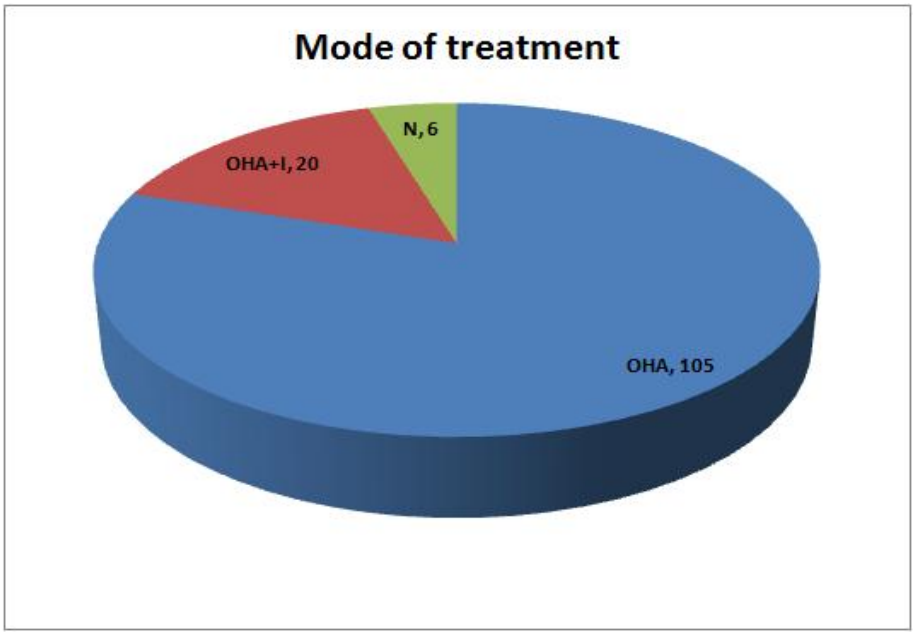


In the present study, we observed in 69.5% patients the duration of diabetes was 0 to 4 years. In 25.9 of patients the duration of diabetes was 5 to 9 years. In 3.8% of patients the duration of diabetes was 10 to 14 years. The mean duration of diabetes was 7.04 ± 3.25 years.

Table - 3. Mode of treatment

Mode of treatment	Distribution (n=131)	
	Number	Percentage
Oral hypoglycemic agents only	105	80.16%
OHA + Insulin	20	15.26%
Newly detected	6	4.58%
Total	**131**	**100%**

Graph - 4. Mode of treatment

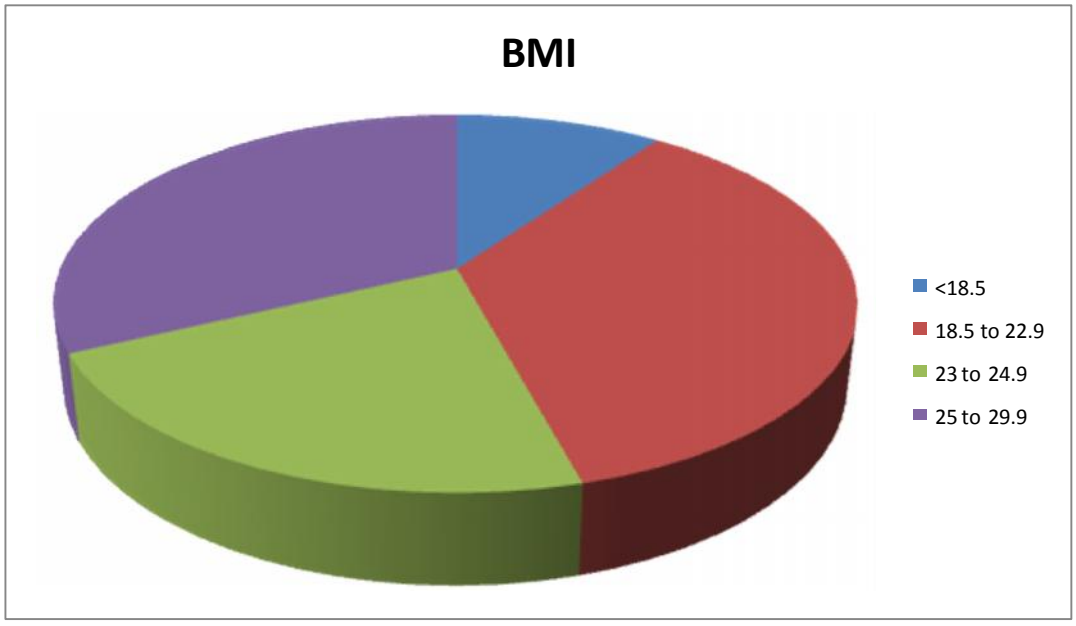


We observed 105 patients (80.16%) were on oral hypoglycemic agents, 6 patients(4.58%) were newly detected and 20 patients (15.26%) were on combination of insulin and oral hypoglycemic agents.

Table - 4. Body mass index (BMI)

Body mass index(kg/m^2)	Distribution (n=125)	
	Number	Percentage
<18.5	13	9.92%
18.5 to 22.99	47	35.88%
23.0 to 24.99	29	22.14%
25.0 to 29.99	42	32.06%
Total	**131**	**100%**

Graph - 5. Body mass index (BMI)

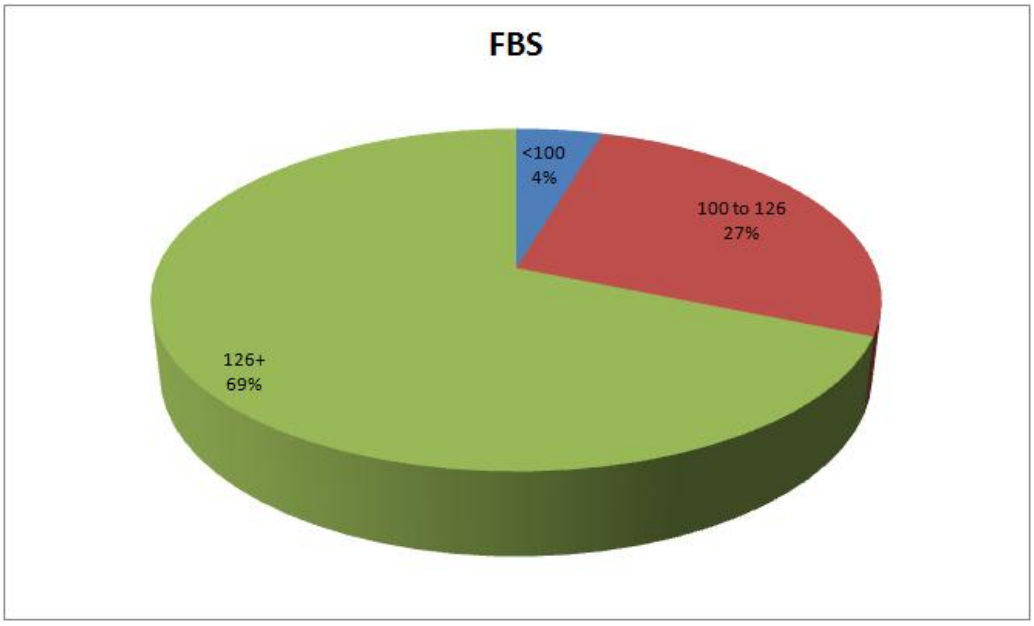


We observed in 47 patients (35.88%) BMI of 18.5 to 22.99, 42 patients (32.08%) BMI of 25 to 29.99, 29 patients (22.14%) BMI of 23 to 24.9 and 13 patients (9.92%) BMI was <18.5. The mean BMI was 23.95 ± 3.15.

Table - 5. Fasting blood sugar (FBS)

FBS(mg/dl)	Number	Percentage
≤100	6	4%
101 to 126	35	27%
>126	90	69%
Total	**131**	**100%**

Graph - 6. Fasting blood sugar (FBS)

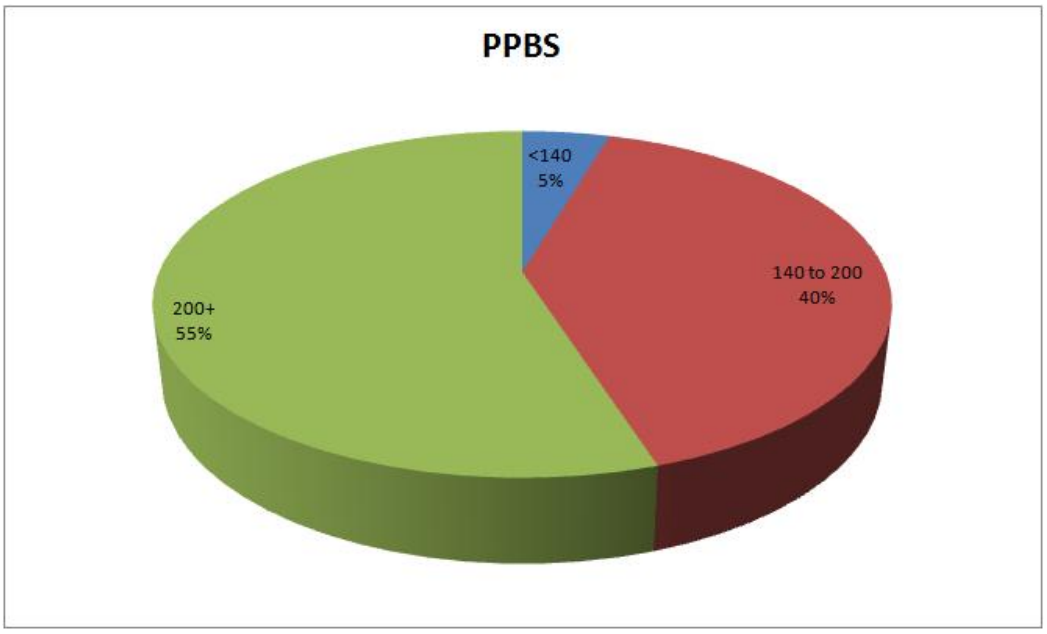


We observed 90 patients(69%) had above 126, 35 patients(27%) had 100 to 126 range and 6 patients(4%) had normal fasting sugar levels.

