"STUDY OF SERUM FERRITIN IN METABOLIC SYNDROME AND ITS COMPONENTS – A ONE YEAR CROSS SECTIONAL STUDY AT KLE UNIVERSITY, BELGAUM"

REG NO. BG0110005

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.

in

GENERAL MEDICINE

DEPARTMENT OF MEDICINE,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELGAUM, KARNATAKA

APRIL - 2013

KLE UNIVERSITY, BELGAUM,

KARNATAKA

ENDORSEMENT

This is to certify that the dissertation entitled **"STUDY OF SERUM FERRITIN IN METABOLIC SYNDROME AND ITS COMPONENTS – A ONE YEAR CROSS SECTIONAL STUDY AT KLE UNIVERSITY, BELGAUM"** is a bonafide research work done by **CANDIDATE REG NO. BG0110005.**

Dr. V. A. Kothiwale **MD, Ph.D**
Professor and Head,
Department of Medicine,
J. N. Medical College,
Nehru Nagar, Belgaum – 10

Date:
Place: Belgaum

Dr. A. S. Godhi **MS**
Principal,
J. N. Medical College,
Nehru Nagar, Belgaum – 10

Date:
Place: Belgaum

LIST OF ABBREVIATIONS USED

%	– Percentage
ACE	– Angiotensin converting enzyme
AHA	– American Heart Association
Apo B	– Apolipoprotein B
Asp35	– Aspartate35
AT II	– Angiotensin II
ATP III	– adult treatment panel III
BMI	– Body mass index
BP	– Blood pressure
CAD	– Coronary Artery Disease
CHD	– Coronary heart disease
cms	– Centimeters
CRP	– C-reactive protein
CVD	– Cardiovascular Disease
DM	– Diabetes Mellitus
FBS	– Fasting blood glucose
FFA	– Free Fatty acids
FPG	– Fasting plasma glucose
HDL	– High density Lipoprotein
HDL-C	– High density Lipoprotein Cholesterol
HIV	– Human Immunodeficiency Virus
Hs-CRP	– Highly sensitive C-Reactive protein
HTN	– Hypertension
ICAM-1	– Intracellular adhesion molecule-1

ICE – Interleukin 1β converting enzyme

IDF – International Diabetes Federation

IFG – Impaired fasting glucose

IFN-γ – Interferon γ

IGT – Impaired glucose tolerance

IL-1 – Interleukin 1

IL-18 – Interleukin 18

IL-18 BP – Interleukin 18 binding protein

IL-6 – Interleukin 6

kD – kilodalton

kg/m^2 – Kilograms per square metre

LPL – Lipoprotein Lipase

MetS – Metabolic syndrome

mg – milligrams

mg/dL – Milligrams per deciliter

MI – Myocardial Infarction

min – Minute

mm Hg – Millimeters of mercury

mMol/L – Millimoles per litre

MMP – Matrix metalloproteinase

NAFLD – Non-alcoholic fatty liver disease

NCEP – National cholesterol education programme

NPDR – Non proliferative diabetic retinopathy

NHLBI – National Heart, Lung, and Blood Institute

NO – Nitric oxide

OGTT	– Oral glucose tolerance test
OSA	– Obstructive sleep apnoea syndrome
PCOS	– Polycystic ovarian syndrome
SD	– standard deviation
TG	– Triglycerides
Th0	– T-helper cell 0
Th1	- T-helper cell 1
Th2	- T-helper cell 2
TNF-α	– Tumor necrosis factor – α
TZD	– Thiazolidinediones
U.S.	– United states
VCAM-1	– Vascular cell adhesion molecule 1
VLDL	– Very low density lipoprotein
WHO	– World Health Organization
µg/min	– Microgram per minute
µU/L	– Microunit per litre

ABSTRACT

Background and objectives

The metabolic syndrome is a cluster of risk factors for cardiovascular disease (CVD), including obesity, hypertension, elevated triglycerides and low levels of HDL Cholesterol. Elevated serum ferritin levels have positive correlation between elevated ferritin levels. The present study was aimed to know the relationship between serum ferritin and metabolic syndrome and to evaluate its relationship with individual component of metabolic syndrome.

Methodology

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the study period from January 2011 to December 2011. Based on NCEP ATP III criteria, 100 cases with metabolic syndrome were enrolled. Serum ferritin was measured on fasting blood samples using radio-immunoassay.

Results

In this study, male preponderance was noticed. The most common age group was 51 to 60 years (30%) and overall, the mean age was 55.84 ± 15.04 years. The commonest component of metabolic syndrome was hypertension 88%. Overall mean duration of hypertension was 9.68 ± 8.52 years and mean duration of diabetes was 9.52 ± 9.52 years. In this study lipid abnormalities revealed raised serum cholesterol, LDL, TG and low HDL among 61%, 15%, 86% and 81% respectively. Results of the present study showed serum ferritin was elevated significantly in individual components of metabolic syndrome (Hypertension,

waist circumference, low HDL levels) though serum ferritin were elevated in patients of FBS and Triglyceride abnormality, but it was of statistically insignificant.

Conclusion and interpretation

Serum ferritin as a marker of metabolic syndrome was inconclusive but was raised significantly in individual components of metabolic syndrome.

Keywords

Lipid abnormalities; Metabolic syndrome; Serum ferritin;

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

Chapter 1

Introduction

INTRODUCTION

The metabolic syndrome is a cluster of risk factors for cardiovascular disease (CVD), including obesity, hypertension, elevated triglycerides and low levels of HDL Cholesterol.

Metabolic syndrome is a burgeoning global problem. Approximately one fourth of the adult European population is estimated to have metabolic syndrome, with a similar prevalence in Latin America.[1] MetS is also considered an emerging epidemic in developing East Asian countries including China, Japan, and Korea. The prevalence of metabolic syndrome may range from 8 to 13% in men and 2 to 18% in women depending on the population and definitions used.[2-4] In Japan, the Ministry of Health, Labor, and Welfare has instituted a screening and interventional program.[5] Metabolic syndrome has been recognized as a highly prevalent problem in many other countries worldwide.[6-11]

The clinical manifestations of this syndrome may include hypertension, hyperglycemia, hypertriglyceridemia, reduced high-density lipoprotein cholesterol (HDL-C), and abdominal obesity.

Abundant data suggest that patients meeting these diagnostic criteria have a greater risk of having significant clinical consequences, the two most prominent and dreaded of which are the development of coronary heart disease[12] and diabetes mellitus (DM).[13] It also increases risk of stroke, fatty liver disease, and several cancers.[14]

Initial laboratory studies in patients suspected of having metabolic syndrome include standard biochemical parameters to assess for hyperglycemia, renal dysfunction and lipid studies to assess for hypertriglyceridemia or low HDL levels.

Obesity and insulin resistance are considered central to the pathophysiology of this metabolic and cardiovascular syndrome.[15,16]

Several studies have observed a positive association between elevated iron stores, measured by serum ferritin levels, and the prevalence of the metabolic syndrome. Studies have also reported positive correlation of ferritin levels with individual components of the metabolic syndrome, particularly serum triglycerides and plasma glucose, as well as markers of insulin resistance.[17,18]

There is increasing evidence that moderately elevated body iron stores, below levels commonly found in genetic hemochromatosis, may be associated with adverse health outcomes. Elevated serum ferritin levels independently predicted incident type 2 diabetes in prospective studies in apparently healthy men and women.[19,20] Several cross-sectional studies have previously examined the association between iron stores and individual metabolic cardiovascular risk factors, including hypertension,[21] dyslipidemia,[22,23] elevated fasting insulin and blood glucose,[24] and central adiposity.[25] These studies have reported positive correlation between elevated ferritin levels with hypertension,[21] dyslipidemia,[22,23] elevated fasting insulin and blood glucose,[24] and central adiposity.[25]

Although the mechanisms for the potential effect of iron on the risk of metabolic syndrome are unclear, it has been hypothesized that elevated iron

stores may interfere with hepatic insulin extraction leading to peripheral hyperinsulinemia.[26,27] Others have suggested that iron may catalyze the formation of hydroxyl radicals, which contribute to the development of insulin resistance.[28,29] The association between elevated iron stores and the metabolic syndrome, however, has been less well explored.[18]

Even in the face of compelling evidence in favour of this theory, there are very few studies done in this area especially in India. Hence, the present study was undertaken to know the relationship between serum ferritin and metabolic syndrome and to evaluate the relationship between serum ferritin and individual component of metabolic syndrome.

Chapter 2

Objectives

OBJECTIVES

The objectives of the present study were

1. To know the relationship between serum ferritin and metabolic syndrome.
2. To evaluate the relationship between serum ferritin and individual component of metabolic syndrome.

Chapter 3

Review of Literature

REVIEW OF LITERATURE

Most of the developing countries including India are undergoing an epidemiological transition. Infectious and nutritional diseases are receding among adults while non communicable diseases are becoming increasingly common, as the cause of morbidity and mortality. Demographic projections indicate a major increase in cardiovascular disease mortality in India due to increase in life expectancy and change in the age structure of the growing population.[30]

Non-communicable or chronic diseases are diseases of long duration and generally slow in progression such as heart disease, stroke, cancer, chronic respiratory diseases, hypertension and diabetes. These diseases are now the leading cause of mortality in the world, representing 63% of all deaths. Out of the 36 million people who died from chronic disease in 2008, nine million were under 60 and ninety per cent of these premature deaths occurred in low- and middle-income countries.[31]

The hallmark of management of Non-communicable diseases is Primary and Secondary prevention. The essence of prevention lies in risk factor identification and reduction. Metabolic syndrome has emerged as an important constellation of risk factors that has been shown to effectively predict the development of Type II Diabetes Mellitus and Cardiovascular Disease.[12,13]

The 'Metabolic Syndrome' is a widely prevalent and multi-factorial disorder that presents in a distinct, albeit heterogenous phenotype.[32]

Although obesity and insulin resistance are not synonymous with the metabolic syndrome, they are integral features in this derangement of adipocyte physiology and carbohydrate metabolism.[32]

Metabolic syndrome was initially observed in 1923 by Kyln, who described the clustering of hypertension, hyperglycemia and gout as the syndrome. Subsequently, several other metabolic abnormalities have been associated with this syndrome, including obesity, microalbuminuria, and abnormalities in fibrinolysis and coagulation.[33]

In 1988, Gerald Reaven reintroduced the concept of Syndrome X for the clustering of cardiovascular risk factors like hypertension, glucose intolerance, high triglycerides and low HDL concentration.[15]

The syndrome has been given several names, including the 'metabolic syndrome', the 'insulin resistance syndrome', the 'plurimetabolic syndrome', and the 'deadly quartet'.[33]

In 1998, WHO proposed a unifying definition for the syndrome and chose to call it the 'metabolic syndrome' rather than the 'insulin resistance syndrome'.[34]

This name was chosen primarily because it was the cause of all the components of the syndrome.

The Third Report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III) included clinical diagnosis guidelines for the Metabolic Syndrome. Compared with findings from earlier studies and WHO

guidelines, the new ATP III defines criteria which can be measured in clinical practice.[32]

The metabolic syndrome (MetS) is a multiplex risk factor that arises from insulin resistance accompanying abnormal adipose deposition and function.[35] It is a risk factor for coronary heart disease (CHD), as well as diabetes, fatty liver and several cancers. The clinical consequences of this syndrome may include hypertension, hyperglycemia, hypertriglyceridemia, reduced high-density lipoprotein cholesterol (HDL-C) and abdominal obesity.

Diagnosis

Under recent guidelines, revised in 2005 by the National Heart, Lung, and Blood Institute (NHLBI) and the American Heart Association (AHA),[36] metabolic syndrome is diagnosed when a patient has at least three of the following five conditions:

1. Fasting glucose ≥ 100 mg/dL (or receiving drug therapy for hyperglycemia)
2. Blood pressure ≥ 130/85 mm Hg (or receiving drug therapy for hypertension)
3. Triglycerides ≥ 150 mg/dL (or receiving drug therapy for hypertriglyceridemia)
4. HDL-C < 40 mg/dL in men or < 50 mg/dL in women (or receiving drug therapy for reduced HDL-C)

5. Waist circumference ≥ 102 cm (40 in) in men or ≥ 88 cm (35 in) in women; if Asian American, ≥ 90 cm in men (35 in) or ≥ 80 cm in women (32 in)

Other Definitions of Metabolic syndrome:[34,37,38]

	WHO 19996[34]	**EGIR 1999**[37]	**NCEP ATP III 2001**[38]
	Glucose intolerance, IGT or diabetes and /or insulin resistance together with two or more of the following	Insulin resistance (Defined as hyperinsulinemia – Top 25% of fasting insulin values among the non diabetic population) plus two of the following	Three or more of the following five risk factors
Fasting plasma glucose	≥ 140 / 90 mm Hg	≥ 6.1 mmol/L (110 mg/dL) but non diabetic	≥ 5.6 mmol/L 100 mg/dL
Blood pressure triglycerides	Raised plasma triglycerides ≥ 1.7 mmol/L (150 mg/dL) and / or	≥ 140/90 mm Hg or treatment	≥ 130 /≥ 85 mm Hg 1.7 mmol/L (150 mg/dL)
HDL cholesterol	Men < 0.9 mmol/L (39 mg/dL)	≥ 2.00 mmol/L (178 mg/dL0 or treatment and/or < 1.0 mmol/L (39 mg/dL) or treatment	Men <1.03 mmol/L (40 mg/dL) Women: < 1,29 mmol/L (< 50 mg/dL)
Obesity	Men WC ratio > 0.90	Men: WC ≥ 94 Cms	Men WC > 102 Cm
	Women WC ratio > 0.85 And/or BMI > 30 Kg/m2	Women: WC ≥ 80 Cms	Women WC > 88 Cms
Microalbuminuria	Urinary albumin excretion rate ≥ 20 µg/min or albuminacreatiine ratio ≥ 30 mg/g		

In 2005, the International Diabetes Federation (IDF) published its definition of the metabolic syndrome in adults. The intention was to rationalize the existing multiple definitions of the syndrome and to have a single, universally accepted diagnostic tool that is easy to use in clinical practice and that does not rely upon measurements only available in research settings.[39]

IDF criteria for the diagnosis of metabolic syndrome[39]

According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have:

- Central obesity (defined as waist circumference ≥ 94 cm for Europid men and ≥ 80 cm for Europid women, with ethnicity specific values for other groups)

Plus any two of the following four factors:

- Raised TG level: ≥ 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality
- Reduced HDL cholesterol: <40 mg/dL (1.03 mmol/L) in males and < 50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality
- Raised blood pressure: Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg, or treatment of previously diagnosed hypertension
- Raised fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes

If FBS is above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome.

Country /Ethnic group		Waist circumference
Europids: In USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purposes	Male	≥ 94 cms
	Female	≥ 80 cms
South Asians: Based on Chinese, Malay and Asian Indian population	Male	≥ 90 cms
	Female	≥ 80 cms
Chinese	Male	≥ 90 cms
	Female	≥ 80 cms
Japanese	Male	≥ 90 cms
	Female	≥ 80 cms
Ethnic South and Central Americans	Use South Asian recommendation until more specific data are available	
Sub Saharan Africans	Use European data until more data are available	
Eastern Mediterranean and middle east (Arab) populations	Use European data until more data are available	

Abundant data suggests, that patients meeting these diagnostic criteria have a greater risk of having significant clinical consequences, the 2 most prominent and dreaded of which are the development of diabetes mellitus (DM) and coronary heart disease. In addition, pooled data from 37 studies involving more than 170,000 patients have shown that metabolic syndrome doubles the risk of coronary artery disease.[1] It also increases risk of stroke, fatty liver disease, diabetes[12] and cancer.[13]

Epidemiology

Frequency

Metabolic syndrome is increasing in prevalence, paralleling an increasing epidemic of obesity. In the United States, data from a 1999-2000 survey showed

that the age-adjusted prevalence of metabolic syndrome among adults aged 20 years or older had risen from 27% (data from 1988-1994) to 32%.[40]

Metabolic syndrome is a burgeoning global problem. Approximately one fourth of the adult European population is estimated to have metabolic syndrome, with a similar prevalence in Latin America.[36] It is also considered an emerging epidemic in developing East Asian countries including China, Japan, and Korea, the prevalence of metabolic syndrome may range from 8-13% in men and 2-18% in women depending on the population and definitions used.[2,3,4] Metabolic syndrome has been recognized as a highly prevalent problem in many other countries worldwide.[6-10,41] In a recent study done on Indian urban population, Metabolic syndrome was present in 31.6%; prevalence was 122 (22.9%) in men and 223 (39.9%) in women.[9]

With the formulation of NCEP/ATP III guidelines, some uniformity and standardization has occurred in the definition of metabolic syndrome and has been very useful for epidemiological purposes. At present, metabolic syndrome is an all or none diagnosis.[42]

The prevalence of the metabolic syndrome depends on age, ethnic background, and gender. It rises linearly from 20 to 50 years and plateaus thereafter. Looking at various studies around the world, which included population samples, aged from 20 to 25 and upwards, the prevalence varies from 8% (India) to 24% (United States) in men and from 7% (France) to 46% (India) in women.[43] Two Indian studies, which differed in their definition of obesity: the first[44] used the obesity criteria suitable for Indians, while the second[45] used the

standard ATP III definition of obesity. Both studies used population based samples within the age range but reported prevalence of 13% in Jaipur[36] and 41% in Chennai.[44] Although, the prevalence of obesity in the two study groups was quite similar (31% versus 33%), despite the different definitions used, far larger differences were observed between the two studies for the prevalence of elevated triglycerides (46% vs. 30%), hypertension (55% vs. 39%) and elevated fasting plasma glucose (27% vs. 5%); indicating a far larger impact of these risk factors than obesity alone.