Table - 6. Post prandial blood sugar (PPBS)

Post prandial blood sugar (mg/dl)	Distribution (n=131)	
	Number	Percentage
≤140	7	5%
141 to 200	52	40%
>200	72	55%
Total	**131**	**100%**

Graph - 7. Post prandial blood sugar (PPBS)

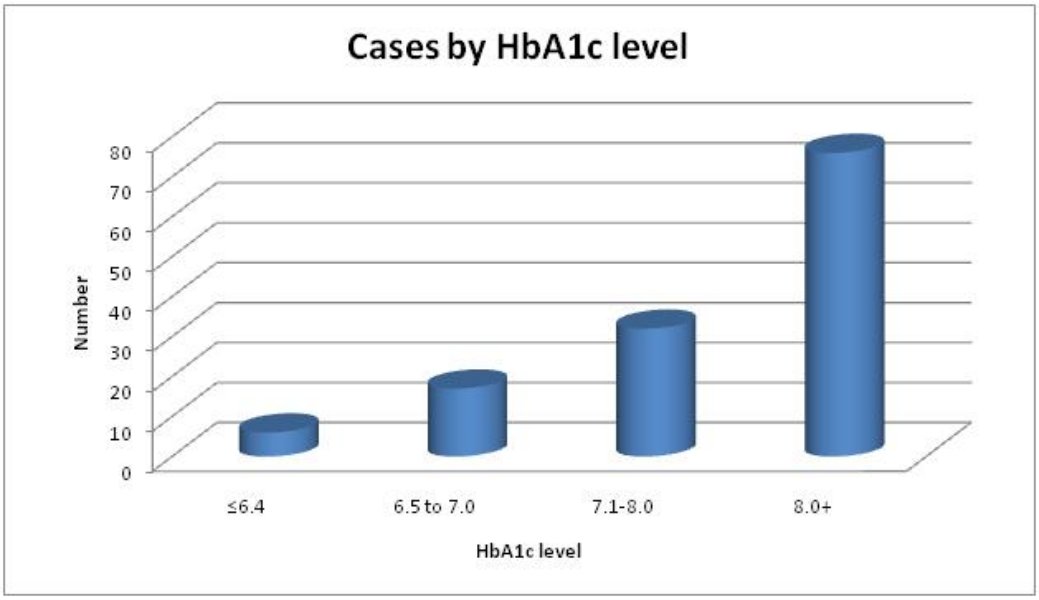


We observed 72 patients(55%) had above 200, 52 patients(40%) had 140 to 200 range and 7 patients(5%) had normal postprandial sugar levels.

Table - 7. HbAlc levels

HbAlc (%)	Distribution (n=131)	
	Number	**Percentage**
≤6.4	06	4.5%
6.5 - 7.0	17	12.9%
7.1 - 8.0	32	24.4%
>8.0	76	58%
Total	**131**	**100%**

Graph - 8. HbAlc levels

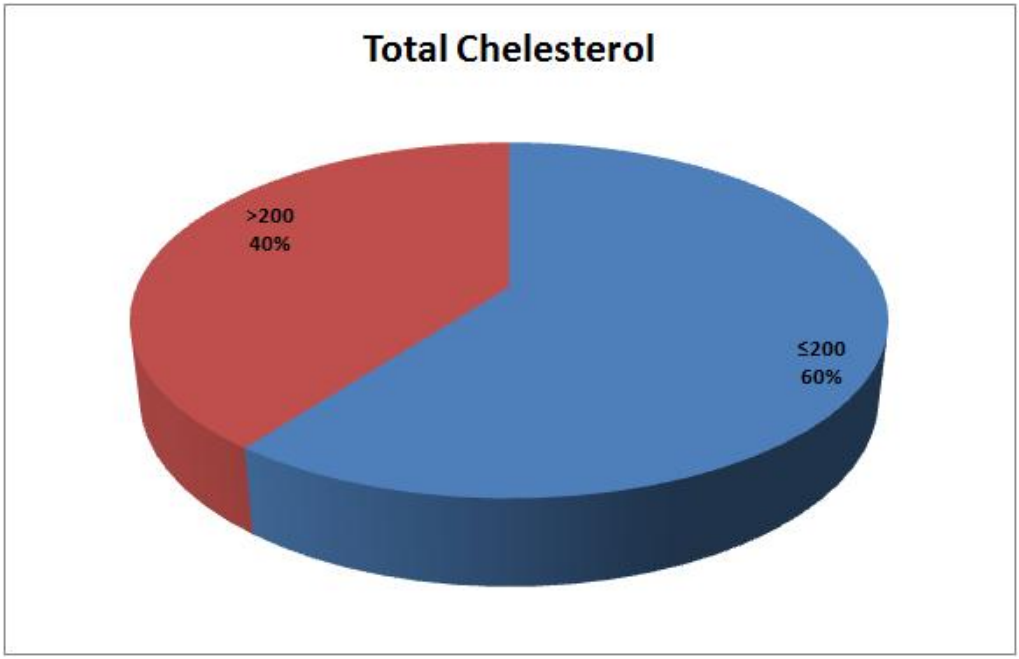


In our present study 76 patients (58%) had HbAlc of more than 8.0%, 32 patients (24.4%) had HbAlc between 7.1 to 8.0% and 17 patients (12.9%) were between 6.5 to 7.0%. The mean HbAlc level was 8.34± 0.84.

Table - 8. Fasting lipids - Total cholesterol

Total cholesterol (mg/dL)	Distribution (n=125)	
	Number	Percentage
≤200	79	60%
>200	52	40%
Total	**131**	**100%**

Graph - 9. Fasting lipids - Total cholesterol

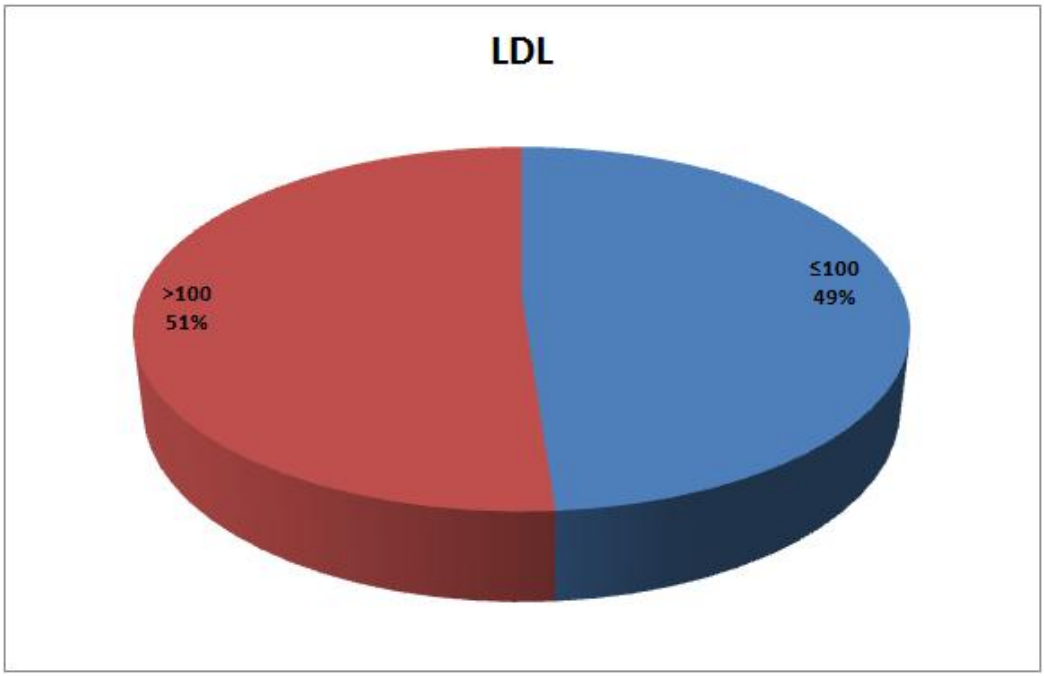


79 patients (60%) had cholesterol ≤ 200 mg/dL and remaining 52 patients (40%) had >200 mg/dL.

Table - 9. Fasting lipids - Low density lipoprotein (LDL)

Low density lipoprotein (mg/dL)	Distribution (n=125)	
	Number	Percentage
≤100	64	49%
>100	67	51%
Total	**125**	**100.0**

Graph - 10. Fasting lipids - Low density lipoprotein (LDL)

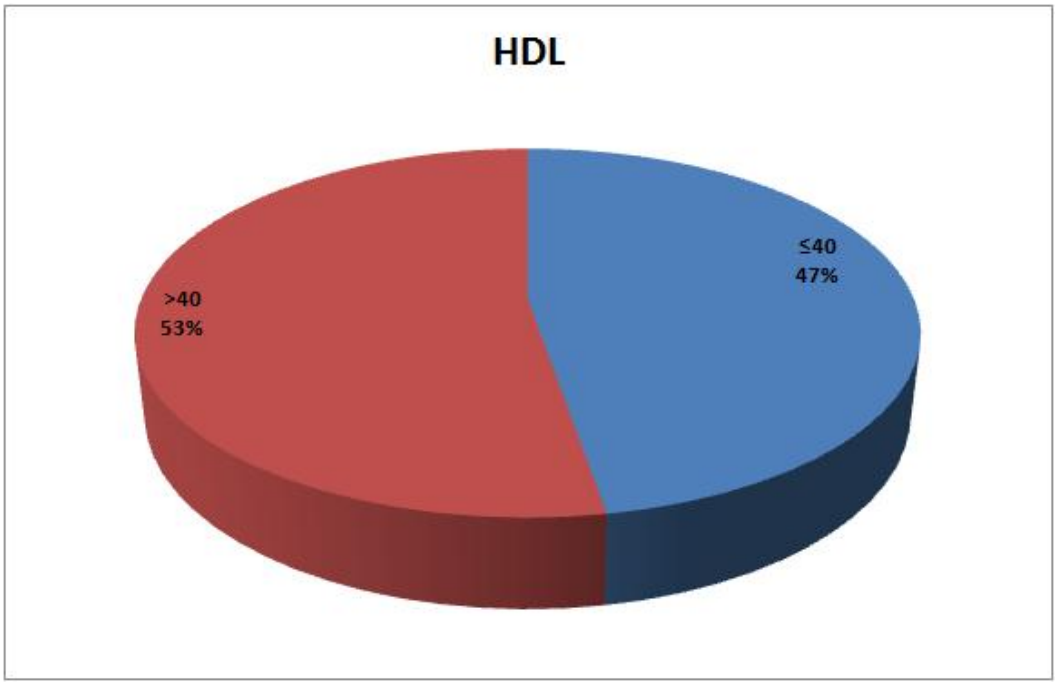


LDL of ≤ 100 mg/dL was observed in 64 patients (49%) and in 67 patients (51%) >100 mg/dL.

Table - 10. Fasting lipids - High density lipoprotein (HDL)

How density lipoprotein (mg/dL)	Distribution (n=131)	
	Number	Percentage
≤40	62	47%
> 40	69	53%
Total	**131**	**100%**

Graph - 11. Fasting lipids - High density lipoprotein (HDL)

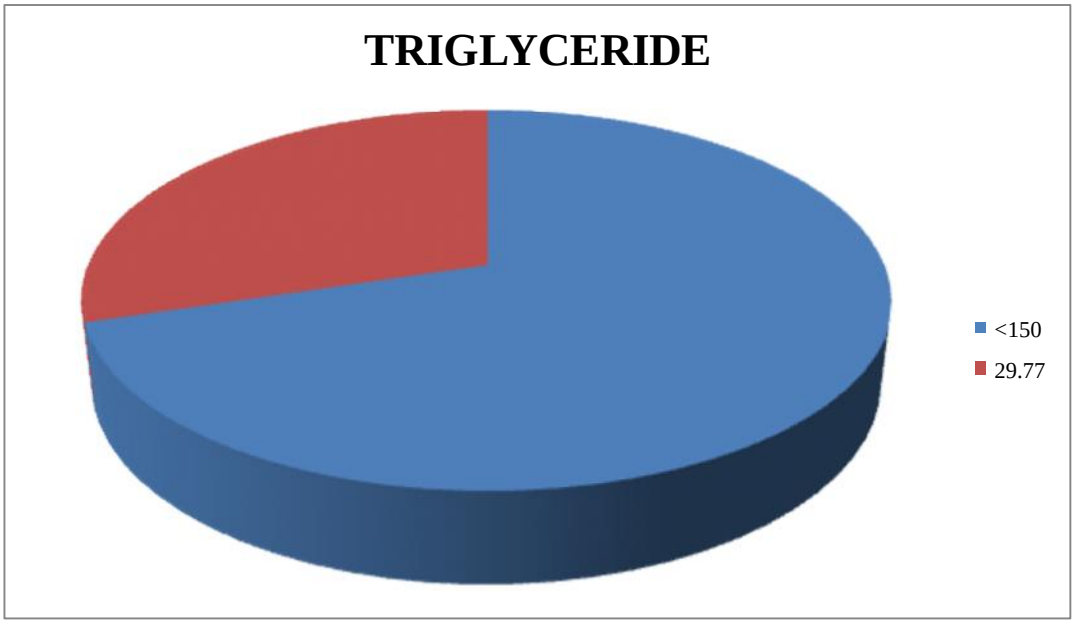


In majority of patients (53%) HDL was >40 mg/dL, in 62 patients (47%) it was ≤40 mg/dL.

Table - 11. Fasting lipids - Triglycerides

Triglycerides (mg/dL)	Distribution (n=131)	
	Number	Percentage
≤ 150	92	70.22%
>150	39	29.77%
Total	**131**	**100%**

Graph - 12. Fasting lipids - Triglycerides

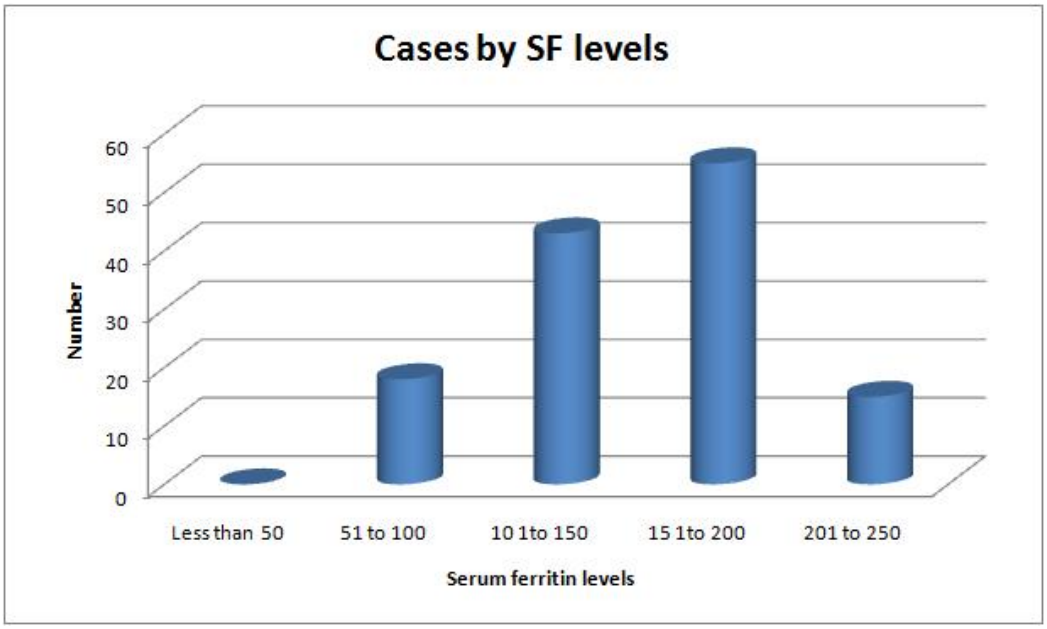


In 92 patients (70.22%) Triglycerides were ≤150 mg/dL, and in remainder 39 patients (29.77%) it was ≥150 mg/dL.

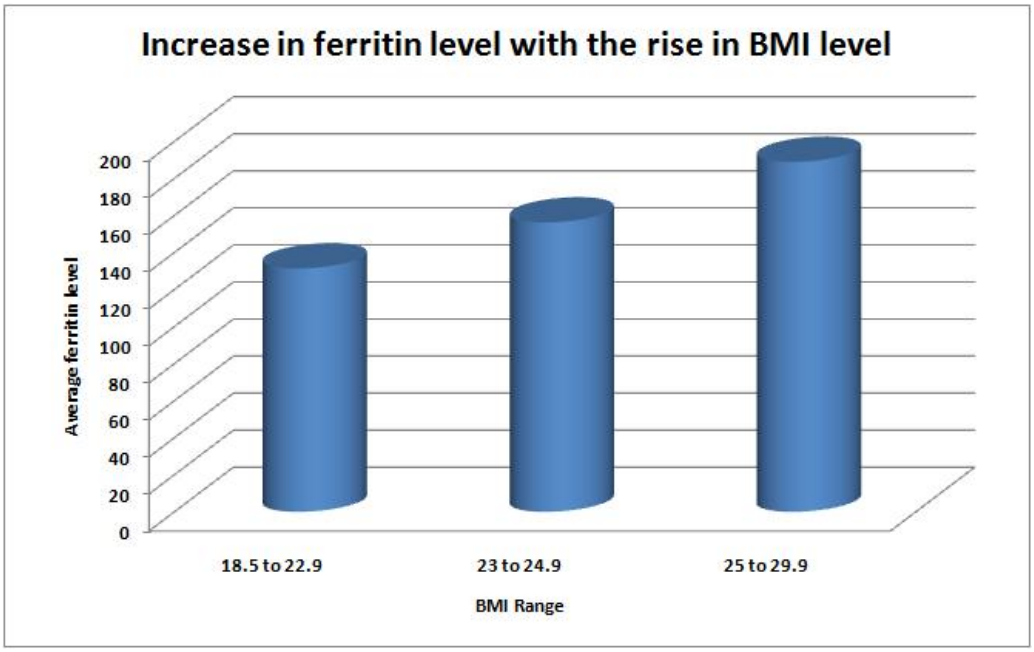
Table - 12. Serum ferritin level

Serum ferritin (ng/ml)	Distribution (n=131)	
	Number	Percentage
≤50	0	0
51 to 100	18	13.7%
101 to 150	43	32.8%
151 to 200	55	41.9%
201 to 250	15	11.5%
Total	**131**	**100%**

Graph - 13. Serum ferritin level



Graph - 14. Correlation of serum ferritin with BMI.