Various Factors contributing to increasing prevalence of metabolic syndrome have been identified:

1. Atherogenic dyslipidemia, Elevated Triglycerides, apolipoprotein B and small low-density lipoprotein, low HDL.
2. Elevated plasma glucose.
3. Elevated blood pressure.
4. Pro-thrombotic state.
5. Pro-inflammatory state

Many studies[46,47,48] have reported that low socio-economic status is associated with a higher mortality rate due to cardiovascular disease. A low education level links cardiovascular disease with risk factors such as smoking, hypertension, impaired glucose tolerance, diabetes mellitus, physical inactivity and overweight associated with other metabolic abnormalities.

Age

The prevalence of metabolic syndrome increases with age, with about 40% of people older than 60 years meeting the criteria.[40] The metabolic syndrome affects 44% of the U.S. population older than age 50. A greater percentage of women older than age 50 have the syndrome than men. The age dependency of the syndrome's prevalence is seen in most populations around the world.

However, metabolic syndrome can no longer be considered a disease of only adult populations. Alarmingly, metabolic syndrome and diabetes mellitus are increasingly prevalent in the pediatric population, again in parallel with a rise in obesity.[49] In the United States, children are becoming obese at triple the rate compared with the 1960s, making the study and treatment of this problem vital. The epidemic of metabolic syndrome in children and adolescents is an international phenomenon, which lead the International Diabetes Foundation to publish an updated consensus statement to guide diagnosis and further study of the condition.[50,51]

Aetiopathogenesis

Risk factors

Overweight/Obesity

Central adiposity is a key feature of the syndrome, reflecting the fact that the syndrome's prevalence is driven by the strong relationship between waist circumference and increasing adiposity. However, despite the importance of

obesity, patients who are normal weight may also be insulin-resistant and have the syndrome.

Sedentary Lifestyle

Many components of the metabolic syndrome are associated with a sedentary lifestyle, including increased adipose tissue (predominantly central); reduced HDL cholesterol; and a trend toward increased triglycerides, blood pressure, and glucose intolerance in the genetically susceptible.

Diabetes Mellitus

DM is included in both the NCEP and International Diabetes Foundation (IDF) definitions of the metabolic syndrome. It is estimated that the large majority (~75%) of patients with type 2 diabetes or impaired glucose tolerance (IGT) have the metabolic syndrome. The presence of the metabolic syndrome in these populations relates to a higher prevalence of CVD compared to patients with type 2 diabetes or IGT without the syndrome.

Coronary Heart Disease

The approximate prevalence of the metabolic syndrome in patients with coronary heart disease (CHD) is 50%, with a prevalence of 37% in patients with premature coronary artery disease.

Lipodystrophy

Lipodystrophic disorders in general are associated with the metabolic syndrome. Both genetic (Berardinelli-Seip congenital lipodystrophy, Dunnigan

familial partial lipodystrophy) and acquired (HIV-related lipodystrophy in patients treated with highly active antiretroviral therapy) forms of lipodystrophy may give rise to severe insulin resistance and many of the metabolic syndrome's components.

Risk factors occur in isolation only 30% of the time, and clustering of three or more factors occurs 17% of the time in both genders. Clustering of the factors was related to baseline obesity and weight gain during adulthood.[52]

In Framingham and in other observational studies, the central core of metabolic risk factors were found to be highly related, including triglycerides, HDL-C, BMI, waist circumference, or fasting insulin levels, to insulin levels after an oral glucose challenge test. In addition to a central metabolic syndrome core, there has been a hypertension cluster with shared variance components that included BMI, systolic pressure and diastolic pressure.[52]

Pathogenesis

Metabolic syndrome is a heterogeneous condition characterized by visceral adiposity, dyslipidemia, hypertension, and insulin resistance. The metabolic syndrome with its clustering of metabolic and atherosclerotic risk factors is a strong determinant of type 2 diabetes and cardiovascular disease (CVD). Obesity and insulin resistance are considered central to the pathophysiology of this metabolic and cardiovascular syndrome.[53]

Insulin resistance

The most accepted and unifying hypothesis to describe the pathophysiology of the metabolic syndrome is insulin resistance, caused by an incompletely understood defect in insulin action. The mechanisms underlying the metabolic syndrome are not fully known; however resistance to insulin stimulated glucose uptake seems to modify biochemical responses in a way that predisposes to metabolic risk factors.[43]

An early major contributor to the development of insulin resistance is an overabundance of circulating fatty acids which are derived predominantly from adipose tissue triglyceride stores released by hormone-sensitive lipase. Fatty acids are also derived through the lipolysis of triglyceride-rich lipoproteins in tissues by lipoprotein lipase (LPL). Insulin mediates both antilipolysis and the stimulation of LPL in adipose tissue. Thus, when insulin resistance develops, increased lipolysis produces more fatty acids, which further decrease the antilipolytic effect of insulin. Excessive fatty acids enhance substrate availability and create insulin resistance by modifying downstream signaling. Fatty acids impair insulin-mediated glucose uptake and accumulate as triglycerides in both skeletal and cardiac muscle, whereas increased glucose production and triglyceride accumulation are seen in liver.

Both adipose cell enlargement and infiltration of macrophages into adipose tissue result in the release of proinflammatory cytokines and promote insulin resistance. Insulin resistance appears to be the primary mediator of metabolic syndrome.[45] Insulin promotes glucose uptake in muscle, fat, and liver

cells, and can influence lipolysis and production of glucose by hepatocytes. Additional contributors to insulin resistance include abnormalities in insulin secretion and insulin receptor signaling, impaired glucose disposal, and proinflammatory cytokines. These abnormalities, in turn, may result from obesity with related increases in free fatty acid levels and changes in insulin distribution (insulin accumulates in fat).

The distribution of adipose tissue appears to affect its role in metabolic syndrome. Fat that is visceral or intra-abdominal correlates with inflammation whereas subcutaneous fat does not.[55] Abdominal fat is known to produce potentially harmful levels of cytokines, such as tumor necrosis factor, adiponectin, leptin, resistin, and plasminogen activator inhibitor.[56]

Dyslipidemia

In general, FFA flux to the liver is associated with increased production of apoB–containing, triglyceride-rich very low density lipoproteins (VLDLs). The effect of insulin on this process is complex, but *hypertriglyceridemia* is an excellent marker of the insulin-resistant condition.

The other major lipoprotein disturbance in the metabolic syndrome is a *reduction in HDL cholesterol*. This reduction is a consequence of changes in HDL composition and metabolism which results in an increased clearance of HDL from the circulation.

In addition to HDL, LDLs are also modified in composition. With fasting serum triglycerides >2.0 mM (~180 mg/dL), there is almost always a

predominance of small dense LDLs. Small dense LDLs are thought to be more atherogenic. They may be toxic to the endothelium and also have increased susceptibility to oxidation and are selectively bound to scavenger receptors on monocyte-derived macrophages.

Glucose Intolerance

The defects in insulin action lead to impaired suppression of glucose production by the liver and kidney and reduced glucose uptake and metabolism in insulin-sensitive tissues, i.e., muscle and adipose tissue. To compensate for defects in insulin action, insulin secretion and/or clearance must be modified to sustain euglycemia. Ultimately, this compensatory mechanism fails, usually because of defects in insulin secretion, resulting in progress from IFG and/or IGT to DM.

Hypertension

The relationship between insulin resistance and hypertension is well established. In the setting of insulin resistance, the vasodilatory effect of insulin is lost, but the renal effect on sodium reabsorption is preserved. Insulin also increases the activity of the sympathetic nervous system, an effect that may also be preserved in the setting of the insulin resistance. Finally, insulin resistance is characterized by pathway-specific impairment in phosphatidylinositol 3-kinase signaling which may cause an imbalance between the production of nitric oxide and secretion of endothelin-1, leading to decreased blood flow.

Role of Inflammation

Abundant in vitro and in vivo data implicate inflammation in atherogenesis, leading to the current understanding of atherosclerosis as a chronic inflammatory disease. Recent evidence demonstrates that a complex network of cytokines orchestrates the underlying immunologic and inflammatory processes.[58]

A central role has been attributed to the pro-inflammatory cytokines, tumor necrosis factor a (TNF- α) and interleukin (IL)-6, supported by the fact that both are produced in substantial amounts by human adipose tissue. TNF- α impairs insulin-stimulated glucose uptake in a variety of cells and decreases lipoprotein lipase activity. Both cytokines increase hepatic lipogensis and elicit a systemic acute- phase response.[43]

Furthermore, various aspects of the acute-phase response, such as fibrinogen and plasminogen activator inhibitor-1 levels, whole-blood viscosity, and white blood cell count, have recently been found to correlate positively with the metabolic syndrome. This is of particular interest because inflammation plays an important role in the pathogenesis of atherothrombosis.[43]

Moreover, C-reactive protein (CRP), the classic and exquisitively sensitive acute phase reactant, shows a strong independent association with the risk of Coronary Heart Disease and other atherothrombotic events. CRP levels have also been found to correlate with BMI and some features of the metabolic syndrome.[40]

Clinical Relevance

The clinical relevance of the metabolic syndrome is related to its role in the development of cardiovascular disease. Two recent prospective population-based studies confirmed that the metabolic syndrome identified a high-risk group of persons who would have been missed by only consideration of the conventional risk factors. The incidence of coronary disease along with carotid atherosclerosis is higher in patients with metabolic syndrome along with higher mortality from all such causes.[43]

Although for many obese patients the risk of developing metabolic syndrome is quiet evident, but studies[43] also show that the risk of having the metabolic syndrome increases steeply even within the overweight or the "preobese" range. Detecting these overweight individuals and the 6% of normal weight individuals with the metabolic syndrome and implementing preventive lifestyle interventions-diet education, physical activity, weight control, smoking cessation, and related behavior modification- is of a high clinical priority.

Other Associated Conditions

In addition to the features specifically associated with metabolic syndrome, insulin resistance is accompanied by other metabolic alterations

Nonalcoholic Fatty Liver Disease

Fatty liver is relatively common. However, in NASH, both triglyceride accumulation and inflammation coexist.

Hyperuricemia

Hyperuricemia reflects defects in insulin action on the renal tubular reabsorption of uric acid, whereas the increase in asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, relates to endothelial dysfunction. Microalbuminuria may also be caused by altered endothelial pathophysiology in the insulin-resistant state.

Polycystic Ovary Syndrome

PCOS is highly associated with the metabolic syndrome, with a prevalence between 40 and 50%. Women with PCOS are 2–4 times more likely to have the metabolic syndrome compared to women without PCOS.

Obstructive Sleep Apnea

OSA is commonly associated with obesity, hypertension, increased circulating cytokines, IGT, and insulin resistance.

Presentation

History

As with other diseases, careful history taking is important in metabolic syndrome. Even though the condition is diagnosed based on physical and laboratory features, it may be suspected if symptoms of any of the component disorders are present, such as the increased hunger, thirst, or urination that may accompany hyperglycemia. Patients reporting a history of hypertension, dyslipidemia warrant screening for metabolic syndrome. Symptoms suggesting

the rise of cardiovascular and other complications, such as chest pain or shortness of breath, must be investigated carefully. As lifestyle changes can ameliorate the condition, attention should be paid to the patient's dietary habits and exercise routines so that areas for improvement can be identified.

The social history is important for identifying additional risks, such as tobacco use, which may exacerbate the increased cardiovascular complications associated with metabolic syndrome. A family history should be obtained because genetics may play an important role in metabolic syndrome. This feature of the disease is under active investigation; however, currently no gene or group of genes has been implicated consistently, suggesting that the environment exerts substantial influence.[59] Finally, a thorough review of systems may help identify related problems, such as menstrual irregularities that can be seen in polycystic ovarian syndrome.

Physical

The physical examination is crucial in patients with metabolic syndrome as the findings of elevated blood pressure and abdominal obesity are 2 of the 5 diagnostic criteria. Measurement and documentation of waist circumference are important routines when screening for metabolic syndrome. Additionally, the examination may reveal findings reflective of the other criteria. For example, patients with insulin resistance and hyperglycemia or diabetes mellitus may have acanthosis nigricans, hirsutism, peripheral neuropathy, and retinopathy. Patients with severe dyslipidemia may have xanthomas or xanthelasmas. The presence of arterial bruits may portend a higher risk of cardiovascular complications.

Laboratory Studies

Fasting lipids and glucose are needed to determine if the metabolic syndrome is present. The measurement of additional biomarkers associated with insulin resistance must be individualized. Such tests might include apo B, high-sensitivity CRP, fibrinogen, uric acid, urinary microalbumin, and liver function tests. A sleep study should be performed if symptoms of OSA are present. If PCOS is suspected based on clinical features and anovulation, testosterone, luteinizing hormone, and follicle-stimulating hormone should be measured.

The Metabolic Syndrome: Treatment

Lifestyle

Obesity is the driving force behind the metabolic syndrome. Thus, weight reduction is the primary approach to the disorder. With weight reduction, the improvement in insulin sensitivity is often accompanied by favorable modifications in many components of the metabolic syndrome. In general, recommendations for weight loss include a combination of caloric restriction, increased physical activity, and behavior modification. The tendency for weight regain after successful weight reduction underscores the need for long-lasting behavioral changes.

Diet

Before prescribing a weight-loss diet, it is important to emphasize that it takes a long time for a patient to achieve an expanded fat mass; thus, the correction need not occur quickly. Diets restricted in carbohydrate typically

provide a rapid initial weight loss. However, after one year, the amount of weight reduction is usually unchanged. Thus, adherence to the diet is more important than which diet is chosen. Diets restricted in saturated fats (<7% of calories), trans fat (as few as possible), and cholesterol (<200 mg daily) should be applied aggressively.

Physical Activity

Before a physical activity recommendation is provided to patients with the metabolic syndrome, it is important to ensure that this increased activity does not incur risk.For the inactive participant, gradual increases in physical activity should be encouraged to enhance adherence and to avoid injury. Although increases in physical activity can lead to modest weight reduction, 60–90 min of daily activity is required to achieve this goal. Even if an overweight or obese adult is unable to achieve this level of activity, they still derive a significant health benefit from at least 30 min of moderate intensity daily activity.

Obesity

In some patients with the metabolic syndrome, treatment options need to extend beyond lifestyle intervention. Weight-loss drugs come in two major classes: appetite suppressants and absorption inhibitors. Appetite suppressants approved by the Food and Drug Administration include phentermine (for short-term use only, 3 months) and sibutramine. Orlistat inhibits fat absorption by ~30% and has been shown to reduce the incidence of type 2 diabetes, an effect that was especially evident in patients with baseline IGT.

Bariatric surgery is an option for patients with the metabolic syndrome who have a body mass index (BMI) of >40 kg/m^2 or >35 kg/m^2 with comorbidities.

Pharmacologic therapy:

Statins (HMG-CoA reductase inhibitors), which produce a 20–60% lowering of LDL cholesterol, are generally the first choice for medication intervention. Side effects are rare and include an increase in hepatic transaminases and/or myopathy.

The cholesterol absorption inhibitor ezetimibe is well tolerated and should be the second choice. Ezetimibe typically reduces LDL cholesterol by 15–20%. The bile acid sequestrants cholestyramine and colestipol are more effective than ezetimibe but must be used with caution in patients with the metabolic syndrome because they often increase triglycerides. Side effects include gastrointestinal symptoms (palatability, bloating, belching, constipation, anal irritation). Nicotinic acid has modest LDL cholesterol–lowering capabilities (<20%). Fibrates are best employed to lower LDL cholesterol when both LDL cholesterol and nontriglycerides are elevated. Fenofibrate may be more effective than gemfibrozil in this group.

Triglycerides

In general, the response of fasting triglycerides relates to the amount of weight reduction achieved. A weight reduction of >10% is necessary to lower fasting triglycerides.

A fibrate (gemfibrozil or fenofibrate) is the drug of choice to lower fasting triglycerides and typically achieve a 35–50% reduction. Although several additional clinical trials have been performed, these have not shown clear evidence that fibrates reduce CVD risk as a consequence of triglyceride lowering.

Other drugs that lower triglycerides include statins, nicotinic acid, and high doses of omega-3 fatty acids.

HDL Cholesterol

Beyond weight reduction, there are very few lipid-modifying compounds that increase HDL cholesterol. Statins, fibrates, and bile acid sequestrants have modest effects (5–10%), and there is no effect on HDL cholesterol with ezetimibe or omega-3 fatty acids. Nicotinic acid is the only currently available drug with predictable HDL cholesterol-raising properties.