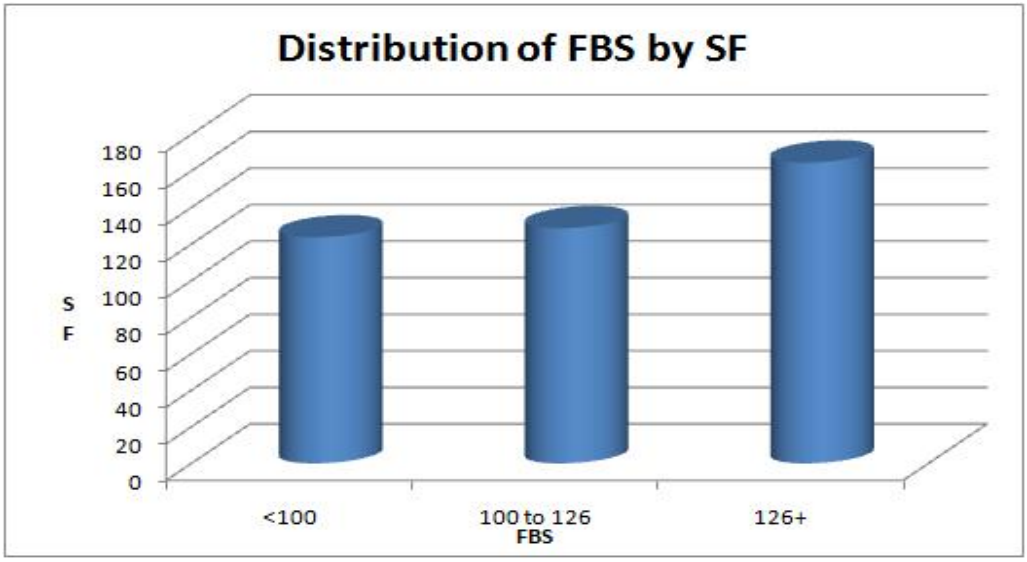


We found 47 patients whose BMI was in the range of 18.5 to 22.9 the mean serum ferritin was 131.4 ng/dl , 29 patients in the range of 23 to 24.9 mean ferritin was 156.2ng/dl and 42 patients in the range of 25 to 29.9 mean ferritin was 188.9ng/dl. We obtained a p-value of < 0.001 when we correlated serum ferritin levels with BMI. This p value is statistically significant.

Table - 13. Correlation of serum ferritin levels with fasting blood sugar

FBS(mg/dL)	Number	Serum Ferritin
≤100	6	123.75
101 to 126	35	128.68
>126	90	164.56
Total	**131**	

Graph – 15. Correlation of serum ferritin levels with fasting blood sugar

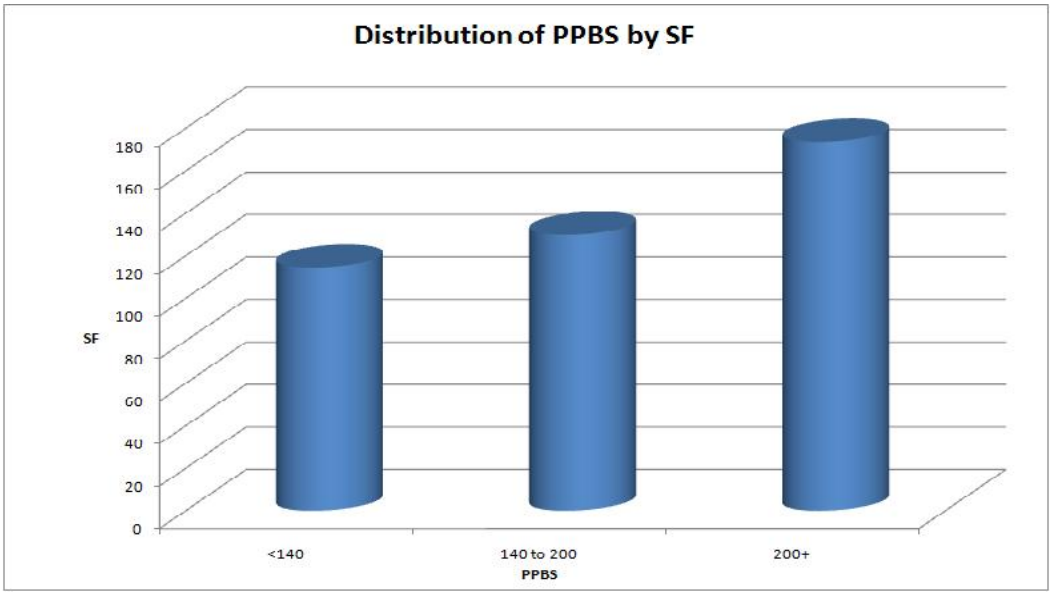


We found 90 patients whose FBS was >126 the mean serum ferritin was 164.56 ng/dl , 35 patients in the range of 100 to 126 mean ferritin was 128.68ng/dl and 6 patients with FBS ≤100 mean ferritin was 123.75ng/dl. We obtained a p-value of ≤ 0.001 when we correlated serum ferritin levels with FBS. This p value is statistically significant.

Table - 14. Correlation of serum ferritin levels with post prandial blood sugar

PPBS(mg/dL)	Number	Serum ferritin
≤140	7	114.53
141 to 200	52	129.77
>200	72	173.49
Total	**131**	

Graph–16. Correlation of serum ferritin levels with post prandial blood sugar

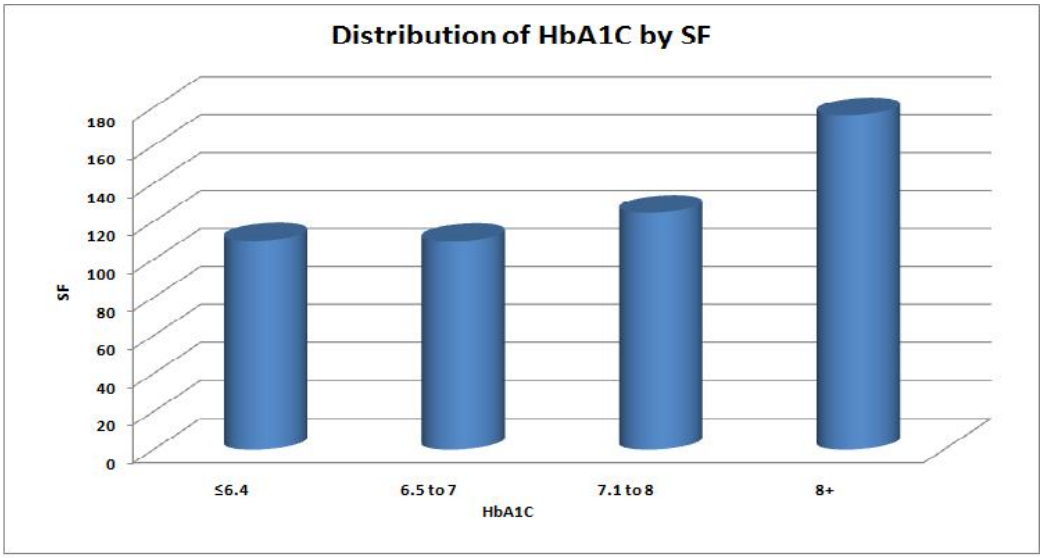


We found 72 patients whose PPBS was >200 the mean serum ferritin was 173.49ng/dl , 52 patients in the range of 140 to 200 mean ferritin was 129.77ng/dl and 6 patients with PPBS ≤140 mean ferritin was 114.53ng/dl. We obtained a p-value of ≤ 0.001 when we correlated serum ferritin levels with PPBS. This p value is statistically significant.

Table 15. Correlation of serum ferritin(SF) levels with HbAlc

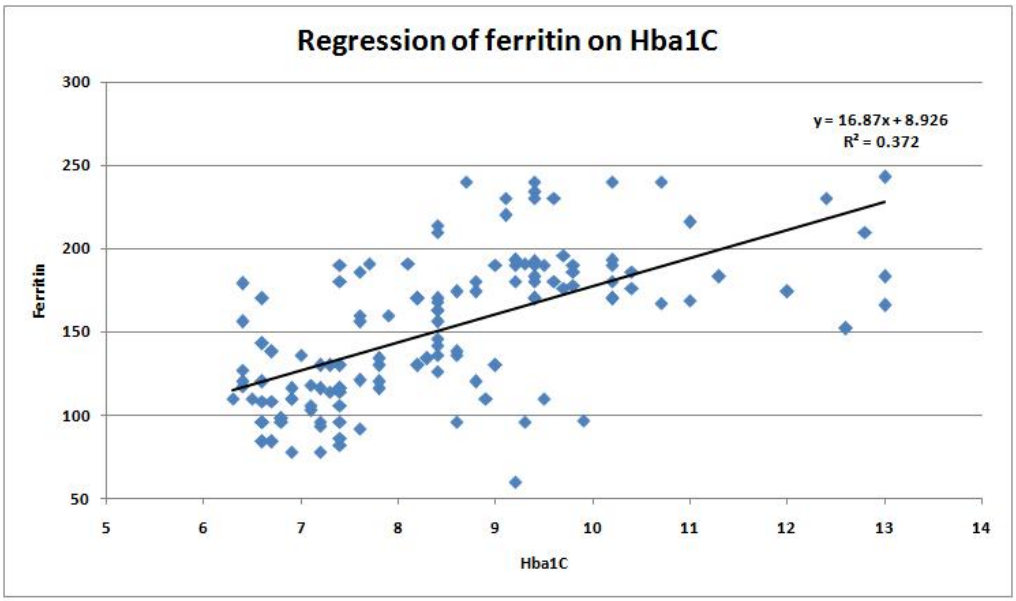
HbAlc (%)	Number	Serum ferritin
≤6.4	06	110
6.5 - 7.0	17	109.7
7.1 - 8.0	32	124.71
>8.0	76	176.06
Total	**131**	

Graph–16. Correlation of serum ferritin(SF) levels with HbAlc



We found 76 patients whose HbAlc was >8 the mean serum ferritin was 176.06ng/dl , 32 patients in the range of 7.1 to 8 mean ferritin was 124.71ng/dl, 17 patients in the range of 6.5 to 7 mean ferritin was 109.7 ng/dl and 6 patients with HbAlc ≤6.4 mean ferritin was 110ng/dl. We obtained a p-value of ≤ 0.001 when we correlated serum ferritin levels with HbAlc. This p value is statistically significant.

Graph–18. Regression of serum ferritin on Hba1C



Correlation analysis: The correlation between Hba1C and ferritin = r=0.6099

Graph–19. Regression of Hba1C on serum ferritin.