Blood Pressure

In patients with the metabolic syndrome without diabetes, the best choice for the first antihypertensive should usually be an ACE inhibitor or an angiotensin II receptor blocker, as these two classes of drugs appear to reduce the incidence of new-onset type 2 diabetes. In all patients with hypertension, a sodium-restricted diet enriched in fruits and vegetables and low-fat dairy products should be advocated.

Impaired Fasting Glucose

In patients with the metabolic syndrome and type 2 diabetes, aggressive glycemic control may favorably modify fasting triglycerides and/or HDL cholesterol. In those patients with IFG without a diagnosis of diabetes, a lifestyle intervention that includes weight reduction, dietary fat restriction, and increased physical activity has been shown to reduce the incidence of type 2 diabetes. Metformin has also been shown to reduce the incidence of diabetes, although the effect was less than that seen with lifestyle intervention.

Insulin Resistance

Several drug classes [biguanides, thiazolidinediones (TZDs)] increase insulin sensitivity. Both metformin and TZDs enhance insulin action in the liver and suppress endogenous glucose production. TZDs, but not metformin, also improve insulin-mediated glucose uptake in muscle and adipose tissue. Benefits of both drugs have also been seen in patients with NAFLD and PCOS, and they have been shown to reduce markers of inflammation and small dense LDL. In general, the beneficial effects of TZDs appear superior to those of metformin.

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.[60]

In 1972, using an immunoradiometric assay, Addison *et al.*. convincingly demonstrated that ferritin could be reliably detected in human serum.[61] To determine the relationship between serum ferritin level and total body iron stores, the authors measured serum ferritin in a 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.[60]

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.".[62] 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 (see below). 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.[60]

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 on 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 about 50%; sequence conservation between subunit types is even greater (among mammalian H subunits, there is approximately 90% homology; among L subunits, approximately 80% homology).[60]

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-β (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.[60]

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.[60]

The decreased serum iron, increased macrophage iron, and decreased dietary iron absorption of anemia of inflammation are explained by increases 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.[60]

Extracellular ferritin in physiological and pathological processes[60]

Due to difficulties in isolating serum ferritin in quantity, few if any experiments have directly assessed effects of exogenous administration of serum ferritin. However, several 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 signaling 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 transferrin, 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[63] 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 160,000 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 ferritin uptake by erythroid cells has not been not characterized, receptor-mediated endocytosis might be involved in this process. However, since a primary defect in the development of TfR knockout mice is a failure of erythropoiesis, it is likely that the transferrin-mediated pathway plays the primary role in iron delivery to the developing erythrocyte.[60]

In order for extracellular ferritin to carry out a physiological role, a cell surface receptor must be envisioned. Indeed, saturable binding of ferritin to a

variety of 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 CD+ 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 for 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.[60]

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 transferrin must be present.

A study[64,65] 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[66] 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 a 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.[60]

Although many studies[67,68] 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 ferritin 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.[60]

A study[69] 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 is no detectable Tf-TfR pathway for iron delivery in oligodendrocytes, ferritin-TIM2 was suggested to be the primary mechanism for iron uptake by these cells.

Recently a study[70] identified another cell surface receptor for ferritin, Scara5. Scara5 is a scavenger receptor that can bind various ligands. In contrast to TIM-2, which is a ferritin H receptor, Scara5 preferentially binds ferritin L.

Scara5 plays an important role in kidney organogenesis, presumably by delivering iron to cells. Identification of Scara5 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 unlabeled TfR1 wild type cells and $TfR^{-/-}$ cells tagged with green fluorescent protein, 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, Scara5. 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 transferrin-dependent and ferritin-dependent iron delivery, and to explore the role of Scara5 in the adult animal.[60]

A human ferritin receptor was recently identified. Using expression cloning, a study[70] 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 the 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.[60]

A study[71] 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 in the proteasome and iron release from ferritin before its degradation. It will be

interesting to identify pathways of iron trafficking following its release from ferritin.

Signaling molecule

Very recently, a study[72] proposed a new role for extracellular ferritin as a pro-inflammatory signaling 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 NFκB. Activation of NFkB in turn enhanced the expression of pro-inflammatory mediators, including interleukin 1 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 modulates 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.[60]

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[70] 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 ERk1/2 activation, while ferritin H knockdown enhanced ERK 1/2 activation.

The signaling 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 have been found in human TIM-1 and TIM-3 and these 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 at least 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.[60]

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 increased 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 tumors. Still's disease and hemophagocytic syndrome represent two clinical entities in which serum ferritin elevations are particularly remarkable.[60]

Angiogenesis

The search 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 byproduct of HK cleavage, HKa, is anti-angiogenic.[60]

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 tumor 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.[60]

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.[60]

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 tumor model where HKa reduces tumor blood vessel growth, the addition of ferritin counteracts the effects of HKa, leading to significantly increased intratumor blood vessel density.[60]

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 tumor growth.[60]

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(α_2M), anti-ferritin autoantibody, and fibrinogen. By binding to apolipoprotein B, ferritin posttranslationally inhibits its secretion. α_2M is a large plasma protein that can bind many ligands and remove them from blood circulation by α_2M receptor mediated endocytosis. The identification of α_2M 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.[60]

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.[60]

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, remain 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 signaling 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.[60]

Recent literature on serum ferritin and metabolic syndrome

A cross-sectional study[17] on 6,044 adults >20 years of age who participated in the Third National Health and Nutrition Examination Survey from Baltimore, USA examined the relationship among iron stores, the metabolic syndrome, and insulin resistance. Metabolic syndrome was defined as the presence of at least three of the following: elevated blood pressure, low HDL cholesterol, elevated serum triglycerides, elevated plasma glucose, and abdominal obesity. Insulin resistance was estimated using homeostasis model assessment (for insulin resistance), fasting insulin, and triglyceride-to-HDL cholesterol ratio. After excluding individuals with likely hemochromatosis, mean serum ferritin values in premenopausal women, postmenopausal women, and men were 33.6, 93.4, and 139.9 microg/l, respectively. Metabolic syndrome was more common in those with the highest compared with the lowest levels of serum ferritin in premenopausal women (14.9 vs. 6.4%, P = 0.002), postmenopausal women (47.5 vs. 28.2%, P < 0.001), and men (27.3 vs. 13.8%, P < 0.001). Insulin resistance also increased across quartiles of serum ferritin for men and postmenopausal women and persisted after adjustment for age, race/ethnicity, C-reactive protein, smoking, alcohol intake, and BMI. Study concluded that, elevated iron stores were positively associated with the prevalence of the metabolic syndrome and with insulin resistance.

Another a cross-sectional study[73] of 1,444 adults over age 40 and under age 70 that lived in a rural area and participated in a survey conducted as part of the Korean Rural Genomic Cohort Study (KRGCS) examined the relationship between serum ferritin and the metabolic syndrome (MS). The MS was defined as the presence of at least three of the followings: elevated blood pressure, low high density lipoprotein cholesterol, elevated serum triglycerides, elevated plasma glucose, or abdominal obesity. After adjustment for age, alcohol intake, menopausal status, body mass index (BMI), high sensitivity C-reactive protein (hs-CRP), and alanine aminotransferase (ALT), odds ratios (ORs) for the prevalence of the MS by sex were calculated for quartiles of serum ferritin using logistic regression analysis. The MS was more common in those persons with the highest levels of serum ferritin, compared to persons with the lowest levels, in men (37.1% vs. 22.4%, p=0.006) and women (58.8% vs. 34.8, p<0.001). In both sexes, the greater the number of MS components presents, the greater the serum ferritin levels. After adjustment for age, alcohol intake, and menopausal status, the OR for metabolic syndrome, comparing the fourth quartile of ferritin with the first quartile, was 2.21 (95% confidence interval; CI=1.26-3.87; p-trend=0.024) in men and 2.10 (95% CI=1.40-3.17; p-trend=0.001) in women. However, after further adjustment for BMI, hs-CRP, and ALT, the ORs were statistically attenuated in both sexes. Study showed that, moderately elevated serum ferritin levels were not independently associated with the prevalence of the MS after adjusting for other risk factors. Further studies are needed to obtain evidence concerning the association between serum ferritin levels and the MS.

A cross-sectional study[74] of 6311 adults older than 20 years who participated in the 2008 Korean National Health and Nutrition Examination Survey examined the association of serum ferritin levels with metabolic syndrome (MS) and diabetes mellitus in a representative sample of the adult South Korean population using data from the 2008 Korean National Health and Nutrition Examination Survey. Metabolic syndrome was defined as the presence of at least 3 of elevated blood pressure, low high-density lipoprotein cholesterol, elevated serum triglycerides, elevated plasma glucose, and abdominal obesity. In a representative sample of the adult Korean population, MS was more prevalent in the highest quartile compared with the lowest quartile of serum ferritin concentrations in women following adjustments for age, education, smoking, alcohol intake, body mass index, aspartate aminotransferase, and alanine aminotransferase. Diabetes mellitus was more prevalent in the highest quartile compared with the lowest quartile of serum ferritin concentrations in premenopausal women and men. The geometric means of fasting insulin and insulin resistance determined using the homeostasis model assessment of insulin resistance in the fourth serum ferritin quartiles of postmenopausal women and men were significantly higher compared with those in the first quartile of the respective groups. This study demonstrated that, elevated serum ferritin concentrations are associated with an increased risk of MS and diabetes mellitus in a representative sample of the adult South Korean population.

In a cross-sectional study,[75] serum ferritin level and metabolic syndrome and its components were measured from 18,581 men from January to December in 2008 to investigate the relationship between serum ferritin level and metabolic

syndrome and its components in healthy Korean men. The presence of metabolic syndrome was determined according to the most recent consensus report of the National Cholesterol Education Program's Third Adult Treatment Panel. Logistic regression models were applied to examine the relationship between the serum ferritin level and the metabolic syndrome and its components. After adjusting for clinical covariates, the odds ratio and 95% confidence intervals of metabolic syndrome with respect to Q2, Q3 and Q4 were 1.34 (1.14-1.57), 1.49 (1.24-1.70) and 1.99 (1.70-2.33), respectively (p for trend < 0.001). The multivariable-adjusted model also showed a significantly graded relationship between individual components of metabolic syndrome and the quartile groups of serum ferritin. In this study, elevated ferritin concentration was independently associated with metabolic syndrome and its components among healthy Korean men.

A study[76] reported that, ferritin, a marker of total body iron stores, is known to be associated with the risk of having metabolic syndrome and has been demonstrated to increase after the onset of menopause. Postmenopause status is an important determinant of metabolic syndrome.

A cross-sectional study[76] aimed to perform a menopause status-specific analysis of the association between ferritin levels and metabolic syndrome included 3,082 participants (1,691 premenopausal women and 1,391 postmenopausal women), all of whom were enrolled in the Korean National Health and Nutrition Examination Survey 2007. Premenopausal and postmenopausal women with metabolic syndrome had higher ferritin levels than did those without metabolic syndrome. After adjustments for age; body mass

index; alcohol intake; smoking history; exercise; hormone therapy use; hemoglobin, aspartate aminotransferase, and alanine aminotransferase levels; and intake of energy and iron, multivariate logistic regression analysis revealed that postmenopausal women with ferritin levels in the third tertile had an increased risk of having metabolic syndrome (odds ratio, 1.62; 95% CI, 1.04-2.81) compared with postmenopausal women with levels in the first quartile. No such association was detected in premenopausal women. Study concluded that, increased ferritin levels may be a determinant for metabolic syndrome in postmenopausal women but not in premenopausal women.

Chapter 4

Methodology

METHODOLOGY

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on patients with metabolic syndrome during the period of January 2011 to December 2011.

Study design

The study design was one year cross sectional study.

Study period and duration

The present one year study was conducted during the period of January 2011 to December 2011.

Method of collection of data

Source of Data

Patients with metabolic syndrome admitted in the wards of Medicine Department or attending the Medicine at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum were studied.

Sample size

A total of 100 patients with metabolic syndrome were selected for the study.

Sampling procedure

The sample size was calculated based on the formula as mentioned below.

$n = 4 X p X q / d^2$

Where p = Prevalence (Prevalence of the disease which was taken as 50% as no records were available regarding the study).

q = 100 – p

d = Absolute error taken as 10%

$n = 4 X 50 X 50 / 10^2$

n = 100

Considering the above variable the sample size was calculated as 100 patients.

Selection criteria

Inclusion Criteria

- Patients with metabolic syndrome diagnosed as per National Cholesterol Education Program Adult Treatment Panel III (2001) requires at least three of the following:
    - *Central obesity*: waist circumference ≥ 102 cm or 40 inches (male), ≥ 88 cm or 36 inches (female)
    - *Dyslipidaemia:* TG ≥ 1.695 mmol/L (150 mg/dL)
    - *Dyslipidaemia:* HDL-C < 40 mg/dL (male), < 50 mg/dL (female)
    - *Blood pressure:* ≥ 130/85 mm Hg
    - *Fasting plasma glucose:* ≥ 6.1 mmol/L (110 mg/dL)

Exclusion Criteria

- Anemic or who received treatment for anemia in last three months.
- Patients with hemolytic anemia.
- Patients who donated blood in last four months.
- Positive inflammatory markers (WBC>11,000/mm^3 or WBC<3000/mm^3).

Ethical clearance

Prior to the commencement, the study was approved by the Ethical and Research Committee, Jawaharlal Nehru Medical College, Belgaum.

Informed Consent

All the patients fulfilling selection criteria were explained about the purpose of study and a written informed consent was obtained to participate in the study before enrollment (Annexure I).

Method of collection of data

Before the enrollment, demographic data such as age, sex, occupation, history regarding hypertension, diabetes mellitus and complications, cerebrovscular events viz, angina pain, myocardial infarction, ischemic disease were recorded. A thorough physical examination such as anthropometry, vitals and systemic examination was conducted. These findings were recorded on a predesigned and pretested proforma (Annexure II).

Procedure

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 in the range of less than 18.5 kg/m^2 were considered as underweight, 18.5 to 24.9 kg/m^2 were considered as normal, 25.0 to 29.9 kg/m^2 were considered as overweight and more than 30 kg/m^2 were considered as obese.[77-79]

The waist circumference was measured using a standard measuring tape in Cms. Waist circumference of ≥ 102 cm or 40 inches (male), ≥ 88 cm or 36 inches (female) was considered as abnormal.

Investigations such as fasting blood sugar and lipid profile (total cholesterol, triglycerides, HDL, LDL) levels were done and the findings were recorded. Further these findings were interpreted according to NCEP ATP III criteria.[38]

Estimation of serum ferritin levels

Serum ferritin was measured on fasting blood samples using radio-immunoassay.[80] The serum ferritin levels were interpreted as below;

Males

- 18 to 30 years – 18.7 to 323 ng/mL
- 31 to 60 years – 16.4 to 294 ng/mL

Females

- Premenopaual – 6.9 to 282.5 ng/mL
- Post menopausal – 14.0 to 233 ng/mL

Statistical analysis

The data obtained was coded and entered into Microsoft Excel Worksheet (Annexure III). The categorical data was expressed as rates, ratios and proportions and comparison was done using chi-square test. The continuous data was expressed as mean ± standard deviation (SD) and comparison was done by student 't' test. A probability value ('p' value) of less than or equal to 0.05 was considered as statistically significant.

Chapter 5

Results

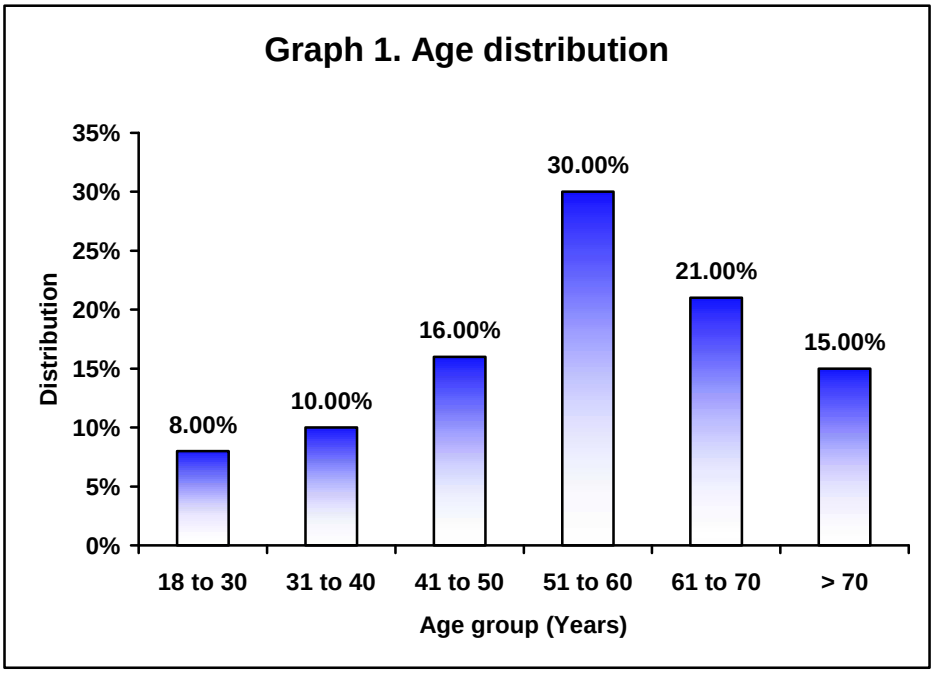
RESULTS

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the study period from January 2011 to December 2011. Based on NCEP ATP III criteria, hundred (100) cases were studied, and the findings obtained are tabulated as below.

`

Table 1. Age distribution

Age group (Years)	Patients (n=100)	
	Number	**Percentage**
18 to 30	8	8.00
31 to 40	10	10.00
41 to 50	16	16.00
51 to 60	30	30.00
61 to 70	21	21.00
> 70	15	15.00
Total	**100**	**100.00**

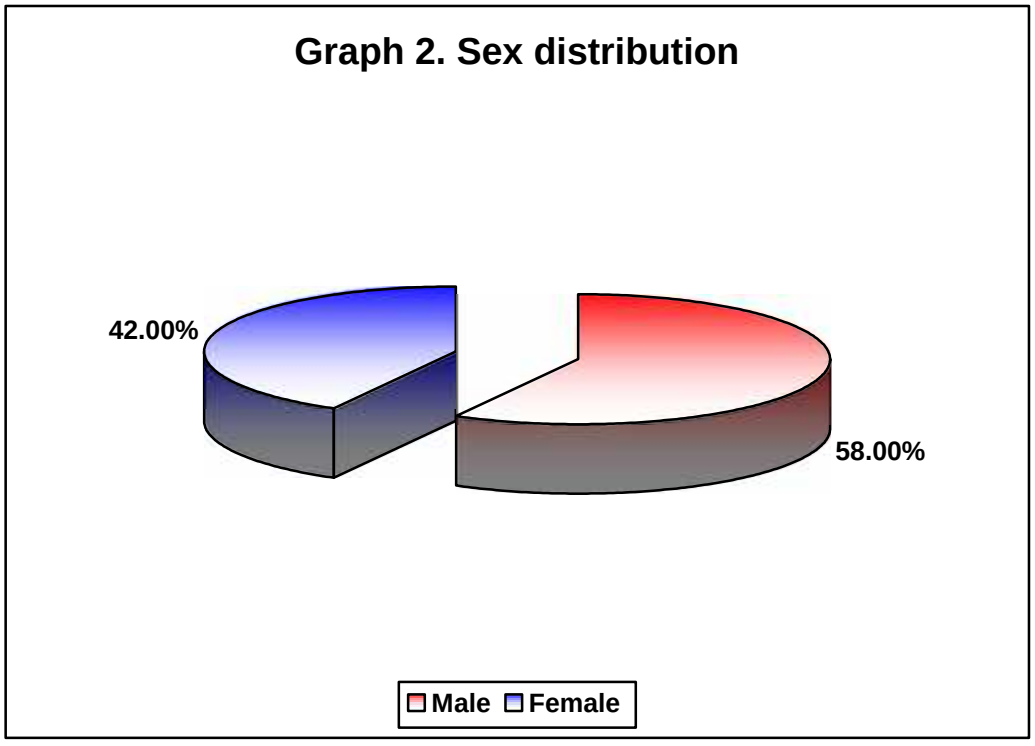


Patients age ranged from 18 to 70 years. Maximum number of cases were in the age group of 51 to 60 (30%) and 21 cases were in the age group of 61 to 70(21%). Average age at presentation was 55.84 ± 15.04 years.

Inference: Majority of the patients were in 5^{th} and 6^{th} decade.

Table 2. Sex distribution

Sex	Patients (n=100)	
	Number	**Percentage**
Male	58	58.00
Female	42	42.00
Total	**100**	**100.00**

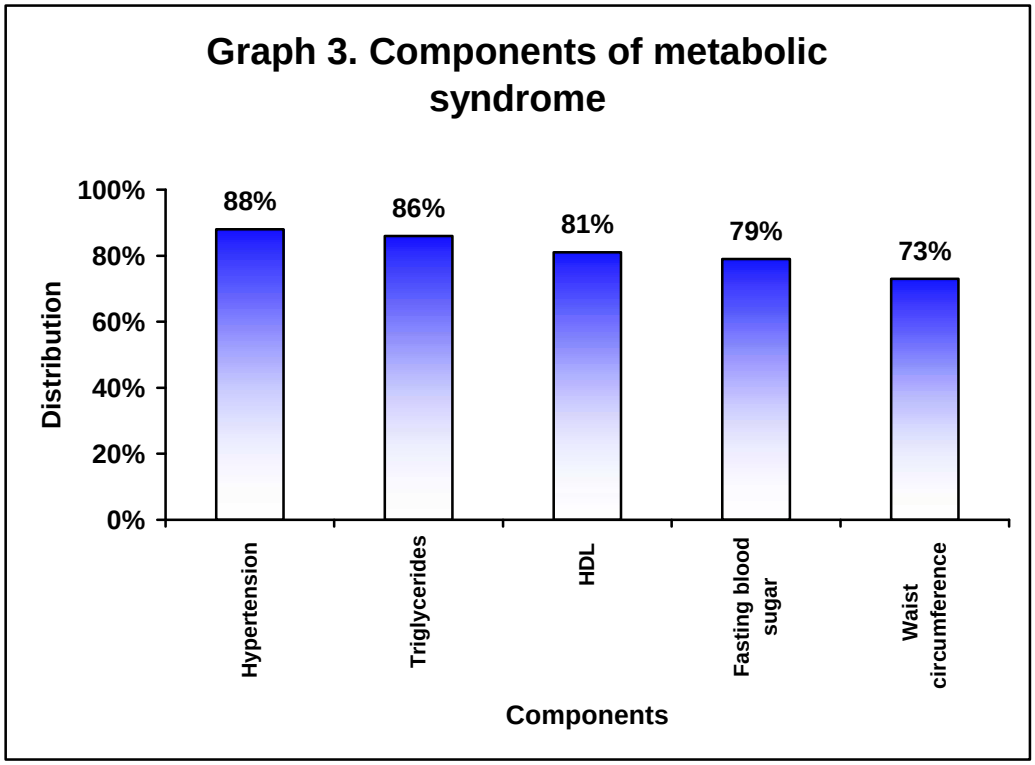


Out of 100 patients 58 (58%) were males and 42 patients (42%) were females, accounting a ratio of male to female was 1.38:1.

Inference: Male preponderance was noticed.

Table 3. Components of metabolic syndrome

Components	Patients (n=100)	
	Number	Percentage
Hypertension	88	88.00
Triglycerides	86	86.00
HDL	81	81.00
Fasting blood sugar	79	79.00
Waist circumference	73	73.00



The commonest component of metabolic syndrome was hypertension 88%, followed by triglyceridemia 86%, low HDL 81%, fasting blood sugar 79%,waist circumference 73%.

Inference: Majority of the patients were having hypertension, triglyceridemia and low HDL levels

Table 4. Duration of hypertension

Duration (Years)	Patients (n=83)	
	Number	**Percentage**
Upto 5	22	26.50
6 to 10	34	40.96
11 to 15	15	18.07
> 15	12	14.45
Total	**83**	**100.00**

34 (40.96%) patients had duration of hypertension between 6 to 10 years. overall mean duration of hypertension was 9.68 ± 8.52 years.

Table 5. Duration of Type 2 diabetes

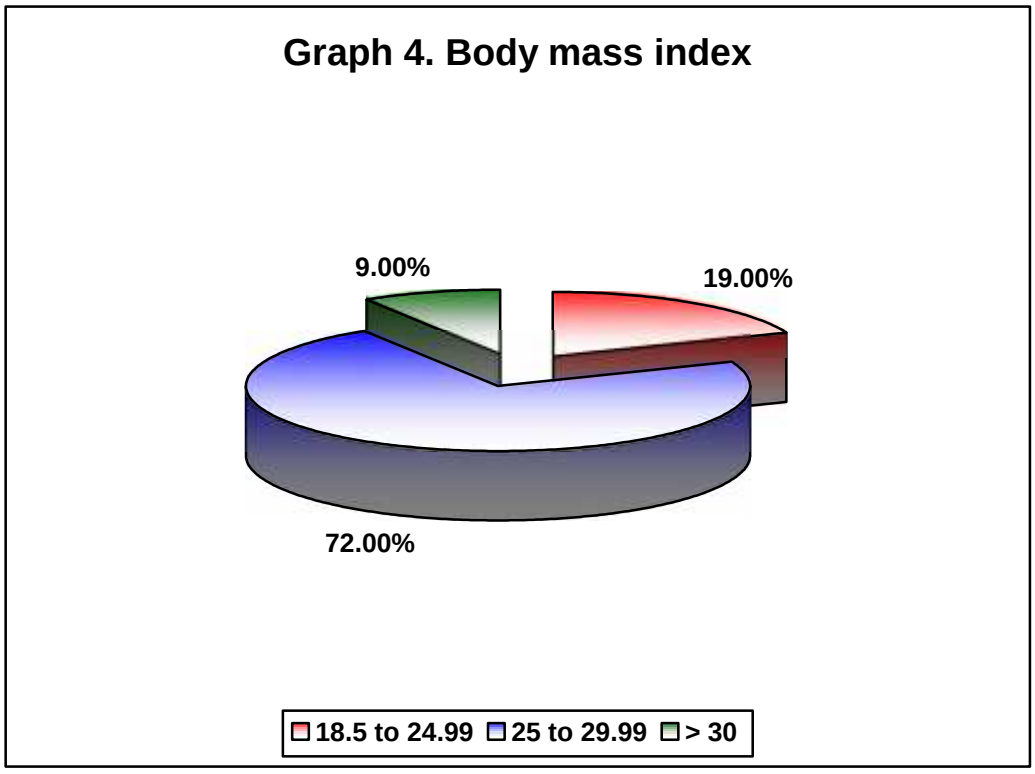
Duration (Years)	patients (n=60)	
	Number	**Percentage**
Upto 5	13	21.67
6 to 10	30	50.00
11 to 15	9	15.00
> 15	8	13.33
Total	**60**	**100.00**

30 patients had duration of type 2 diabetes mellitus between 6 to 10 years.overall mean duration of diabetes was 9.52 ± 9.52 years.

Individual components metabolic syndrome

Table 6. Body mass index

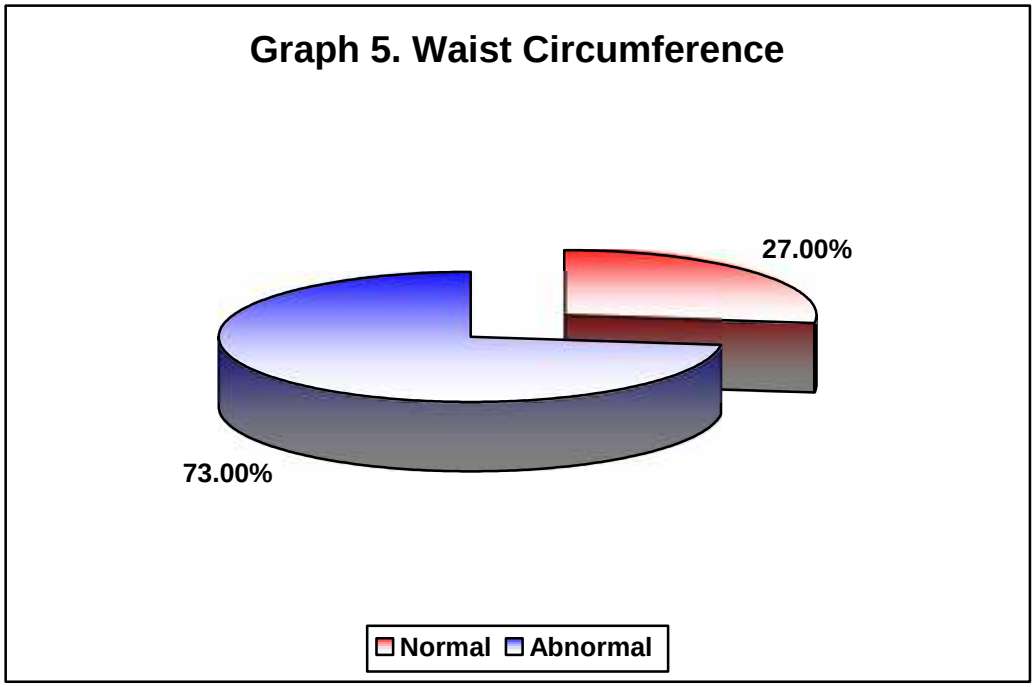
Body mass index (Kg/m^2)	patients(n=100)	
	Number	**Percentage**
18.5 - 24.99	19	19.00
25 - 29.99	72	72.00
> 30	9	9.00
Total	**100**	**100.00**



Majority of patients with BMI between 25 to 29.99 Kg/m^2 were observed 72% followed by 19% between 18.5 to 24.99 Kg/m^2

Table 7. Waist Circumference

Waist circumference	patients (n=100)	
	Number	**Percentage**
Normal	27	27.00
Abnormal	73	73.00
Total	**100**	**100.00**

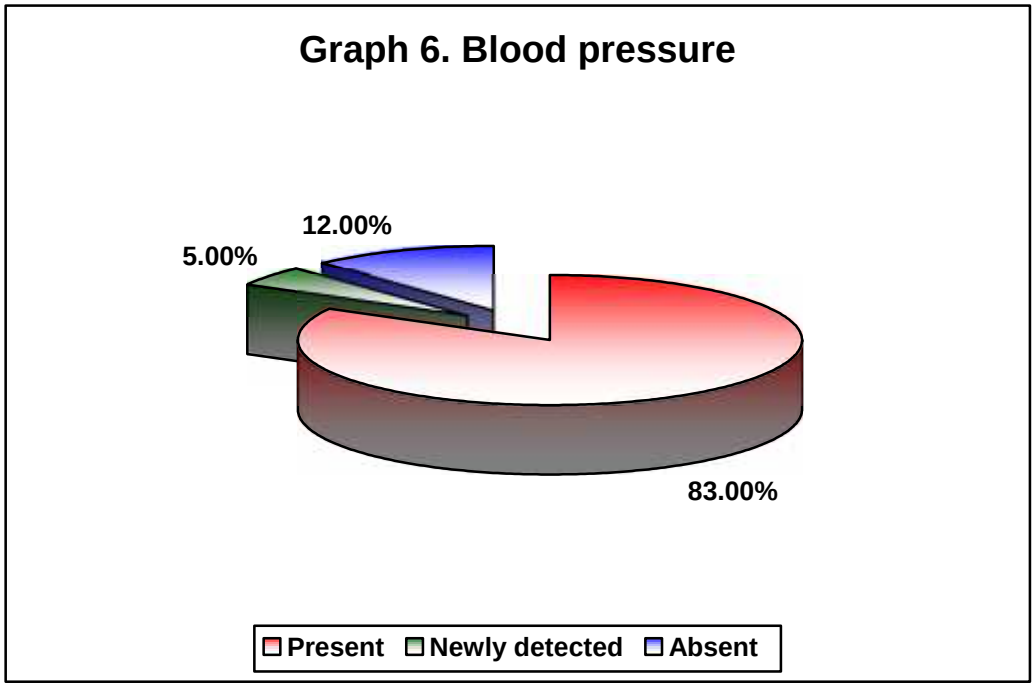


Majority of the patients had waist circumference abnormality 73% and was normal in 27%.

Observations of individual metabolic components

Table 8. Blood pressure

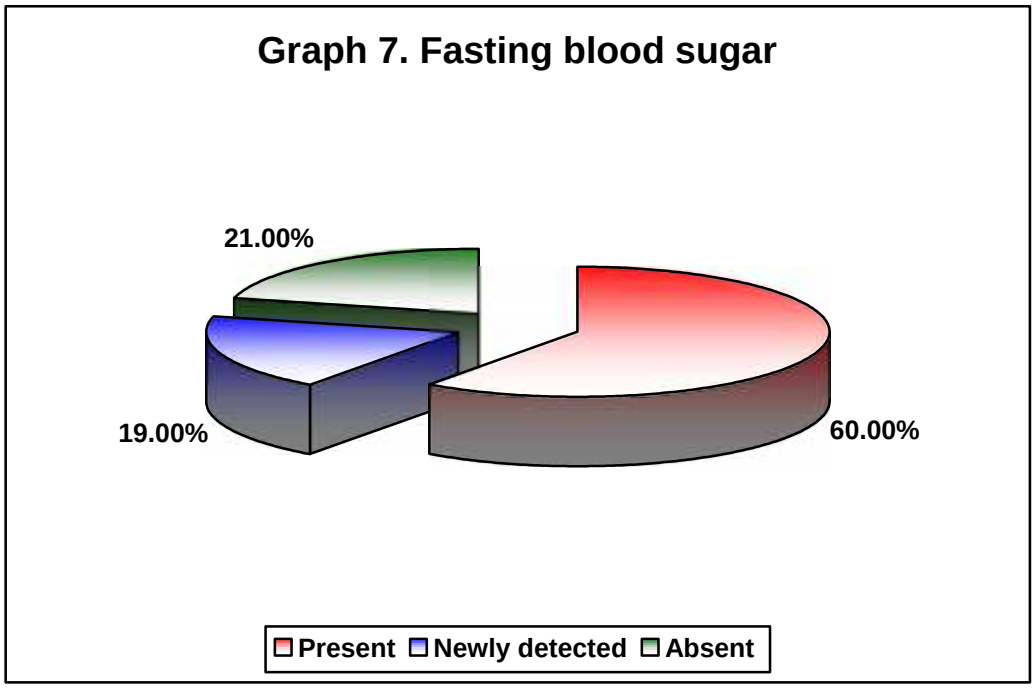
Hypertension	patients (n=100)	
	Mean	**SD**
Present (Presented with HTN)	83	83.00
Newly detected	5	5.00
Absent	12	12.00
Total	**100**	**100**



83% of the patients were known hypertensive, 5% were newly detected, 12% of the patients were with normal blood pressure.