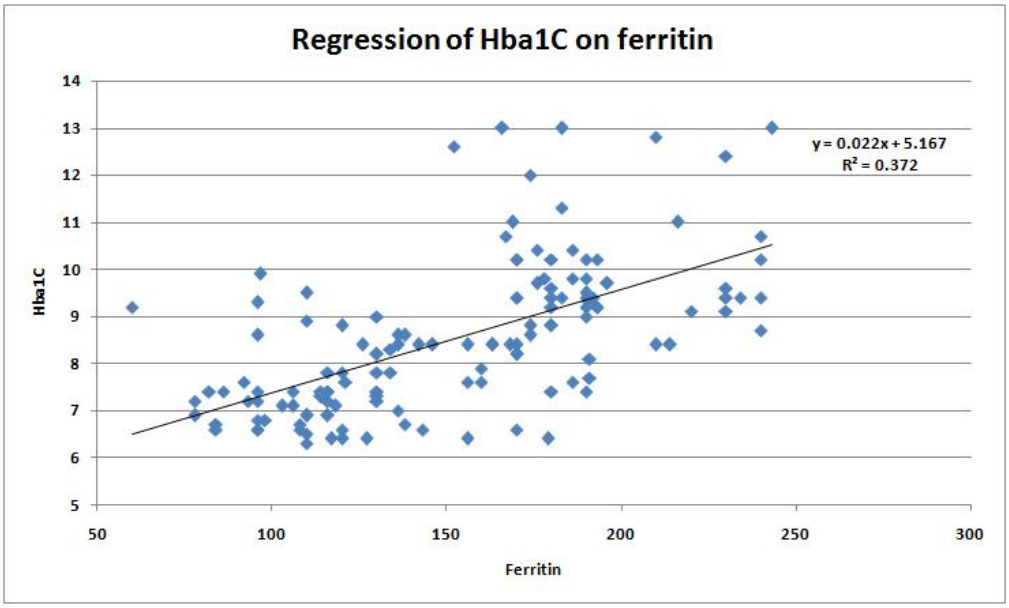
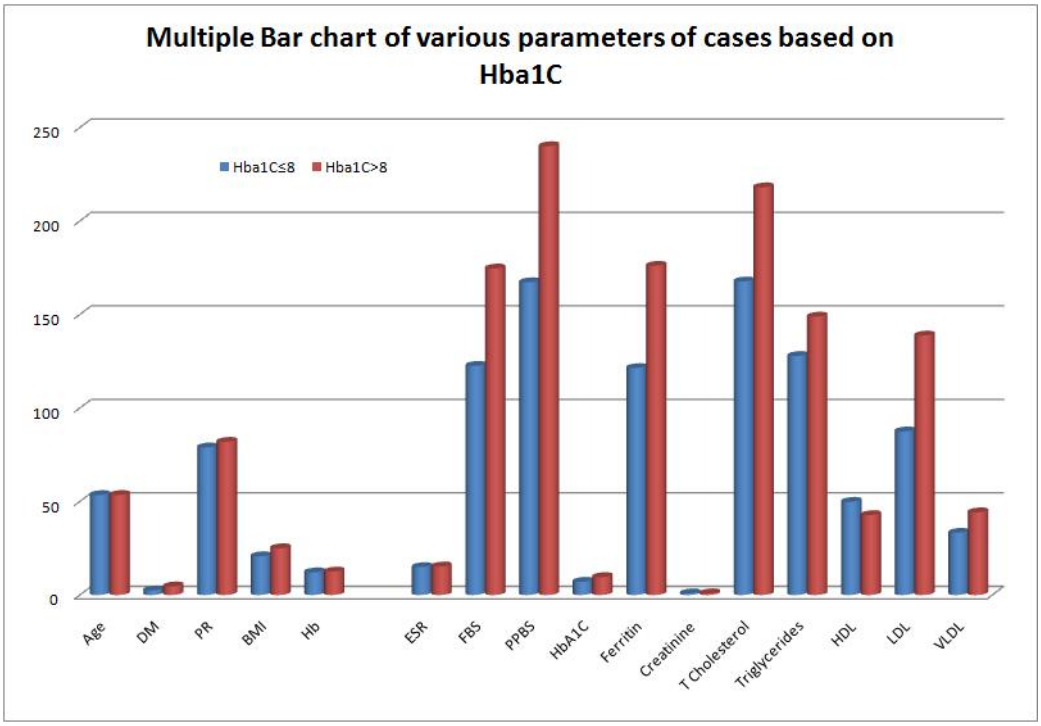


Table 16. Comparison between various parameters of two groups

	Hba1C less than equal to 8				Hba1C greater than 8				Significance test		
	Mean	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**	**Min**	**Z**	**P-value**	**Conclusion**
Age	53.46296	10.74703	85	35	53.5	7.462797	75	30	-0.02201	0.984	NS
DM	2.472727	2.267766	8	0	4.572368	3.250337	20	0	-4.35432	0.0001	HS
PR	78.92727	9.386465	108	62	81.92105	9.057981	100	64	-1.82823	0.0688	S
BMI	20.73818	2.623222	28.9	16.7	25.01842	3.057306	29.8	17.8	-8.59315	0.0001	HS
Hb	12.13818	1.505114	15	9.4	12.54447	1.61375	16.2	9.6	-1.4791	0.1416	NS
WBC	9079.636	2293.331	13200	4400	9421.316	2326.904	13200	4600	-0.83644	0.4066	NS
ESR	14.94545	8.70806	40	4	15.27632	8.3132	42	6	-0.21873	0.8336	NS
FBS	122.5455	25.12695	243	70	174.6053	35.42319	301	110	-9.84016	0.0001	HS
PPBS	167.2545	31.26838	270	100	239.9868	41.44924	360	160	-11.4454	0.0001	HS
HbA1C	7.090909	0.456749	7.9	6.3	9.592105	1.243196	13	8.1	-16.1019	0.0001	HS
Ferritin	121.3855	29.32565	191	78	176.0618	38.02774	243	60	-9.28668	0.0001	HS
Creatinine	0.887273	0.196313	1.2	0.6	0.921053	0.208049	1.4	0.6	-0.9478	0.472	NS
T Cholesterol	167.6909	23.83343	240	90	218.0263	41.31891	280	130	-8.79005	0.0001	HS
Triglycerides	127.8545	25.59038	210	80	148.8816	30.61545	273	59	-4.27086	0.0001	HS
HDL	49.83636	10.41147	66	25	42.75	9.762684	64	27	3.946036	0.0001	HS
LDL	87.49091	23.237	154	36	138.75	43.55047	199	40	-8.69257	0.0001	HS
VLDL	33.29091	9.344582	66	16	44.22105	14.80456	77	10	-5.16889	0.0001	HS

Test of significance between respective parameters of cases having Hba1c less than or equal to 8 and above

Graph–20. Bar chart on various parameters of cases based on Hba1C



DISCUSSION

Management of type 2 diabetes mellitus is a challenge for health care workers, patients and their families. Current management standards focus on optimizing glycaemic control to reduce the risks of long term complications. The main aim of our investigation was to clarify, whether there exists association between serum ferritin levels and glycated hemoglobin in patients of type 2 diabetes mellitus.

In the present study of 131 patients with type 2 diabetes mellitus, we observed the levels of serum ferritin and compared it to various factors. In this cross sectional study, we found an linear association between serum ferritin levels and HbAlc.

In the present study, there were 70 males & 61 females with mean Age distribution of 53.48±8.56. Majority of patients (41%) were in their sixth decade of life. In a study conducted by Raj S et al[62], the mean age distribution among study population was 54.3±9.2 yrs, male and females were 57 and29 respectively . In a study done by Bansal P et al[64] mean age of the patients was 45.70±1.6years.

In our study, the duration of diabetes varied from 0 to >15 yrs. We did not find any association between serum ferritin levels and duration of diabetes, but Kundu D et al[74] found there is inverse correlation between serum ferritin and duration of diabetis.

Some of our patients were on treatment either with oral hypoglycaemic agents (80.15%), combination of oral hypoglycemic drugs and insulin (15.26%) and 4.58 % were newly detected diabetics. All of these patients when presented had either fasting blood sugar or post prandial blood sugar abnormality reflecting poor diabetic status. Most of our patients were having higher normal levels of serum

ferritin. Same conclusion was drawn by Raj S et al.[62] This issue needs further evaluation by comparing the diabetic patients with healthy non-diabetic individuals.

In our study when levels of serum ferritin were compared with BMI, We found 47 patients whose BMI was in the range of 18.5 to 22.9 the mean serum ferritin was 131.4 ng/dl , 29 patients in the range of 23 to 24.9 mean ferritin was 156.2ng/dl and 42 patients in the range of 25 to 29.9 mean ferritin was 188.9ng/dl. This signifies there is a postitve correlation between serum ferritin and BMI (p value $\leq$0.001). Same conclusion was drawn by Raj S et al[62] and Sultan S et al[16].

When correlated with HbA1c, the serum ferritin levels were definitely affected with HbA1c of 8% and above. There is direct correlation between serum ferritin and HbA1c levels, p-value being significant (p=0.0001). Similar observation was made by Raj S et al[62] and Kundu D et al[74].

When serum ferritin levels were compared with fasting blood sugar, most of patients with fasting glucose abnormality had either higher or upper limit of normal serum ferritin levels (p=0.001; significant). In their study by Raj S et al[62] (sample size 86 cases), observed elevated serum ferritin levels are associated with fasting glucose abnormality (p=0.01; significant). Similarly study done by Sultan S et al[16] (sample size 40 patients) found a positive correlation of serum ferritin levels with fasting glucose abnormality (p-0.001; significant).Study by Mukesh G et al[63] found higher levels of serum ferritin in patients with increasing fasting blood sugars.