Table 9. Fasting blood sugar

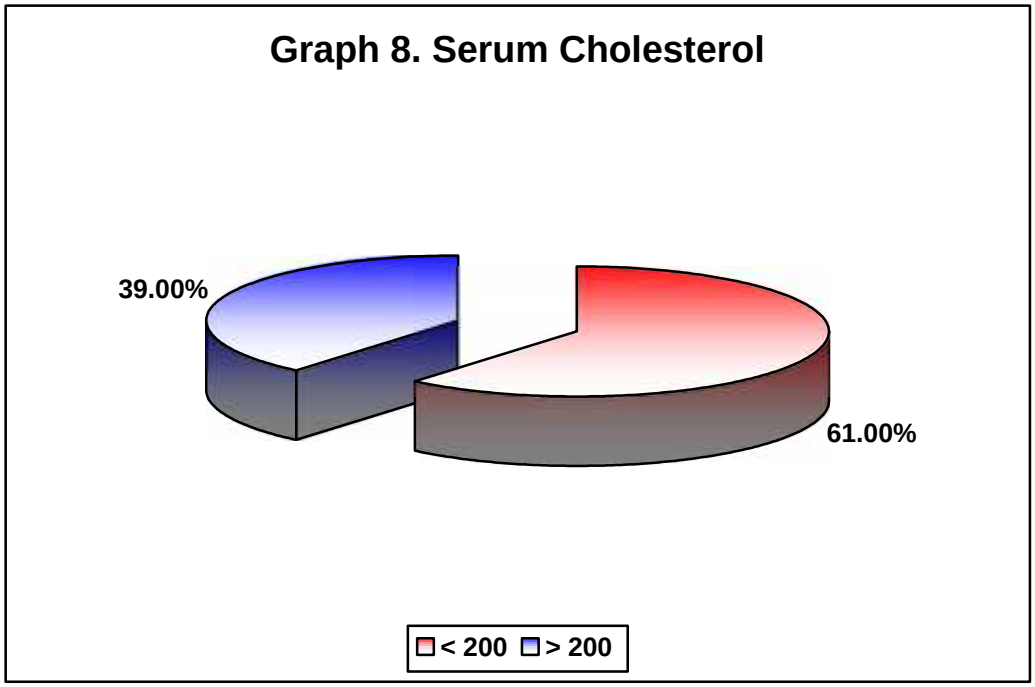
Fasting blood sugar (mg/dL)	patients (n=100)	
	Number	**Percentage**
Present (Presented with DM)	60	60.00
Newly detected	19	19.00
Absent	21	21.00
Total	**100**	**100**



60% of the patients were known diabetic, 19% of patients were having FBS abnormality, 21% had no FBS abnormality.

Table 10. Serum Cholesterol

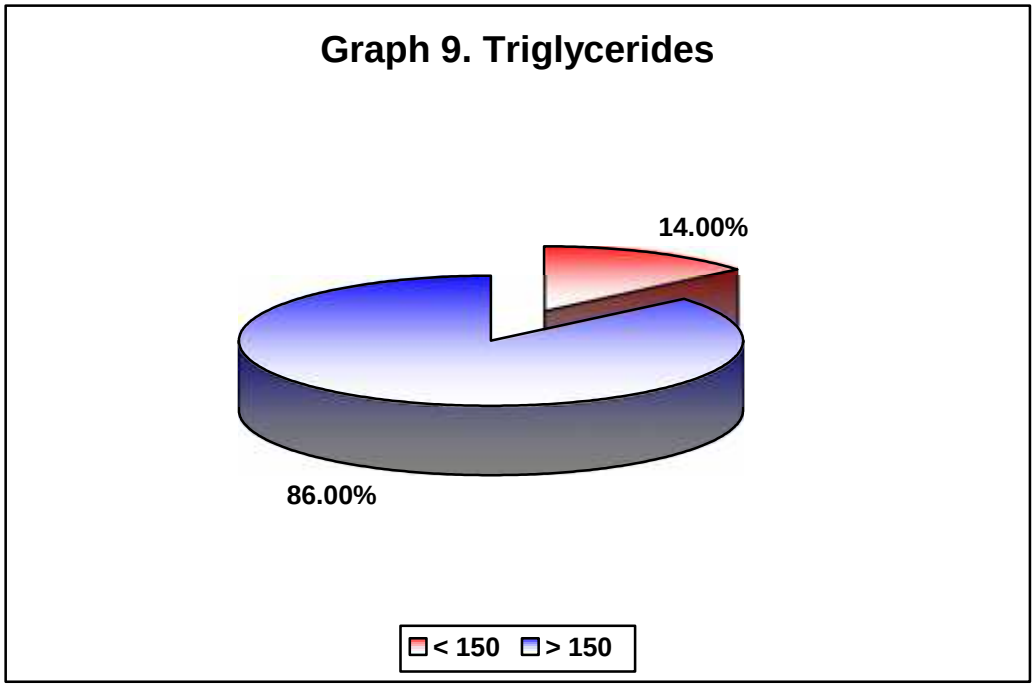
Serum cholesterol levels (mg/dL)	patients (n=100)	
	Number	**Percentage**
< 200	61	61.00
> 200	39	39.00
Total	**100**	**100.00**



39% of patients had serum cholesterol levels > 200 mg/dl and 61% had <200 mg/dl.

Table 11. Triglycerides

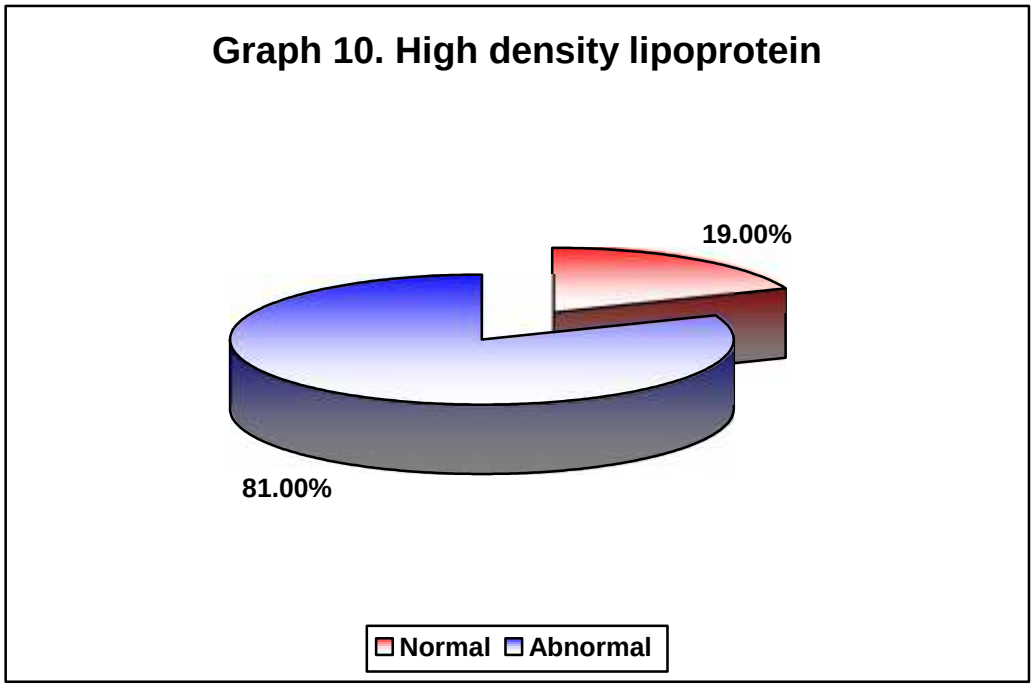
Triglyceride levels (mg/dL)	patients (n=100)	
	Number	**Percentage**
< 150	14	14.00
≥ 150	86	86.00
Total	**100**	**100.00**



Majority of patients 86% had triglycerides equal or > 150 mg/dl, 14% had <150 mg/dl.

Table 12. High density lipoprotein

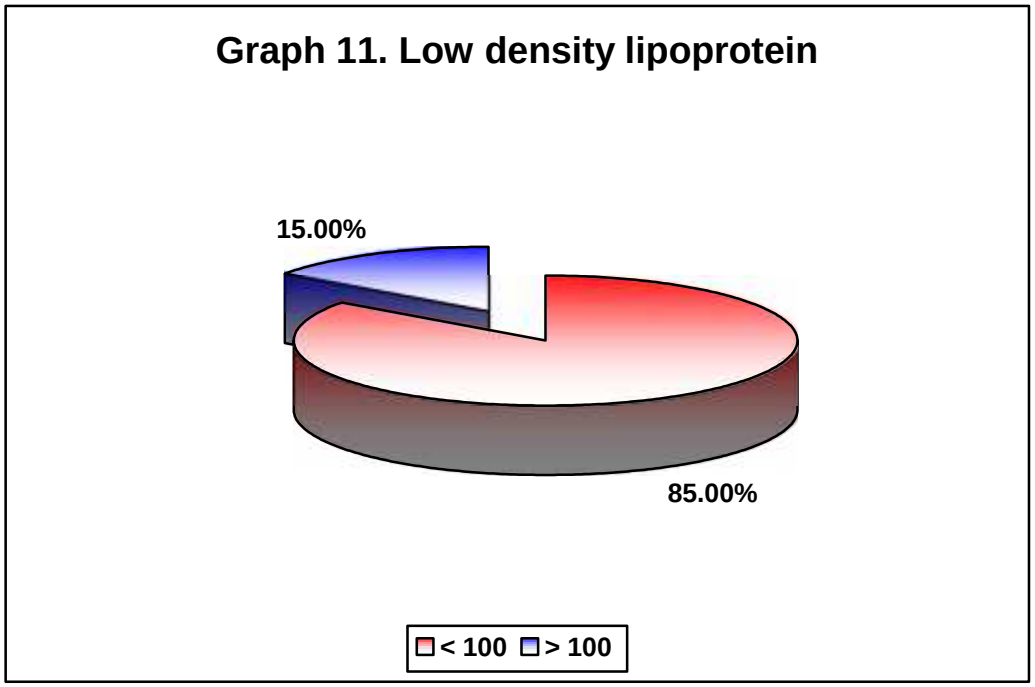
HDL Levels (mg/dL)	patients(n=100)	
	Number	**Percentage**
Normal	19	19.00
Abnormal	81	81.00
Total	**100**	**100.00**



81% of the patients had HDL abnormality and in 19% it was normal.

Table 13. Low density lipoprotein

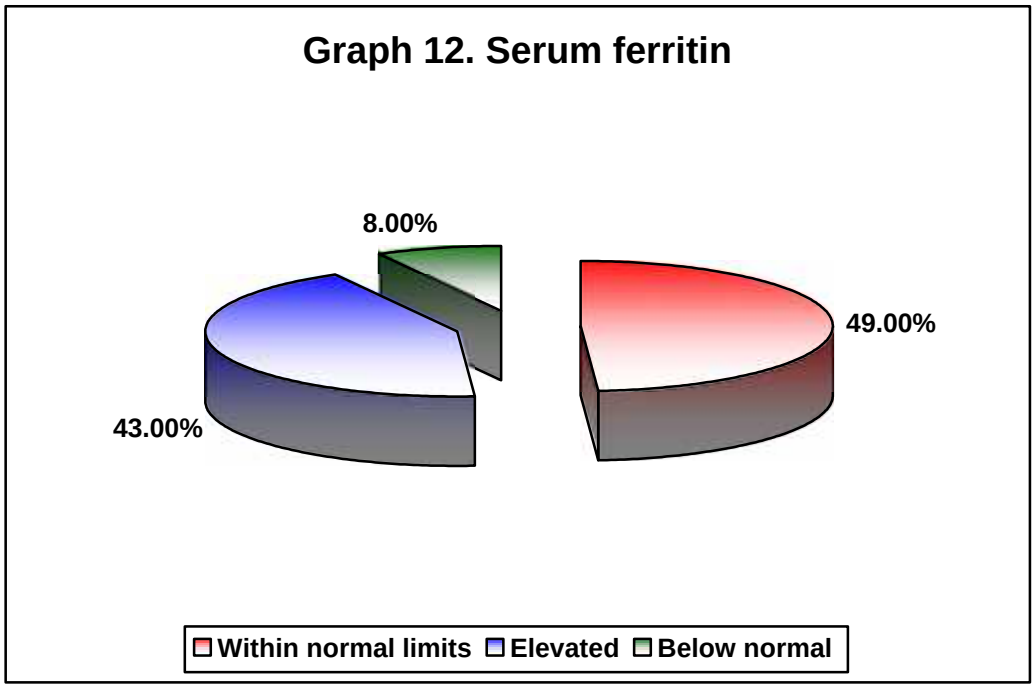
Low density lipoprotein (mg/dL)	Patients (n=100)	
	Number	Percentage
< 100	85	85.00
> 100	15	15.00
Total	**100**	**100.00**



In majority of patients 85% LDL was below 100mg/dl and in only 15% it was >100 mg/dl.

Table 14. Serum ferritin

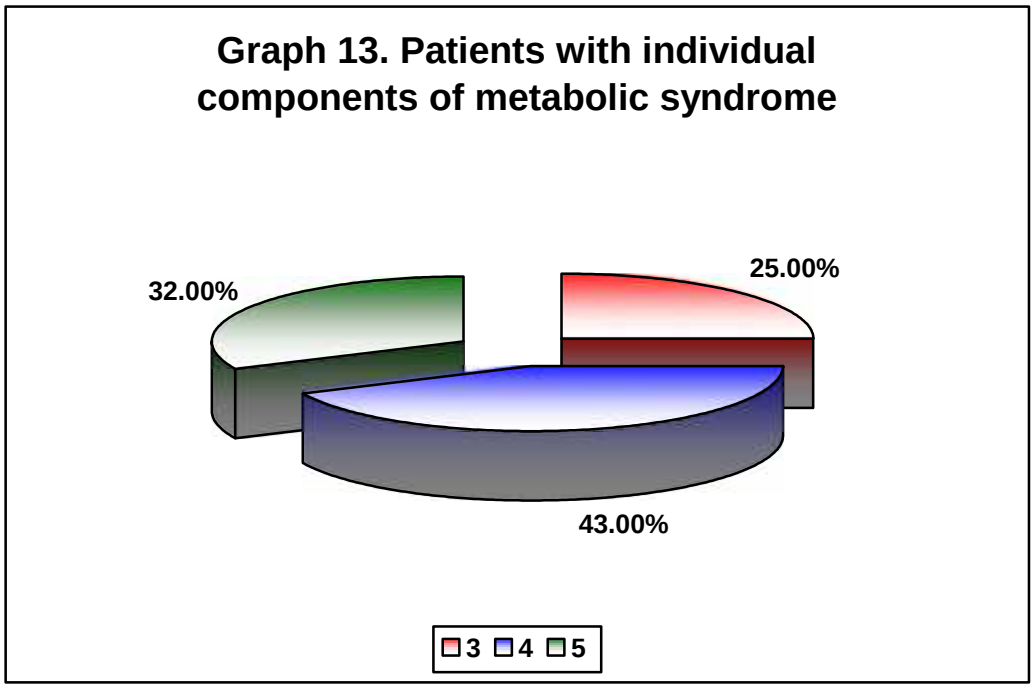
Serum Ferritin levels (ng/mL)	patients (n=100)	
	Number	**Percentage**
Within normal limits	49	49.00
Elevated	43	43.00
Below normal	8	8.00
Total	**100**	**100.00**



Serum ferritin was elevated in 43%, normal in 49% and below normal in 8%.

Table 15. Patients with individual components of metabolic syndrome

No of Components	Patients (n=100)	
	Number	Percentage%
3	25	25.00
4	43	43.00
5	32	32.00
Total	**100**	**100.00**



In the present study 43% patients had metabolic syndrome with four components and 32% had five components. However 25% of the patients were noted with three components of metabolic syndrome.

Table 16. Comparison of serum ferritin levels and metabolic syndrome components

No of Components	Serum ferritin levels						Total	
	Normal limits		Elevated		Below normal			
	No	%	No	%	No	%	No	%
3	9	36.00	14	56.00	2	8.00	25	100.00
4	20	46.51	20	46.51	3	6.98	43	100.00
5	20	62.50	9	28.13	3	9.38	32	100.00

x^2=4.991 **DF=4** **p=0.288**

Patients with 3 components of metabolic syndrome elevated serum ferritin abnormality was seen in 14 (56%), in 9 (36%) it was within normal limits and in only 2 (8%) it was below normal.

In patients with 4 components the elevated serum ferritin was seen in 20 (46.51%), normal in 20 (46.51%), and below normal in 3 (6.98%).

In patients of all 5components of metabolic syndrome elevated was seen in 9(28.13%), normal in 20(62.5%) and below normal in 3 (9.38%).

Inference: It was observed that in 43 patients serum ferritin levels were elevated, in 49 patients it was within normal limits and in 8 it was below normal.

Table 17. Comparison of other parameters with serum ferritin in individual components of metabolic syndrome

Components of metabolic syndrome		Serum ferritin levels						Total	
		Within normal limits		Elevated		Below normal			
		No.	%	No.	%	No.	%	No.	%
Waist Circumference	Normal	8	29.63	17	62.96	2	7.41	27	27.00
	Abnormal	41	56.16	26	35.62	6	8.22	73	73.00
		x^2=6.276; DF=2; p=0.043 (significant)							
Hypertension	Normal	12	100.00	0	0.00	0	0.00	12	12.00
	Abnormal	37	42.05	43	48.86	8	9.09	88	88.00
		x^2=14.192; DF=2; p=0.0008 (significant)							
Fasting blood Sugar	Normal	6	28.57	13	61.90	2	9.52	21	21.00
	Abnormal	43	54.43	30	37.97	6	7.59	79	79.00
		x^2=4.550; DF=2; p=0.102							
Triglycerides	Normal	7	50.00	5	35.71	2	14.29	14	14.00
	Abnormal	42	48.84	38	44.19	6	6.98	86	86.00
		x^2=1.008; DF=2; p=0.604							
HDL	Normal	5	26.32	13	68.42	1	5.26	19	19.00
	Abnormal	44	54.32	30	37.04	7	8.64	81	81.00
		x^2=6.208; DF=2; p=0.045(significant)							

Waist circumference: Patients with abnormal waist circumference 73 (73%) serum ferritin was elevated in 26 (35.6) normal in 41 (56.16%) below normal in 6 (8.2%).

Hypertension: Patients with hypertension 88 (88%), Serum ferritin was elevated in 43 (48.86%) normal in 37 (42.05%) below normal in 8 (9.0%).

Fasting blood sugar: Patients with abnormal FBS 79 (79%), 30 (37.9%) had elevated levels,43 (54.4%) had normal levels, 6 (7.59%) had below normal levels.

Triglcerides: Out of 86 (86%) patients of triglyceridemia 38 (44.19%) had elevated levels, 42 (48.8%) had normal levels, 6 (6.98%) had below normal.

Low HDL; In patients with HDL abnormalities 81(81%), 30 (37%) had elevated level, 44 (54.3%) had normal levels, 7 (8.6%) had below normal levels.

Table 18. Comparison of mean serum ferritin levels with metabolic syndrome components

No of Components	Males (n=58)		Females (n=42)		Overall (n=100)	
	Mean	SD	Mean	SD	Mean	SD
3	328.85	371.77	566.92	621.34	405.03	466.46
4	391.58	387.05	225.21	201.45	325.81	333.77
5	365.67	468.58	242.18	369.81	300.07	416.73

In patients with 3 components of metabolic syndrome, as well 4 components and in patients with all 5 components the mean serum ferritin levels were elevated in both males/females.

Table 19. Comparison of mean metabolic components with mean serum ferritin

No of Components	Mean serum ferritin levels (ng/mL)					
	normal limits (n=49)		Elevated (n=43)		Below normal (n=8)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
WC (Cms)	96.67	6.84	97.39	8.12	93.62	5.95
SBP (mm Hg)	140.57	16.87	146	10.98	147.75	8.71
DBP (mm Hg)	87.87	8.14	92.04	6.64	91	5.75
FBS (mg/dL)	150.48	122.00	131.62	40.72	125.12	27.52
TGA (mg/dL)	189.79	95.69	186.14	35.41	168.37	48.28
HDL (mg/dL)	41.95	10.85	39.9	12.04	37.75	6.73

Mean Systolic blood pressure, diastolic blood pressure, FBS, triglycerides, low HDL abnormality was seen in 43 patients with elevated mean serum ferritin levels.

Complication of individual component.

Table 20. Hypertension

Complications	Patients (n=100)	
	Number	**Percentage**
Angina pain	9	9.00
Cerebrovascular events	4	4.00

Complication of hypertension presenting as angina was observed in 9 (9%) and CVA 4 (4%).

Table 21. Diabetes mellitus

Diabetic complications	Patients (n=100)	
	Number	**Percentage**
Diabetic retinopathy	35	35.00
Diabetic nephropathy	2	2.00
Diabetic neuropathy	1	1.00

Complication of type 2 diabetes presenting as retinopathy 35 (35%), nephropathy 2 (2%), peripheral neuropathy 1 (1%).

Inference: Majority of patients had retinopathy as a complication of diabetes mellitus.

Chapter 6

Discussion

DISCUSSION

In the present study of 100 patients with metabolic syndrome we observed various abnormalities associated with different components were observed.

Patients with waist circumference (WC) abnormality 73 (73 %) as a component of metabolic syndrome elevated serum ferritin abnormality was seen in 26(35.62%), with significant p value 0.043.

Patient with hypertension 88 (88%), Serum ferritin was elevated in 43 (48.86%) with significant p value of 0.0008.

Patient with HDL abnormality 81 (81%), Serum ferritin was elevated in 30 (37.04%) with significant p value of 0.045.

In other components of metabolic syndrome that is FBS abnormality and Triglyceridemia we did not find reflection of serum ferritin levels,The p value in these both components was insignificant.

The most common age group in metabolic syndrome was 51 to 60 years (30%) and overall, the mean age was 55.84 ± 15.04 years.

The Indian study[81] it was observed, significantly higher rates of metabolic syndrome in older age groups. The incidence of metabolic syndrome would increase with increasing age 6.7% (in the age group of 20 -29 years) peaking to 65.6% (in the age group of 60-69 years)

In a study done in Australia, in 2005, the mean age for the cohort of patients with metabolic syndrome was 57.1 (55.3–58.9)[82]

In the present study, males out numbered females with male to female ratio of 1.38:1,with male preponderance of 58%.

In a study[82] done in Perth, (Australia) using four groups of subjects according to the presence of the individual components of the metabolic syndrome, 60.7% were males in the cohort of subjects with metabolic syndrome as diagnosed by the NCEP-ATP III criteria.

This is in sharp contrast with one study[81] done in Andhra Pradesh which showed metabolic syndrome rates are significantly higher among females with 52.2% (n=307) than in males at 34.2% (n=202).

This difference could be because of number of males and females patients studied (total of 509).

In the present study 83% of patients were known hypertensive, 5% were newly detected, remaining 12% were with normal blood pressure. The mean SBP was 143.48 ± 14.25 mm Hg and DBP was 89.92 ± 7.57 mm Hg.

In a study[83] done in the University of Oslo, among patients with metabolic syndrome, 40% had hypertension and 28% had previous cardiovascular disease (composite of coronary heart disease, cerebrovascular disease and peripheral vascular disease).

In the CURES-34 study[84] done in Chennai, India, 430 subjects diagnosed with metabolic syndrome by the ATPIII criteria had raised blood pressure, which was seen in 31.2% (males, 35.3% and females, 27.6%)

A study[81] of metabolic syndrome in Asian Indians, all the individual components of metabolic syndrome increased significantly with age, elevated blood pressure (63.1%) was the commonest abnormality observed.

In our study 60% of patients were known type 2 diabetes mellitus, 19% were newly detected. Out of 60% with known diabetes mellitus 50% had duration of diabetes mellitus ranged between 6 to 10 years. Overall, the mean duration of diabetes was 9.52 ± 9.52 years.

In a study[83] done in the University of Oslo, among patients with metabolic syndrome, 29% had diabetes, on the other hand, in a prevalence study of metabolic syndrome and its components in urban India, Diabetes was found in 82.01% of patients.

In the CURES-34 study[84] done in Chennai, India, of 430 subjects 18.3% diagnosed with metabolic syndrome by the ATPIII criteria had DM in 14.7% of individuals (males, 17.8% and females, 12.0%)

An Indian study[81] the high prevalence of Diabetes and Hypertension, is supported by the fact that Diabetes and Hypertension have been found to be more widely prevalent in South Asians, thus, conferring a large and very real risk of adverse cardiovascular events and other attending complications. The possible explanations for this inordinately high prevalence can be attributed to a high prevalence of obesity, insulin resistance and a body fat structure that favours the development of metabolic syndrome, Diabetes and Hypertension.[85,86]

In our study, 72% patients had BMI between 25 to 29.99 Kg/m^2 and 19% patients had more than 30 Kg/m^2. Overall the mean BMI was 27.18 ± 2.49 $Kg/m^{2.}$

In a study[87] done in Norway, IL 18 was correlated with cardiovascular events in metabolic syndrome patients, the mean BMI in patients with metabolic syndrome was found to be 28.4 kg/m^2 (26.6-30.6).

In the National health statistics report[88] of 2009, 29.8% of subjects were overweight and 65% of subjects were obese. In a study of urban Indian population, they found 79.01% of subjects with a BMI of > 24.9.

An Indian study[81] showed Metabolic syndrome and cardiovascular risk in Asian Indians/South Asians are also heightened by their relative increase in body fat mass, truncal subcutaneous fat mass, intra-abdominal fat mass, and also in ectopic fat deposition. Cardiovascular risk cluster also manifests at a lower level of adiposity and abdominal obesity 9 kg/m. South Asians also seem to have a peculiar body phenotype known as South Asian Phenotype, characterized by increased waist circumference, increased waist hip ratio, excessive body fat mass.

In the present study the waist circumference was abnormal in 73% of patients and 27% had normal waist circumference. The mean waist circumference was 96.78 ± 7.33 cms.

Another study[89] done in Taiwan according to the Third Report of the National Cholesterol Education Program Adult Treatment Panel III criteria, waist circumference abnormality was seen in 40% in 11411 adults.

This sharp contrast in our study and study reported by thaiwan group may be because of number of patients in study group.

Study done[90] in china showed Waist circumference (WC) and body mass index (BMI) were good markers for MetS, WC was a good marker for T2DM and dyslipidemia, and BMI was a good marker for hypertension. The optimal BMI cut-off value of MetS was 24 kg/m², and the optimal WC cut-offs were 86 cm and 78 cm in men and women, respectively.

Regional body fat distribution has an important influence on metabolic and cardiovascular risk factors. Increased abdominal (visceral) fat accumulation is a risk factor for coronary artery disease (CAD), dyslipidemia, hypertension, stroke, and type 2 diabetes.[91]

In our study lipid profile revealed raised serum cholesterol, Triglycerides, and low levels of HDL in patients of metabolic syndrome constituting a percentage of 39%86% and 81% respectively. LDL was elevated in 15%.

In a survey[88] done by the US Department of Health and Human services, Triglyceride levels and HDL levels were found to be abnormal in 31.4% and 24.7% respectively.

In another study[91] done in New Delhi, India, the age-adjusted HDL levels were found to be low in 64.91% of subjects.

In a study done on Asian-Indians (Chennai), abnormal TG and HDL levels were seen in 76% and >90% of the study population. Asian Indians were found not only to have low HDL, but also have a preponderance of small, dense,

dysfunctional HDL particles that are associated with less efficient reverse cholesterol transport and less protection against CAD.[83] In a sub-study of the Chennai rural epidemiology study, abnormal Triglycerides and HDL was found in 25.2% and 63.5% of patients.[84] However, in a study[92] done only on urban south Indian men, abnormal TG was seen in 45.2%, whereas abnormal HDL was seen in 70.3% of subjects.

In our study various complications of metabolic syndrome observed were angina pectoris 9 (9%), cerebrovascular accident 4 (4%).

A study[93] showed that, the proportion of subjects with the Metabolic Syndrome was very high (92.3%). At the baseline, 31.7% of subjects were coded positive for CVD, which was more prevalent in subjects with the Metabolic Syndrome (32.9 vs. 17.8%, p=0.005).

Metabolic syndrome is very common with ≈ 44% of the US population over 50 years of age meeting the NCEP criteria. hose with metabolic syndrome without diabetes had higher CHD prevalence (13.9%), and those with both metabolic syndrome and diabetes had the highest prevalence of CHD (19.2%).[94]

A study[95] in Finland showed coronary heart disease was more prevalent in MS patients Of 48%compared to non MS patients of 18%.

In our study retinopathy was observed in 35% followed by nephropathy in 2 % and peripheral neuropathy 1%.

A study[95] done in Finland showed retinopathy was commonly associated with MS 48% vs 41% in non MS patients, neuropathy in 16% of patients were as 6% was seen in non MS patients.

In the present study serum ferritin levels were normal among 49% patients and elevated serum ferritin levels were noted among 43% patients. The mean serum ferritin among males were 366.50±398.93 and in females it was 297.17±390.80 ng/mL.

In the present study, based on NCEP III criteria 43% of patients had four components of metabolic syndrome, and 32% had five components. And 25% of the patients were with three components of metabolic syndrome.

In patients with 3, 4 and all 5 components of metabolic syndrome in both males and females mean serum ferritin levels were elevated. But p value was insignificant in all these patients of metabolic syndrome.

Elevated serum ferritin levels were noted in 56% patients with three components, 46.51% with four components and 28.13% patients with five components. However, no statistically significant difference was observed between elevated serum ferritin levels and metabolic syndrome components.

The correlation of lipid profile with serum ferritin showed, significantly elevated serum ferritin levels in patients with abnormal waist circumference, hypertension and high density lipoprotein whereas no statistically significant difference was seen in patients with abnormal fasting blood sugar and triglycerides.

Patient with Waist circumference abnormality 73 (73 %) as a component of metabolic syndrome elevated serum ferritin abnormality was seen in 26(35.6%), with significant p value 0.043.

Patients with hypertension 88(88%), Serum ferritin was elevated in 43 (48.8%) with significant p value of 0.0008.

Patients with HDL abnormality 81 (81%), serum ferritin was elevated in 30 (37.04%) with significant p value of 0.045.

In other components of metabolic syndrome that is FBS abnormality and Triglyceride abnormality of serum ferritin levels did not show significant reflection as far as statistical p value was concerned.

Serum ferritin is a widely used marker of iron status in epidemiological studies[96] and accurately reflects differences in body iron stores by age and sex.[97] However, serum ferritin is an acute-phase reactant and may be artificially elevated in the presence of inflammation.[98] Several cross-sectional studies have previously examined the association between iron stores and individual metabolic cardiovascular risk factors, including hypertension,[21] dyslipidemia,[22,23] elevated fasting insulin and blood glucose,[24] and central adiposity .

It is unclear whether elevated iron stores may simply be another marker of insulin resistance or whether elevated iron stores may contribute to the pathogenesis of altered metabolic states. Iron is a transition metal capable of causing oxidative tissue damage by catalyzing the formation of free radicals.[29]

Iron overload may contribute to insulin resistance through mechanisms related to both reduced extraction of insulin and impaired insulin secretion.[99]

In a US study[17] mean reported moderately elevated iron levels were associated with an increased prevalence of metabolic syndrome and marker of insulin resistance. These associations were evident at moderately elevated iron levels, high levels associated with hemochromatosis. Given the high prevalence of elevated iron stores, especially in older ages, prospective studies are needed to determine whether moderately elevated iron stores precede the development of insulin resistance and contribute to the increased risk associated with it.

A cross-sectional study[17] on 6,044 adults >20 years of age who participated in the Third National Health and Nutrition Examination Survey from Baltimore, USA examined the relationship among iron stores, the metabolic syndrome, and insulin resistance. Study concluded that, elevated iron stores were positively associated with the prevalence of the metabolic syndrome and with insulin resistance.

Another a cross-sectional study[73] of 1,444 adults over the age of 40 and under the age of 70 years lived in rural area showed that, moderately elevated serum ferritin levels were not independently associated with the prevalence of the MS after adjusting for other risk factors.

Another cross-sectional study[74] of 6311 adults older than 20 years demonstrated that, elevated serum ferritin concentrations are associated with an increased risk of MS and diabetes mellitus in a representative sample of the adult South Korean population.

In a cross-sectional study,[75] serum ferritin levels and metabolic syndrome and its components studied in 18,581 men and it was observed that elevated ferritin concentrations was, independently associated with metabolic syndrome and its components among healthy Korean men.

We analysed the data both qualitatively and quantitatively to find out correlation of elevated serum ferritin with individual component of metabolic syndrome. We found significant correlation of serum ferritin levels with hypertension, waist circumference and low HDL levels (significant p value for hypertension 0.0008, waist circumference 0.043, low HDL levels 0.045).This may not be a true reflection because of small sample size and other confounding factors.

Studies with large sample size with proper adjustment of confounding variables may reflect significance of elevated serum ferritin in patients of metabolic syndrome or its individual components.