In our study when levels of serum ferritin were compared with post prandial blood sugars, we found elevated serum ferritin levels with higher post prandial blood sugar levels($p<0.001$), similar results observed by Mukesh G et al[63]. But study by Raj S et al[62] found no correlation between serum ferritin and postprandial sugar levels.

An attempt was made to correlate serum ferritin levels with fasting lipids. We found positive association between total cholesterol ($p = 0.1$, not significant), triglycerides($p \leq 0.05$) and low density lipoprotein ($p = 0.001$)with serum ferritin levels, there was inverse relation between serum ferritin levels and high density lipoproteins(p value < 0.001). One study by Raj S et al[62] found no correlation between serum ferritin and total cholesterol, low density lipoproteins and serum triglycerides. But study done by Sumeet et al[66] found significant relationship between increased serum ferritin and increased total cholesterol, triglyceride and decreased serum HDL levels.

To overcome the bias with confounding factors and co-morbid conditions, may be large sample size is required. And also comparison of diabetic individuals with non-diabetic healthy individuals is essential to find out the true correlation of serum ferritin levels with other variables.

CONCLUSION

- There was positive association between serum ferritin and body mass index measurements.
- We found direct association between serum ferritin and fasting, postprandial blood sugars.
- We found positive association between serum ferritin levels and glycosylated hemoglobin as glycemic control.
- We also observed there was direct association between serum ferritin levels and fasting lipids (triglyceride and low density lipoproteins) but inverse association with high density lipoproteins.
- When correlated with other variables like age, gender, duration of diabetes, total cholesterol, we found no significant correlation (p-value being statistically insignificant).
- Further studies to compare patients with type 2 diabetes mellitus and non-diabetic healthy individuals may be necessary to know the relationship serum ferritin levels with other variables.

SUMMARY

Diabetes and its associated complications are significant public health problems. In our study we found that there was positive association between serum ferritin levels and HbA1c in patients with type 2 diabetes mellitus. Higher serum ferritin levels were associated with poor glycemic control. In addition, we observed a significant correlation of serum ferritin levels with variable factors like BMI, fasting blood sugar, post prandial sugar levels, triglycerides, low density lipoprotein and high density lipoproteins. However, we did not find significant correlation with variable factors like age, gender, duration of diabetes and total cholesterol.

Overall, these findings suggest a potential beneficial role of serum ferritin in diabetes risk. Confirmation of these findings in future observational studies and specifically, well-designed randomized controlled trials potentially has significant public health implications given the high prevalence of higher serum ferritin status in the general population and the relative ease and low cost at which such an intervention may be implemented.

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ANNEXURE-I

ETHICAL CLEARANCE CERTIFICATE

Institutional Ethical Committee BIJAPUR-586 103. OUTWARD No. SD/13 Date 14.11.13 B.L.D.E.U's Shri B.M. Patil Medical College

B.L.D.E.UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Study of association between Serum ferritin and glycated hemoglobin in type 2 Diabetes Mellitus" —x—x—

Name of P.G. student Dr. Timmanna Giraddi, Department of Medicine

Name of Guide/Co-investigator Dr L.S. Patil. Professor of Medicine

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-II

CONSENT FORM

A STUDY ON THE ASSOCIATON BETWEEN SERUM FERRITIN AND HbA1C LEVELS IN TYPE 2 DIABETES MELLITUS

Objective and purpose of the study:

This research is intended to estimate the serum ferritin levels in patients with Type 2 Diabetes Mellitus. The principal investigator of the study is Dr Timmanna Giraddi under the guidance of Dr. L.S.Patil

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 sample for the same study.

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, bruising or infection (rarely happens) at the site from where the blood is drawn.

Alternatives

Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part you can later change my 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 sponsorer may stop your participation in this study any time. If you choose not to take part in the study you will receive the standard treatment for patients with your condition.

VOLUNTARY PARTICIPATION/ WITHDRAWAL:

Your participation in this study is entirely voluntary and you may withdraw from the study at any time.

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.

Financial incentives for participation

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

Authorization to publish the results

The results of the study would be forwarded to the B L D E University, Vijayapur as part of requirement towards the completion of MD degree, review and publishing.

CONSENT FORM

I voluntarily agree to take part in this study by signing on the line below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicated that I have read this entire consent form or it has been read to me and has been explained to me in my vernacular language and had all my questions answered. I will be given a copy of this consent form.

Signature /Left Thumb print of the Participant or legally authorized representative.

Participant's Name/ :_____

Signature/ Left Thumb Impression of the participant's :_____

Name of the legally authorized representative/ :_____

Guardian Signature/ :_____

Left Thumb Impression. :_____

Witness's Name :_____

Signature/ Left Thumb Impression. :_____

Investigators name and Signature :_____

Date and Place

ANNEXURE-III

PROFORMA

Case No.: IP number:

Patient Name: ID number:

Age: Sex:

Address:

Occupation:

Date of admission:

RESENTING SYMPTOMS _ _ _ __ _ _ _ _

Classical symptoms of diabetes mellitus (Polyphagia, Polyuria, Polydipsia)	Yes / No
Ischaemic heart disease	Yes / No
Peripheral vascular disease	Yes / No
Cerebrovascular accident	Yes / No
Peripheral neuropathy	Yes / No
Retinopathy	Yes / No
Any other relevant history	Yes / No

HISTORY

Diabetic history

Duration:

Medication:

Hypertension Yes / No

Ischaemic heart disease	Yes / No
Cerebrovascular disease	Yes / No
Family history	Yes / No

Personal history	Vegetarian / Mixed
Diet:	Yes / No
Smoking:	Yes / No
Tobacco chewing:	Yes / No
Alcohol:	Yes / No

Menstrual history:

General physical examination:

BMI :

Vitals :

Pulse rate:		
Blood pressure:	Systolic:	Diastolic:
Respiratory rate:		
Temperature:	Febrile / Afebrile	
Pallor	Yes / No	
Icterus:	Yes / No	
Oedema:	Yes / No	
Cyanosis:	Yes / No	
Clubbing:	Yes / No	
Lymphadenopathy:	Yes / No	

Systemic examination:

Respiratory system:

Cardiovascular system:

Per abdomen:

Central nervous system:

Fundoscopy

Investigations

Complete blood count

Haemoglobin (gm%):

Total count:

Differential count:

Platelet count:

ESR:

Red blood cell count:

Renal profile

Urea:

Creatinine

Sodium

Potassium

Urine

Routine

Microscopy

Ketone bodies

Blood sugar levels

Fasting:

Post prandial:

HbA1c:

Serum ferritin

Lipid profile:

Cholesterol

Low density lipoprotein

High density lipoprotein

Triglycerides

ANNEXURE-IV

MASTER CHART

Y	-	Yes
N	-	No
M	-	Male
F	-	Female
Kg/m^2	-	Kilogram per meter square
YRS	-	Years
PR	-	Pulse rate
BP	-	Blood pressure
RBS	-	Random blood sugar
FBS	-	Fasting blood sugar
PPBS	-	Post prandial blood sugar
ng/ml	-	Nanogram per milliliter
mg/dL	-	Milligram per deci litre
mm Hg	-	Millimeter of mercury
OHA+I	-	Oral hypoglycemic drugs and insulin
OHA	-	Oral hypoglycaemic agents
HTN	-	Hypertension