Chapter 7

Conclusion

CONCLUSION

Amongst the patient presenting with metabolic syndrome, serum ferritin was elevated in hypertension (48.86%), waist circumference (35.62%), low HDL levels (37.04%).

Metabolic syndrome was observed in elderly age groups than in younger age groups.

In individual components of metabolic syndrome hypertension was commonly seen followed by triglyceridemia and low HDL levels.

Serum ferritin as a marker of metabolic syndrome was inconclusive but was raised significantly in individual components of metabolic syndrome.

To know significance of elevated serum ferritin levels in patients of metabolic syndrome may be a large sample size with proper adjustment of confounding variables may reflect importance of serum ferritin levels.

Chapter 8

Summary

SUMMARY

The present study was conducted to analyse the serum ferritin as a marker of metabolic syndrome. This study was conducted on 100 patients age above 18 years admitted in medicine wards of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the period from January 2011 to December 2011.

Results of the present study showed serum ferritin was elevated significantly in individual components of metabolic syndrome (Hypertension, waist circumference, low HDL levels) though serum ferritin were elevated in patients of FBS and Triglyceride abnormality ,but it was of statistically insignificant.

Chapter 9

Bibliography

BIBLIOGRAPHY

1. Grundy SM. Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biol. 2008; 28(4): 629-36.

2. Hoang KC, Le TV, Wong ND. The metabolic syndrome in East Asians. J Cardiometab Syndr. 2007; 2(4): 276-82.

3. Hwang LC, Bai CH, Chen CJ. Prevalence of obesity and metabolic syndrome in Taiwan. J Formos Med Assoc. 2006; 105(8): 626-35.

4. Nestel P, Lyu R, Low LP, Sheu WH, Nitiyanant W, Saito I, et al. Metabolic syndrome: recent prevalence in East and Southeast Asian populations. Asia Pac J Clin Nutr. 2007; 16(2): 362-7.

5. Kohro T, Furui Y, Mitsutake N, Fujii R, Morita H, Oku S, et al. The Japanese national health screening and intervention program aimed at preventing worsening of the metabolic syndrome. Int Heart J. 2008; 49(2): 193-203.

6. Kolovou GD, Anagnostopoulou KK, Salpea KD, Mikhailidis DP. The prevalence of metabolic syndrome in various populations. Am J Med Sci. 2007; 333(6): 362-71.

7. Hu G, Lindstrom J, Jousilahti P, Peltonen M, Sjöberg L, Kaaja R, et al. The increasing prevalence of metabolic syndrome among Finnish men and women over a decade. J Clin Endocrinol Metab. 2008; 93(3): 832-6.

8. Erem C, Hacihasanoglu A, Deger O, Topbas M, Hosver I, Ersoz HO, et al. Prevalence of metabolic syndrome and associated risk factors among Turkish adults: Trabzon MetS study. Endocrine. 2008; 33(1): 9-20.

9. Mahadik SR, Deo SS, Mehtalia SD. Increased prevalence of metabolic syndrome in non-obese asian Indian-an urban-rural comparison. Metab Syndr Relat Disord 2007; 5(2): 142-52.

10. Mokan M, Galajda P, Pridavkova D, Tomásková V, Sutarík L, Krucinská L, et al. Prevalence of diabetes mellitus and metabolic syndrome in Slovakia. Diabetes Res Clin Pract. 2008; 81(2): 238-42.

11. Malik M, Razig SA. The prevalence of the metabolic syndrome among the multiethnic population of the United Arab Emirates: a report of a national survey. Metab Syndr Relat Disord. 2008; 6(3): 177-86.

12. Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol. 2007; 49(4): 403-14.

13. Hanley AJ, Karter AJ, Williams K, Festa A, D'Agostino RB, Wagenknecht LE et al. Prediction of type 2 diabetes mellitus with alternative definitions of the metabolic syndrome: the Insulin Resistance Atherosclerosis Study. Circulation. 2005; 112(24): 3713-21.

14. Giovannucci E. Metabolic syndrome, hyperinsulinemia, and colon cancer: a review. Am J Clin Nutr. 2007; 86(3): s836-42.

15. Reaven GM. Role of insulin resistance in human disease. Diabetes. 1988; 37: 1595-606.

16. Haffner S, Taegtmeyer H. Epidemic obesity and the metabolic syndrome. Circulation. 2003; 108: 1541-5.

17. Jehn M, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in U.S. adults. Diabetes Care. 2004;27(10):2422-8.

18. Fernández-Real JM, Ricart-Engel W, Arroyo E, Balançá R, Casamitjana-Abella R, Cabrero D, et al. Serum ferritin as a component of the insulin resistance syndrome. Diabetes Care. 1998;21(1):62-8.

19. Salonen JT, Tuomainen TP, Nyyssonen K, Lakka HM, Punnonen K: Relation between iron stores and non-insulin dependent diabetes in men: case-control study. BMJ 1998;317:727

20. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB: Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA 2004;291:711–7.

21. Piperno A, Trombini P, Gelosa M, Mauri V, Pecci V, Vergani A, et al. Increased serum ferritin is common in men with essential hypertension. J Hypertens 2002;20:1513–8.

22. Halle M, Konig D, Berg A, Keul J, Baumstark MW. Relationship of serum ferritin concentrations with metabolic cardiovascular risk factors in

men without evidence for coronary artery disease. Atherosclerosis 1997;128:235–40.

23. Williams MJ, Poulton R, Williams S. Relationship of serum ferritin with cardiovascular risk factors and inflammation in young men and women. Atherosclerosis 2002;165:179–84.

24. Tuomainen TP, Nyyssonen K, Salonen R, Tervahauta A, Korpela H, Lakka T, et al. Body iron stores are associated with serum insulin and blood glucose concentrations: population study in 1,013 eastern Finnish men. Diabetes Care 20:426–428, 1997.

25. Gillum RF. Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men: the Third National Health and Nutrition Examination Survey. Int J Obes Relat Metab Disord 2001;25:639–45.

26. Ferrannini E. Insulin resistance, iron, and the liver. Lancet 2000;355:2181–2.

27. Niederau C, Berger M, Stremmel W, Starke A, Strohmeyer G, Ebert R, et al. Hyperinsulinaemia in non-cirrhotic haemochromatosis: impaired hepatic insulin degradation? Diabetologia 1984;26:441–4.

28. Ford ES, Cogswell ME. Diabetes and serum ferritin concentration among U.S. adults. Diabetes Care 1999;22:1978–83.

29. Wolff SP. Diabetes mellitus and free radicals: free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull 1993;49:642–52.

30. Omran AR. The epidemiologic transition: a theory of the epidemiology of population change. Millbank Mem Fund Q 1971; 49: 509-38.

31. Non Communicable diseases – Country profiles 2011. Geneva: World Health Organization; 2011.

32. Vega GL. Obesity, the metabolic syndrome, and cardiovascular disease. Am Heart J 2001; 142: 1108-16.

33. Isomaa B, Almgren P, Tuomi T, Torsen B, Lahti K, Nissen M, et al. Cardiovascular Morbidity and Mortality Associated with the Metabolic Syndrome. Diabetes Care 2001; 24: 683-9.

34. Alberti KG, Zimmet PZ. For the WHO Consultation: Definition, diagnosis and classification of diabetes mellitus and its complications. Part I: Diagnosis and classification of diabetes mellitus, provisional report of a WHO Consultation. Diabet Med 1998; 15: 539-53.

35. Olufadi R, Byrne CD. Clinical and laboratory diagnosis of the metabolic syndrome. J Clin Pathol. Jun 2008; 61(6): 697-706.

36. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an

American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005; 112(17): 2735-52.

37. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). Diabet Med 1999; 16: 442-3.

38. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel) JAMA 2001; 285: 2486-97.

39. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med 2006; 23(5): 469-80.

40. Ford ES, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among u.s. Adults. Diabetes Care. 2004; 27(10): 2444-9.

41. Fauci AS, Kasper DS, Longo DL, Braunwald E, Hauser SL, Jameson JL, et al. Harrison's principles of internal medicine. United States; McGraw Hill: 2008

42. Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. Physiol Behav. 2008; 94(2): 206-18.

43. Gogia A. Agarwal PK. Metabolic syndrome. Indian J Med Sci 2006; 60 (2): 72-81.

44. Ramachandran A, Snehalatha C, Satyavani K, Sivasankari S, Vijay V. Metabolic syndrome in urban Asian Indian adults-a population study using modified ATP III criteria. Diabetes Res Clin Pract 2003; 60: 199-204.

45. Gupta A, Gupta R, Sarna M, Rastogi S, Gupta VP, Kothari K. Prevalence of diabetes, impaired fasting glucose and insulin resistance syndrome in an urban Indian population. Diab Res Clin Pract 2003; 61: 69-76.

46. Pekkanen J, Tuomilehto J, Uutela A, Vartiainen E, Nissinen A. Social class, health behaviours and mortality among men and women in eastern Finland. BMJ 1995; 311: 589-93.

47. Davey SG, Neaton JD, Wentworth D, Stamler J. Mortality differences between black and white men in USA: Contribution of income and other risk factors among men screened for the MRFIT. Lancet 1998; 351: 934-9.

48. Kaplan GA, Keil JE. Socioeconomic factors and cardiovascular disease: a review of the literature. Circulation 1993; 88: 1973-98.

49. De Ferranti SD, Osganian SK. Epidemiology of paediatric metabolic syndrome and type 2 diabetes mellitus. Diab Vasc Dis Res. 2007; 4(4): 285-96.

50. Zimmet P, Alberti KG, Kaufman F, F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents - an IDF consensus report. Pediatr Diabetes. 2007; 8(5): 299-306.

51. Morrison JA, Ford ES, Steinberger J. The pediatric metabolic syndrome. Minerva Med. 2008; 99(3): 269-87

52. Wilson PW. Estimating cardiovascular disease risk and the metabolic syndrome: a Framingham view. Endocrinol Metab Clin N Am 2004; 33: 467-81.

53. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005; 365: 1415-28.

54. Lann D, LeRoith D. Insulin resistance as the underlying cause for the metabolic syndrome. Med Clin North Am. 2007; 91(6): 1063-77, viii.

55. Després JP, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. Arterioscler Thromb Vasc Biol. 2008; 28(6): 1039-49.

56. Türkoglu C, Duman BS, Günay D, Cagatay P, Ozcan R, Büyükdevrim AS. Effect of abdominal obesity on insulin resistance and the components of the metabolic syndrome: evidence supporting obesity as the central feature. Obes Surg. 2003; 13(5): 699-705.

57. Zirlik A, Abdullah SM, Gerdes N, MacFarlane L, Schönbeck U, Khera A, et al. Interleukin-18, the metabolic syndrome, and subclinical atherosclerosis: results from the Dallas Heart Study. Arterioscler Thromb Vasc Biol. 2007; 27(9): 2043-9.

58. Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB Sr, Wilson PW. C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study. Circulation 2004; 110: 380–85.

59. Joy T, Lahiry P, Pollex RL, Hegele RA. Genetics of metabolic syndrome. Curr Diab Rep. 2008; 8(2): 141-8.

60. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: Past, present and future. Biochim Biophys Acta. 2010;1800(8):760-9.

61. Addison GM, Beamish MR, Hales CN, Hodgkins M, Jacobs A, Llewellin P. An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. J Clin Pathol 1972;25:326–9.

62. Jacobs A, Worwood M. Ferritin in serum. Clinical and biochemical implications. N Engl J Med 1975;292:951–6.

63. Sibille JC, Kondo H, Aisen P. Interactions between isolated hepatocytes and Kupffer cells in iron metabolism: a possible role for ferritin as an iron carrier protein. Hepatology 1988;8:296–301.

64. Hulet SW, Heyliger SO, Powers S, Connor JR. Oligodendrocyte progenitor cells internalize ferritin via clathrin-dependent receptor mediated endocytosis. J Neurosci Res 2000;61:52–60.

65. Fisher J, Devraj K, Ingram J, Slagle-Webb B, Madhankumar AB, Liu X, Klinger M, Simpson IA, Connor JR. Ferritin: a novel mechanism for delivery of iron to the brain and other organs. Am J Physiol Cell Physiol. 2007;293:C641–C649.

66. Kalgaonkar S, Lonnerdal B. Receptor-mediated uptake of ferritin-bound iron by human intestinal Caco-2 cells. J Nutr Biochem. 2009;20:304–311.

67. Chen TT, Li L, Chung DH, Allen CD, Torti SV, Torti FM, Cyster JG, Chen CY, Brodsky FM, Niemi EC, Nakamura MC, Seaman WE, Daws MR. TIM-2 is expressed on B cells and in liver and kidney and is a receptor for H-ferritin endocytosis. J Exp Med. 2005;202:955–965.

68. Chakravarti S, Sabatos CA, Xiao S, Illes Z, Cha EK, Sobel RA, Zheng XX, Strom TB, Kuchroo VK. Tim-2 regulates T helper type 2 responses and autoimmunity. J Exp Med. 2005;202:437–444.

69. Todorich B, Zhang X, Slagle-Webb B, Seaman WE, Connor JR. Tim-2 is the receptor for H-ferritin on oligodendrocytes. J Neurochem. 2008;107:1495–1505.

70. Li JY, Paragas N, Ned RM, Qiu A, Viltard M, Leete T, Drexler IR, Chen X, Sanna-Cherchi S, Mohammed F, Williams D, Lin CS, Schmidt-Ott KM, Andrews NC, Barasch J. Scara5 is a ferritin receptor mediating non-transferrin iron delivery. Dev Cell. 2009;16:35–46.

71. De Domenico I, Vaughn MB, Li L, Bagley D, Musci G, Ward DM, Kaplan J. Ferroportin-mediated mobilization of ferritin iron precedes ferritin degradation by the proteasome. Embo J. 2006;25:5396–5404.

72. Ruddell RG, Hoang-Le D, Barwood JM, Rutherford PS, Piva TJ, Watters DJ, Santambrogio P, Arosio P, Ramm GA. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. Hepatology. 2009;49:887–900.

73. Ryu SY, Kim KS, Park J, Kang MG, Han MA. Serum ferritin and risk of the metabolic syndrome in some Korean rural residents. J Prev Med Public Health 2008;41(2):115-20.

74. Lee BK, Kim Y, Kim YI. Association of serum ferritin with metabolic syndrome and diabetes mellitus in the South Korean general population according to the Korean National Health and Nutrition Examination Survey 2008. Metabolism 2011;60(10):1416-24

75. Ryoo JH, Kim MG, Lee DW, Shin JY. The relationship between serum ferritin and metabolic syndrome in healthy Korean men. Diabetes Metab Res Rev 2011;27(6):597-603.

76. Cho GJ, Shin JH, Yi KW, Park HT, Kim T, Hur JY, Kim SH. Serum ferritin levels are associated with metabolic syndrome in postmenopausal women but not in premenopausal women. Menopause 2011;18(10):1120-4.

77. Tsai PS, Ke TL, Huang CJ, Tsai JC, Chen PL, Wang SY, et al. Prevalence and determinants of prehypertension status in the Taiwanese general population. J Hypertens 2005; 23: 1355-60.

78. Grotto I, Grossman E, Huerta M, Sharabi Y. Prevalence of prehypertension and associated cardiovascular risk profiles among young Israeli adults. Hypertension 2006; 48: 254-9.

79. Choi KM, Park HS, Han JH, Lee JS, Lee J, Ryu OH, et al. Prevalence of prehypertension and hypertension in a Korean population: Korean National Health and Nutrition Survey 2001. J Hypertens 2006; 24: 1515-21.

80. Barnett MD, Gordon YB, Amess JA, Mollin DL. Measurement of ferritin in serum by radioimmunoassay. J Clin Pathol 1978;31:742-8.

81. Ravikiran M, Bhansali A, Ravikumar P, Bhansali S, Dutta P, Thakur JS, et al. Prevalence and risk factors of metabolic syndrome among Asian Indians: a community survey. Diabetes Res Clin Pract. 2010;89(2):181-8.

82. Hung J, McQuillan BM, Chapman CM, Thompson PL, Beilby JP. Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. Arteriosclerosis, Thrombosis, and Vascular Biology 2005; 25: 1268-73.

83. Enas EA, Mohan V, Deepa M, Farooq S, Pazhoor S, Chennikkara H. The metabolic syndrome and dyslipidemia among Asian Indians: a population

with high rates of diabetes and premature coronary artery disease. J Cardiometabolic Syndrome 2007; 2 (4): 267-75.

84. Deepa M, Farooq S, Datta M, Deepa R, Mohan V. Prevalence of metabolic syndrome using WHO, ATPIII and IDF definitions in Asian Indians: the Chennai Urban Rural Epidemiology Study (CURES-34) Diabetes Metab Res Rev. 2007; 23: 127-34.

85. Misra A, Vikram NK. Insulin resistance syndrome (metabolic syndrome) and Asian Indians. Curr Sci 2002; 83: 1483–96.

86. Yajnik CS, Yudkin JS. The Y-Y paradox. Lancet 2004; 363: 163.

87. Baldassarre D, Porta B, Camera M, Amato M, Arquati M, Brusoni B, et al. Markers of inflammation, thrombosis and endothelial activation correlate with carotid IMT regression in stable coronary disease after atorvastatin treatment. Nutr Metab Cardiovasc Dis 2009; 19: 481-90.