MASTER CHART

Sl no	Name	Age(yrs)	Sex	IP/OP No	Duration of DM(yrs)	Mode of Treatment	HTN	PR	BP(mm of Hg)	BMI(Kg/m2)	Hb(gm)	WBC conut	ESR(mm)	FBS(mg/dl)	PPBS(mg/dl)	HbA1C(%)	Ferritin(ng/ml)	Creatinine(mg/dl)	T Cholesterol(mg/dl)	Triglycerides(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
1	SHIVAPPA	60	M	6715	2	OHA	Y	86	140/90	23.4	16.2	13,200	10	150	190	8.1	191	0.8	263	176	35	192	35
2	SURAYA	60	F	7717	10	OHA+I	Y	100	100/60	26.8	10.5	9500	30	170	268	8.8	180	0.9	280	190	40	180	60
3	VACHALABAI	68	F	7773	20	OHA	N	90	116/70	24.6	10.4	6000	20	165	293	9.9	96.7	0.8	210	150	30	140	40
4	RAKMABAI	65	F	7836	2	OHA	N	96	100/60	20.4	11.9	10700	20	119	131	6.4	117	1	160	110	60	70	30
5	SHIVALINGAWWA	80	F	8126	1	OHA	Y	82	140/90	21.8	11.6	9170	38	70	100	7.2	93.2	0.7	200	167	42	124	34
6	VITTAL	74	M	8123	2	OHA	N	90	110/70	23.4	12.6	6700	30	105	208	6.4	120	1.2	157	191	30	89	38
7	MANTAPPA	56	M	8070	4	OHA	Y	80	150/90	27.6	13.2	6940	10	164	227	8.4	163	0.8	132	183	36	60	36
8	SHRISHAIL	49	M	8947	8	OHA+I	Y	75	130/70	24.3	13.3	12090	25	118	160	7	136	1	142	170	46	50	46
9	GURUSHANT	48	M	9422	2	OHA	N	86	110/70	19.8	15.9	13170	23	118	179	8.6	96	0.8	149	273	37	58	54
10	SHIVANANDAYYYA	72	M	9440	1	OHA	N	90	170/90	20.4	15.4	12800	42	238	224	8.4	214	0.8	273	114	51	199	22
11	REVANSIDDAPPA	85	M	9595	3	OHA	Y	100	140/80	18.4	9.7	11140	30	163	270	6.9	78	0.7	90	80	25	49	16
12	AYUB	57	M	14397	6	OHA	N	86	106/60	24.8	14.7	12560	40	112	170	10.7	167	0.7	226	159	34	160	32
13	NINGAPPA	50	M	10547	0	N	N	84	150/90	27.4	14.3	11940	30	301	336	10.2	193	1.2	230	170	28	161	41
14	AMMANNA	72	M	14688	0	N	Y	90	130/80	16.8	14.5	8900	40	243	210	6.5	110	0.8	222	210	34	146	42
15	AMEERBEE	65	F	14643	8	OHA	N	108	106/80	20.6	10.1	12820	36	181	235	7.1	103	1.2	213	179	52	109	35
16	BASANNA	55	M	17339	3	OHA	N	100	110/70	26.7	13.3	12490	30	200	233	9.7	176	0.9	176	121	48	104	24
17	SRIKANTH	46	M	14020	6	OHA+I	N	90	130/70	29.7	13.7	11790	26	248	320	9.1	220	0.8	209	59	39	158	11.8
18	SHARANAPPA	59	M	179507	1	OHA	N	80	110/70	19.3	14.9	10600	25	99	149	7.9	160	1.2	195	155	31	126	38
19	PARVATI	45	F	2407	3	OHA	N	86	130/70	24.5	12.4	7800	14	160	230	8.2	170	0.6	160	120	36	40	70
20	SIDDAMMA	52	F	3999	1	OHA	N	68	110/70	18.7	14.6	6200	14	110	160	6.8	98	1	160	140	60	66	34
21	BASANNA	64	M	2629	6	OHA	N	70	116/70	20.4	10.2	8900	24	140	180	6.9	110	1.2	180	130	60	80	40
22	PANCHAYYA	48	M	2147	6	OHA+I	Y	86	130/80	26.7	13.6	10200	10	200	280	10.4	186	1.2	260	160	40	160	60
23	DARAMANNA	54	M	2401	0.5	OHA	N	94	130/80	21.4	12.4	8700	16	94	126	6.6	96	0.8	140	120	54	60	26
24	MAHADEVI	48	F	2031	0	N	N	76	130/80	19.7	10.2	8400	6	160	240	7.4	190	1	240	180	46	154	40
25	CHANDRAWWA	56	F	532	5	OHA	N	66	120/80	26.4	10.8	6800	10	160	200	9.4	180	1.2	190	160	34	78	42
26	VEERESH	44	M	2368	0.5	OHA	N	80	110/70	17.8	13.4	10200	12	110	140	6.9	110	0.8	130	120	64	36	30
27	JALANI	40	F	2519	1	OHA	N	88	140/80	16.7	9.8	5600	16	124	140	7.2	116	1.2	160	140	50	80	30
28	SANGAPPA	58	M	2802	14	OHA+I	Y	68	130/70	27.8	13.6	6800	10	160	230	12.8	210	1.2	260	200	30	160	70
29	RAMAPPA	60	M	2298	2	OHA	N	76	110/70	19.6	14.6	6200	14	110	160	6.8	96	1	160	140	36	84	40
30	SIDDRAM	46	M	1296	0.50	OHA	N	80	116/70	17.4	13.4	5800	4	106	130	7.1	106	1	140	120	40	70	30
31	LAXMAN	52	M	2508	5	OHA	N	72	130/80	23.4	10.8	5200	10	160	280	10.2	180	0.9	280	160	46	174	60
32	BABU	44	M	32541	4	N	N	64	116/70	26.4	14.8	11700	8	180	260	9.6	230	1.2	260	150	40	160	60

33	SIDDANAGOUDA	43	M	3494	0.5	N	N	84	130/80	21.4	12.3	12600	6	130	160	7.2	130	0.9	180	120	66	80	34
34	PARAMMA	52	F	2692	10	OHA	N	90	140/80	27.3	10.6	10400	14	240	360	10.2	240	1.2	230	180	40	140	50
35	KASHIBAI	46	F	1992	1	OHA	N	84	124/70	18.6	10.7	12600	16	110	150	7.4	180	1	180	140	54	96	30
36	KASTURI	54	F	2657	4	OHA+I	Y	78	134/80	19.7	12.4	8700	20	140	200	8.8	174	0.8	140	110	40	74	20
37	SHAKUNTALA	56	F	2780	6	OHA	N	68	110/70	28.4	10.4	9300	16	160	260	8.4	142	0.9	240	180	46	160	34
38	RATANSING	64	M	2508	6	OHA	Y	73	120/80	18.3	11.6	10300	8	180	240	9.3	96	0.7	180	120	64	80	36
39	MANOHAR	40	M	31138	0.5	OHA	N	76	116/76	18.9	13	9600	16	140	210	7.4	82	0.8	164	110	40	86	40
40	KASHIBAI	46	F	30866	1	OHA	N	80	126/70	26.4	11.2	7600	8	160	240	8.8	120	0.9	240	130	46	150	44
41	TANAJI	42	M	31258	1	OHA	N	76	100/70	28.6	14	10000	8	180	256	9.2	180	1	200	150	60	80	60
42	SIDRAMAPPA	56	M	31225	5	OHA	N	70	120/70	21.4	12.4	9400	16	150	200	7.8	116	0.6	160	130	50	80	38
43	BANDAGISAB	50	M	30057	4	OHA+I	N	84	130/90	24.6	13.1	8000	10	170	268	8.4	170	0.9	170	130	45	100	35
44	SHARANAPPA	56	M	27303	8	OHA	Y	86	150/90	26.7	11.4	7300	15	190	260	9.4	234	0.7	230	140	60	140	40
45	EGAPPAGOUDA	49	M	30838	4	OHA	N	72	130/60	23.4	14.1	8400	14	170	240	8.4	163	1	170	120	40	110	30
46	MAHENDRA	38	M	30913	1	OHA	N	64	100/70	21.8	12.6	10200	10	130	180	7.3	130	0.6	180	110	46	100	34
47	SIDRAM	44	M	26167	2	OHA	N	84	140/70	23.4	11.6	11400	4	140	180	7.4	96	0.9	190	110	60	120	50
48	NANDAWWA	52	F	28218	2	OHA	Y	72	130/80	27.6	14.8	12400	8	190	260	9.4	230	0.8	280	150	40	180	60
49	ARJUN	54	M	28271	1	OHA	N	84	100/70	24.8	13.2	9400	14	180	270	9	190	1.2	160	110	60	100	30
50	GAJRABAI	46	F	29339	4	OHA	N	70	140/80	19.8	12.4	10200	10	140	180	7.6	92	0.8	180	110	60	100	20
51	NEELAMMA	42	F	29422	1	OHA	N	84	110/70	24.6	14.2	7400	14	190	240	8.4	210	1.2	240	120	60	180	40
52	FATIMA	52	F	25899	5	OHA	N	96	100/60	21.4	13.4	12800	16	160	200	8.2	170	1.2	230	140	56	160	40
53	MALLAWWA	46	F	27163	6	OHA	N	80	1160/70	17.4	11.6	9800	4	130	180	7.8	130	0.6	180	80	66	80	34
54	MALLAPPA	55	M	28535	8	OHA+I	Y	98	130/70	26.7	10.4	10600	20	190	260	12.4	230	1	260	150	30	180	40
55	SHARANAMMA	58	F	27303	3	OHA	N	68	140/70	24.8	11.6	12400	26	140	260	10.2	190	0.8	230	170	40	130	50
56	KASIMSAB	48	M	28745	2	OHA	N	92	120/70	17.8	13.6	9600	12	130	160	7.8	120	1	180	120	64	110	36
57	GOURABAI	60	F	27157	4	OHA	N	84	100/70	26.7	10.4	9200	40	180	240	9.2	190	0.8	240	140	30	160	60
58	AMBAJI	54	M	27777	1	OHA	N	86	110/80	19.4	13.1	11300	10	110	160	7.4	114	1.2	160	110	60	90	20
59	LAXMIBAI	48	F	26366	3	OHA	N	100	110/60	24.8	9.8	11400	34	140	210	9.4	190	0.9	260	150	34	190	60
60	SHANTABAI	44	F	25646	4	OHA	N	76	130/70	27.9	12.2	13000	40	170	250	9.1	230	0.7	210	140	30	180	60
61	FATIMA	56	F	25899	3	OHA	N	70	130/70	24.6	14.4	9600	10	180	260	9.8	190	0.8	280	130	36	190	64
62	MAHALINGAPPA	45	M	26079	4	OHA	N	80	110/70	23.7	12.4	8600	11	160	260	8.9	110	1.2	220	150	30	170	46
63	VIJAYKUMAR	48	M	24520	2	OHA	Y	68	130/70	19.8	13.6	10400	6	130	160	7.6	121	0.9	160	110	60	90	20
64	GANAGABAI	49	F	25923	4	OHA+I	N	62	120/80	17.8	12	10200	6	120	180	7.6	160	0.9	180	150	40	110	46
65	SANGAMMA	54	F	25893	6	OHA	N	80	110/70	21.2	10.2	10600	10	160	220	10.2	170	1	260	180	36	190	56
66	KASHINATH	56	M	26054	3	OHA	N	86	130/70	21.3	12.4	7800	14	160	230	8.3	134	0.6	160	120	40	74	36
67	LALITA	60	F	25585	4	OHA	N	80	110/70	23.4	11.6	12400	20	116	160	6.6	96	1	160	150	60	80	30
68	NEELAMMA	54	F	34597	6	OHA	N	64	120/70	19.8	11.4	9700	12	110	160	8.2	130	1	160	110	50	90	30
69	SHARADA	46	F	34776	1	OHA	N	86	110/70	19.4	13.6	10300	10	128	180	7.4	86	1	170	120	60	80	30
70	GOPINATH	54	M	34120	2	OHA	N	94	140/80	26.7	13.2	12000	8	170	260	9.4	170	0.9	240	160	30	190	60
71	SUMITRA	49	F	2671	4	OHA	N	72	140/80	21.8	11.6	11400	10	140	180	8.4	146	0.6	180	130	40	110	36
72	ILABAI	48	F	2785	3	OHA+I	N	86	120/70	25.8	12.4	4600	8	180	270	9.4	192	1.2	180	130	46	98	34

73	NINGAPPA	54	M	3169	4	OHA	N	80	130/70	21.8	10.6	6400	6	160	230	8.6	136	0.9	230	170	50	180	46
74	TAMANNA	56	M	4746	4	OHA+I	N	70	136/60	19.8	13.6	10400	8	140	160	7.2	78	0.9	150	110	60	80	20
75	LAXMIBAI	60	F	5535	8	OHA	N	68	130/70	21.8	11.6	10200	6	110	170	7.6	156	0.6	180	130	40	110	30
76	PAIVANIBAI	56	F	5660	1	OHA	N	76	110/70	17.6	14.4	6200	12	120	160	6.7	84	1	160	140	36	60	66
77	SHAKUNTALA	46	F	5825	3	OHA	N	86	130/70	23.4	12.8	6700	16	170	240	8.6	138	0.6	160	120	40	80	40
78	MALLAPPA	60	M	6055	4	OHA	Y	80	150/90	23.6	11.6	12400	20	116	160	6.6	84	1	160	150	60	80	20
79	TUKARAM	54	M	6236	6	OHA	N	64	120/70	18.6	11.4	9700	12	114	170	8.4	126	0.9	160	110	50	100	10
80	BASALINGAMMA	54	F	6250	1	OHA	N	84	110/7	17.9	13.6	10600	10	138	190	9.2	60	1	170	120	50	90	30
81	SHREEDEVI	48	F	6653	2	OHA	Y	94	140/90	26.7	14.2	12000	8	170	270	9.4	170	0.9	240	170	40	160	60
82	YAMANAWWA	54	F	6761	6	OHA	N	72	140/80	21.2	11.8	11400	10	140	180	8.4	136	0.6	230	140	40	160	40
83	MRUTUNJAYASWAMI	54	M	8260	2	OHA	N	74	110/70	23.8	14	13200	6	130	210	7.8	134	1.2	160	110	50	90	20
84	GUNDAPPA	46	M	9000	2	OHA	N	80	130/70	17.8	11.4	4900	17	160	240	8.4	156	1	240	180	46	164	60
85	NAGAPPA	54	M	9034	6	OHA	N	84	126/80	29.3	11.8	11200	16	170	210	9.4	191	1.4	190	160	34	123	64
86	SOMANING	54	M	9157	7	OHA	N	84	130/70	21.4	13.6	10200	14	130	240	9.2	193	0.8	130	120	64	60	30
87	AMEERBI	46	F	9176	1	OHA	N	68	110/70	18.6	12.4	9400	24	130	160	7.4	116	0.8	130	110	60	60	20
88	SUGALABAI	54	F	9307	2	OHA	N	84	130/80	24.6	12.6	10000	12	180	260	9.8	178	1.4	220	160	50	180	40
89	KESHAPPA	63	M	9336	2	OHA	N	80	120/80	27.8	14.2	12500	8	170	280	9.4	240	0.9	240	170	30	180	30
90	KAMALABAI	54	F	9544	1	OHA	N	74	110/70	21.8	13.6	9400	14	190	230	9	130	1.2	160	130	40	80	30
91	JANABAI	49	F	9566	4	OHA	N	80	110/70	23.8	12.4	10300	8	110	190	7.6	186	0.8	160	110	60	70	30
92	BANGAREMMA	54	F	9600	3	OHA+I	Y	86	130/80	26.8	14.2	6700	18	170	250	8.6	174	1.2	260	150	60	180	30
93	VEENA	48	F	9690	1	OHA	N	68	106/60	19.6	12.8	9400	12	110	160	7.4	130	0.8	160	110	60	70	30
94	SHARANAWWA	60	F	9730	3	OHA	N	86	130/80	24.7	12.9	10200	16	180	260	9.2	193	1.2	220	160	40	170	30
95	LAXMI	49	F	9792	3	OHA	N	74	116/70	17.6	11.4	6700	14	94	120	7.2	96	0.8	140	110	40	60	40
96	RAMAPPA	56	M	9803	1	OHA	N	86	120/70	23.6	10.4	11200	16	120	170	7.4	106	1.2	160	110	40	80	40
97	BASAVANTARAY	64	M	9821	5	OHA	N	70	130/86	26.9	10.8	6600	21	160	200	9.6	180	0.9	190	110	30	120	40
98	PARVATEWWA	52	F	9860	2	OHA	N	80	130/90	21.6	10.4	10400	16	136	250	8.4	168	0.6	160	110	60	60	40
99	KAVEREMMA	46	F	9969	4	OHA	N	84	124/70	21.6	10.4	5600	8	124	140	6.9	116	1.2	160	110	40	70	30
100	RAMESH	54	M	10130	6	OHA+I	Y	70	140/90	29.8	11.6	10600	10	160	220	10.2	170	1	260	180	40	190	30
101	BHIMANAGOUDA	45	M	23171	1	OHA	N	76	110/70	26.8	15.3	7400	15	130	170	9.8	186	0.9	230	170	46	140	54
102	BASAPPA	58	M	21265	6	OHA	N	84	130/70	21.8	9.9	4660	12	219	270	9.5	190	0.9	210	150	60	190	30
103	KARIYAPPA	65	M	22109	0.5	OHA+I	N	76	120/70	26.7	11.2	6560	14	120	160	9.3	191	1.1	180	110	40	80	50
104	HANAMABAI	75	F	22772	2	OHA	N	80	110/70	24.3	10.4	11120	10	180	240	13	166	0.7	260	150	40	190	70
105	HANAMAWWA	45	F	23263	4	OHA	N	76	130/80	24.9	13.5	7370	12	156	170	12	174	0.6	190	130	60	90	30
106	DARMARAY	30	M	20976	2	OHA	N	80	110/70	27.8	13.4	6050	10	168	240	11	169	0.7	250	130	40	190	30
107	VADIRAJ	58	M	21399	3	OHA	Y	76	140/70	21.3	10.6	6800	16	112	126	6.4	179	0.6	190	150	40	130	30
108	SIDRAYA	54	M	20755	6	OHA	N	80	120/70	26.8	12.4	11700	12	220	290	10.7	240	0.8	190	110	50	90	40
109	JUBEDA	45	F	20355	2	OHA	N	90	116/70	23.4	9.4	7600	16	124	160	7.7	191	0.6	160	110	50	70	40
110	KAMALESHWAR	44	M	23763	0.5	OHA	N	74	110/70	21.6	13.1	4800	10	116	160	6.6	120	0.8	190	120	40	110	40
111	VIJAYAKUMAR	51	M	21103	4	OHA+I	Y	90	150/90	26.7	13.88	9500	16	290	300	11.3	183	0.7	260	140	40	180	30
112	AKSHATA	46	F	21191	4	OHA	N	70	130/80	23.4	9.4	9100	15	90	140	6.6	170	0.6	160	110	40	90	30

113	GOURAMMA	`52	F	23773	1	OHA	N	84	110/70	19.6	13.3	11000	30	100	160	6.4	156	0.7	190	120	50	110	30
114	JAYAMMA	35	F	23777	1	OHA	N	70	110/80	21.6	10.9	5300	15	110	140	6.3	110	0.7	160	110	50	100	30
115	DEVENDRAPPA	50	M	22910	2	OHA	N	84	104/70	23.7	11.6	8700	10	120	160	6.6	143	0.8	150	110	48	80	32
116	MAHADEVI	56	F	22886	0	N	Y	76	150/90	28.9	10.9	6800	16	116	220	7.4	116	1	190	140	64	96	30
117	MAHALAXMI	45	F	22892	2	OHA	N	78	124/70	19.6	11.1	7600	12	124	164	6.7	138	0.9	150	110	43	86	31
118	ASHOK	50	M	22964	6	OHA+I	N	80	130/70	29.7	12.5	5700	12	187	260	8.7	240	0.7	270	160	54	174	62
119	PARVATI	48	F	22916	1	OHA	N	84	116/70	21.4	11.2	6160	13	128	170	7.1	118	0.6	170	140	50	92	28
120	TARABAI	50	F	21739	10	OHA+I	N	90	140/80	26.8	13.6	9020	15	164	212	11	216	0.9	230	170	37	150	43
121	SHIVASHARAN	45	M	22396	2	OHA	N	73	130/80	28.4	14	9030	8	207	187	13	183	0.9	270	190	27	166	77
122	KASHIRAYA	60	M	21695	6	OHA	N	88	136/80	26.4	10.8	11600	12	220	290	10.4	176	1.1	222	170	34	120	68
123	ARAVIND	64	M	21815	9	0HA	Y	96	140/90	29.2	13.5	7400	10	213	280	13	243	0.9	260	190	47	158	55
124	YALLAPPA	58	M	21800	7	OHA	N	68	130/70	20.6	15	8200	5	120	180	7.3	114	1.1	170	140	58	76	36
125	CHANDAMMA	72	F	22914	0.5	OHA	N	76	116/60	18.7	10.6	6900	16	116	144	6.6	108	0.7	150	110	44	70	36
126	SIDDRAMAYYA	52	M	23920	10	OHA+I	N	88	136/70	25.8	14.4	8700	12	220	280	9.7	196	1.2	270	190	37	180	53
127	VITHOBA	63	M	23991	6	OHA	N	96	140/70	28.3	10.8	7220	14	212	240	9.5	110	0.8	190	150	46	110	34
128	RAMAWWA	70	F	23874	7	OHA+I	Y	86	136/90	26.7	12.3	11800	10	190	170	12.6	152	0.6	230	180	37	140	53
129	ISHWARAPPA	53	M	23739	0.5	OHA	N	68	118/70	19.7	10.8	4400	11	120	156	6.7	108	0.7	170	110	54	93	23
130	LAXMI	45	F	19498	8	OHA+I	Y	94	150/90	28.2	9.6	9900	12	200	286	9.4	183	1.2	250	180	57	130	63
131	REVANSIDDAPPA	76	M	22915	8	OHA	N	76	130/70	28.7	13.4	8900	7	84	140	6.4	127	0.7	190	130	48	84	58