88. Ervin RB. Prevalence of Metabolic Syndrome Among Adults 20 Years of Age and Over, by Sex, Age, Race and Ethnicity, and Body Mass Index: United States, 2003–2006. National Health Statistics Reports No. 13. Hyattsville, MD: National Center for Health Statistics; 2009.

89. Hsieh MH, Lin WY, Chien HH, Chien LH, Huang CK, Yang JF, et al. Waist circumference, body mass index, serum uric acid, blood sugar, and triglyceride levels are important risk factors for abnormal liver function tests in the Taiwanese population. Kaohsiung J Med Sci. 2012;28(9):470-6.

90. Feng RN, Zhao C, Wang C, Niu YC, Li K, Guo FC, BMI is strongly associated with hypertension, and waist circumference is strongly associated with type 2 diabetes and dyslipidemia, in northern Chinese adults. J Epidemiol. 2012;22(4):317-23

91. Sawant A, Mankeshwar R, Shah S, Raghavan R, Dhongde G, Raje H, et al. Prevalence of Metabolic Syndrome in Urban India. Cholesterol 2011; 920983 doi:10.1155/2011/ 920983

92. Kaur P, Radhakrishnan E, Rao SR, Sankarasubbaiyan S, Rao TV, Gupte MD. The metabolic syndrome and associated risk factors in an urban industrial male population in South India. J Assoc Physicians India 2010; 58: 363-6, 371.

93. Bonora E, Targher G, Formentini G, Calcaterra F, Lombardi S, Marini F, et al. The Metabolic Syndrome is an independent predictor of cardiovascular disease in Type 2 diabetic subjects. Prospective data from the Verona Diabetes Complications Study. Diabet Med. 2004; 21(1):52-8.

94. A5 Alexander CM, Landsman PB, Teutsch SM, Haffner SM; Third National Health and Nutrition Examination Survey (NHANES III); National Cholesterol Education Program (NCEP). NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. Diabetes. 2003;52(5):1210-4.

95. Isomaa B, Henricsson M, Almgren P, Tuomi T, Taskinen MR, Groop L. The metabolic syndrome influences the risk of chronic complications in patients with type II diabetes. Diabetologia. 2001;44(9):1148-54.

96. Witte DL, Crosby WH, Edwards CQ, Fairbanks VF, Mitros FA: Practice guideline development task force of the College of American Pathologists: hereditary hemochromatosis. Clin Chim Acta 1996;245: 139–200,.

97. Zacharski L, Ornstein D, Woloshin S, Schwartz L: Association of age, sex, and race with body iron stores in adults: analysis of NHANES III data. Am Heart J 2000;140:98–104.

98. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.

99. Wilson JG, Lindquist JH, Grambow SC, Crook ED, Maher JF: Potential role of increased iron stores in diabetes. Am J Med Sci 2003;325:332–9.

Annexures

Annexure I

ANNEXURE I – CONSENT FORM

STUDY OF SERUM FERRITIN IN METABOLIC SYNDROME AND ITS COMPONENTS – A ONE YEAR CROSS SECTIONAL STUDY AT KLE UNIVERSITY, BELGAUM

Objective and purpose of the study

This research is intended to study the association of serum ferritin and metabolic syndrome. This study will be of great help to patients with metabolic syndrome and dyslipidemias and also predict their complications in the future.

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 samples for the necessary investigations.

Risk and Benefits

The only risk and possible discomfort you might get is while taking blood from my 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 I decide to take part I 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 sponsor may stop your participation in this study at any time. If you choose not to take part in the study, you will receive the standard treatment for patients with your condition.

Privacy and Confidentiality

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

Institution / Sponsor's policy

Does not apply to this research

Financial incentives for participation

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

Authorization to publish the results

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

If you have any questions about your rights as a participant, you may call Principal and Chairman, J.N.M.C Ethical Committee for Human Research phone number ****-*******.

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

Principal investigator : Dr. ****** *******

Guide : Dr. ****** *******

Consent Statement

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

Name of the Participant: _____Signature / Thumb print _____

Name of the Witness _____Signature / Thumb print _____

Investigator Name: _____Signature / Thumb print _____

Date: Place:

Annexures

Annexure II

ANNEXURE II – PROFORMA

Case No:

NAME: AGE/SEX:

IP No. ADDRESS:

OCCUPATION:

COMPLAINTS AT PRESENTATION:

HISTORY PERTAINING TO METABOLIC SYNDROME :

k/c/o Type 2 diabetes mellitus yes ☐ no ☐

If yes,

Duration of illness -

Details of treatment –

Past history of cerebrovascular events –

Past history of angina pain/ Myocardial infarction/ Ischaemic heart disease or on any specific medication.

H/o peripheral neuropathy - yes ☐ no ☐

Documented Diabetic retinopathy/surgical management of retinopathy in the past

Diabetic nephropathy yes ☐ no ☐

Diabetic foot yes ☐ no ☐

Autonomic neuropathy yes ☐ no ☐

K/c/o Hypertension or on specific anti-hypertensive medication and duration yes ☐ no ☐

H/o Peripheral vasucular disease/past h/o treatment for the same yes ☐ no ☐

K/c/o of dyslipidemia or on any specific medication yes ☐ no ☐

ON EXAMINATION :

- NUTRITION :

  HEIGHT :

  WEIGHT:

  BMI :

  WAIST CIRCUMFERENCE :

- BLOOD PRESSURE :

INVESTIGATIONS :

- FBS –
- TOTAL TRIGLYCERIDES –
- TOTAL HDL –
- TOTAL CHOLESTEROL-
- ESTIMATED LDL –
- SERUM FERRITIN -

ANNEXURE III - MASTER CHART

Serial Number	In Patient Number	Demography			History																					Clinical examination						Investigations					
					T2 DM			Cerebrovascular events	Cerebrovascular events				Peripheral neuropathy	Diabetic retinopathy		Diabetic nephropathy	Diabetic foot	Autonomic neuropathy	HTN			PVD		Dislipidemia							BP (mm Hg)							
		Sex	Age (Years)	Occupation	History	Duration (Years)	Treatment		Angina pain	MI	IHD	Treatment		History	Treatment				History	Duration (Years)	Treatment	History	Treatment	History	Treatment	Height (Cms)	Weight (Kg)	BMI (Kg/m2)	WC (Cms)	Systolic	Diastolic	FBS (mg/dL)	Total TG (mg/dL)	Total HDL (mg/dL)	Total CHL (mg/dL)	Estimated LDL (mg/dL)	Serum Ferritin (ng/mL)
1	413786	M	60	RT	Y	6	O	N	N	N	N	-	N	N	N	N	N	N	Y	4	T	N	-	N	-	166	60	21.77	84	140	100	186	226	32	139	62	90
2	414534	M	68	SR	Y	10	I	N	N	N	N	-	N	N	-	Y	N	N	Y	6	T	N	-	N	-	169	65	22.76	92	130	90	126	182	48	136	49	10
3	414279	F	58	LB	Y	10	I	Y	N	N	N	-	N	Y	-	N	N	N	Y	13	Y	N	-	N	-	167	80	28.69	96	140	90	146	180	30	148	78	0
4	414523	M	47	SR	Y	6	I	N	Y	N	N	-	N	N	N	N	N	N	N	-	-	N	-	N	-	165	63	23.14	102	140	90	113	136	36	139	79	480
5	414464	M	72	RT	Y	10	I	N	N	N	N	-	N	N	N	N	N	N	Y	8	T	N	-	N	-	168	79	27.99	106	120	70	295	245	36	169	88	1330
6	411869	M	60	BS	Y	0.2	O	N	N	N	N	-	N	N	N	N	N	N	Y	3	T	N	-	N	-	156	67	27.53	104	120	70	112	804	31	237	127	53
7	413196	F	89	HW	Y	15	I	N	N	N	N	-	N	Y	-	N	N	N	Y	16	T	N	-	N	-	160	62	24.22	94	140	90	130	120	48	135	63	84
8	412604	F	74	HW	Y	12	I	N	N	N	N	-	N	N	-	N	N	N	Y	2	T	N	-	N	-	166	65	23.59	90	120	80	125	115	48	168	97	79
9	414226	M	45	FR	Y	6	O	N	N	N	N	-	N	N	-	N	N	N	N	-	-	N	-	N	-	169	98	34.31	130	110	80	125	159	32	290	130	690
10	402639	F	65	HW	Y	12	O	N	N	N	N	-	N	Y	-	N	N	N	Y	14	T	N	-	N	-	166	72	26.13	89	160	80	136	264	34	190	58	232
11	465996	F	50	LB	Y	4	O	N	N	N	N	-	N	Y	-	N	N	N	Y	9	T	N	-	N	-	166	65	23.59	96	140	90	120	263	34	203	58	1500
12	467848	M	33	CL	Y	6	O	N	N	N	N	-	N	N	-	N	N	N	Y	4	T	N	-	N	-	178	88	27.77	103	150	90	136	146	18	165	36	1500
13	469539	M	25	SR	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	18	T	N	-	N	-	182	88	26.57	105	150	100	96	180	38	100	46	1500
14	470081	M	64	FR	Y	12	I	N	N	N	N	-	N	Y	-	N	N	N	N	-	-	N	-	N	-	160	82	32.03	102	120	80	144	214	31	110	36	561
15	469778	M	47	FR	Y	6	O	N	N	N	N	-	N	N	-	N	N	N	Y	2	T	N	-	N	-	168	76	26.93	96	140	100	119	223	48	151	62	431
16	469316	M	55	HW	Y	15	I	N	N	N	N	-	N	Y	BILNPDR	N	N	N	Y	8	T	N	-	N	-	158	92	36.85	92	150	90	176	202	24	150	54	563
17	469322	F	53	HW	Y	4	O	N	N	N	N	-	N	N	-	N	N	N	N	-	-	N	-	N	-	158	66	26.44	90	100	60	148	264	34	203	58	563
18	469792	M	37	FR	Y	4	O	N	N	N	N	-	N	Y	NPDR	N	N	N	Y	10	T	N	-	N	-	178	89	28.09	90	146	90	136	264	34	160	36	317
19	470144	M	52	LB	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	5	T	N	-	N	-	167	68	24.38	90	130	90	90	191	21	106	53	911
20	469358	M	46	FR	Y	9	O	N	N	N	N	-	N	Y	NPDR	N	N	N	Y	22	T	Y	T	N	-	180	90	27.78	98	150	100	138	196	46	115	67	8
21	467858	F	32	HW	Y	4	I	N	N	N	N	-	N	N	-	N	N	N	Y	6	T	N	-	N	-	160	68	26.56	84	140	90	190	154	52	160	36	301
22	450168	M	25	SR	Y	3	I	N	N	N	N	-	N	Y	-	N	N	N	N	-	-	N	-	N	-	180	90	27.78	104	120	80	176	153	35	150	90	58
23	450731	F	22	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	2	T	N	-	N	-	158	60	24.03	88	130	90	108	160	52	132	46	41
24	1896734	M	59	RT	Y	20	I	N	N	N	N	-	N	Y	-	N	N	N	Y	7	T	N	-	N	-	164	68	25.28	104	150	90	138	143	35	150	90	21
25	451307	F	59	HW	Y	9	O	N	N	N	N	-	N	Y	-	N	N	N	Y	8	T	N	-	N	-	168	70	24.80	87	160	90	118	156	39	100	51	6
26	1906813	F	50	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	3	T	N	-	N	-	170	84	29.07	90	140	90	145	155	52	190	64	194
27	451959	M	24	FR	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	6	T	N	-	N	-	180	79	24.38	104	156	90	90	192	26	200	80	2
28	461938	F	53	HW	Y	10	I	N	N	N	N	-	N	Y	-	N	N	N	Y	9	T	N	-	N	-	161	68	26.23	88	146	84	120	180	54	180	68	148

ANNEXURE III - MASTER CHART

Serial Number	In Patient Number	Demography			History																						Clinical examination						Investigations					
					T2 DM			Cerebrovascular events	Cerebrovascular events				Peripheral neuropathy	Diabetic retinopathy		Diabetic nephropathy	Diabetic foot	Autonomic neuropathy	HTN			PVD		Dislipidemia		Height (Cms)	Weight (Kg)	BMI (Kg/m2)	WC (Cms)	BP (mm Hg)		FBS (mg/dL)	Total TG (mg/dL)	Total HDL (mg/dL)	Total CHL (mg/dL)	Estimated LDL (mg/dL)	Serum Ferritin (ng/mL)
		Sex	Age (Years)	Occupation	History	Duration (Years)	Treatment		Angina pain	MI	IHD	Treatment		History	Treatment				History	Duration (Years)	Treatment	History	Treatment	History	Treatment					Systolic	Diastolic						
29	451816	F	46	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	1	T	N	-	N	-	158	66	26.44	94	130	90	120	233	30	170	90	348
30	452191	F	28	LB	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	9	T	N	-	N	-	165	68.5	25.16	89	150	80	78	251	44	202	105	220
31	411941	F	40	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	2	T	N	-	N	-	172	69	23.32	92	140	90	177	242	44	192	76	3
32	4044481	M	60	FR	Y	10	O	N	N	N	N	-	N	Y	-	N	N	N	Y	8	T	N	-	N	-	168	68	24.09	98	148	90	126	154	30	150	97	297
33	458816	M	60	BS	Y	6	O	N	N	N	N	-	N	N	-	N	N	N	Y	14	T	N	-	N	-	163	68	25.59	99	148	100	190	160	57	127	74	350
34	1482102	F	40	SR	Y	2	I	N	N	N	N	-	N	N	-	N	N	N	Y	4	T	N	-	N	-	177	88	28.09	96	156	80	110	75	41	124	68	2
35	1819307	M	58	BS	Y	8	I	N	N	N	N	-	N	Y	-	N	N	N	Y	9	T	N	-	N	-	160	74	28.91	101	146	98	128	140	39	124	64	259
36	409488	M	72	LB	Y	16	O	N	N	N	N	-	N	Y	-	N	N	N	Y	12	T	N	-	N	-	174	78	25.76	104	160	90	38	160	39	209	78	837
37	405589	F	68	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	5	T	N	-	N	-	164	64	23.80	92	140	100	89	208	44	221	120	249
38	1012171	M	64	LB	Y	16	O	N	N	N	N	-	N	Y	-	N	N	N	Y	7	T	N	-	N	-	176	80	25.83	103	160	80	180	200	30	164	64	258
39	753126	M	60	FR	Y	11	I	N	N	N	N	-	N	N	-	N	N	N	Y	12	T	N	-	N	-	174	78	25.76	98	160	110	120	155	52	90	70	1500
40	453070	M	52	BS	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	4	T	N	-	N	-	169	76	26.61	100	170	90	124	190	35	140	50	790
41	455053	F	30	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	20	T	N	-	N	-	161	69	26.59	92	148	100	112	180	34	184	76	58
42	402201	F	40	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	12	T	N	-	N	-	162	60	23.00	89	140	90	102	200	40	208	80	842
43	405254	F	93	FR	Y	10	I	N	N	N	N	-	N	N	-	N	N	N	Y	3	T	N	-	N	-	165	69	25.34	88	188	80	200	190	30	220	100	215
44	605851	F	40	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	6	T	N	-	N	-	166	68	24.68	88	140	92	118	140	38	200	84	4
45	456455	F	60	LB	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	2	T	N	-	N	-	160	67.5	26.37	87	140	100	92	190	30	220	110	758
46	485791	M	42	FR	Y	6	I	N	Y	N	N	-	Y	Y	-	N	N	N	N	-	-	N	-	N	-	175	78	25.62	102	160	80	155	210	90	225	90	502
47	461986	M	42	SR	Y	3	O	N	Y	N	N	-	N	N	-	N	N	N	N	-	-	N	-	N	-	159	68	26.90	103	140	90	156	154	60	196	56	89
48	462475	M	50	FR	N	-	-	N	N	N	N	-	N	N	-	N	N	N	N	-	-	N	-	N	-	176	79	25.50	104	120	70	86	168	38	200	84	117
49	461919	F	40	LB	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	6	T	N	-	N	-	168	70	24.80	99	140	110	138	166	88	155	55	19
50	460686	F	60	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	11	T	N	-	N	-	162	69	26.29	88	160	90	210	204	32	230	86	19
51	405981	F	60	RT	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	2	T	N	-	N	-	160	69	26.95	88	150	90	148	200	38	208	80	136
52	462901	M	44	FR	N	-	-	N	N	N	N	-	N	N	-	N	N	N	N	-	-	N	-	N	-	164	89	33.09	104	110	80	120	160	38	200	84	271
53	462611	M	62	FR	Y	9	I	N	N	N	N	-	N	Y	-	N	N	N	N	-	-	N	-	N	-	164	80	29.74	104	114	80	138	155	55	166	88	178
54	462056	M	44	FR	Y	10	O	N	N	N	N	-	N	Y	-	N	N	N	N	-	-	N	-	N	-	168	84	29.76	102	110	90	122	100	38	198	90	174
55	460956	M	65	BS	Y	6	O	N	N	N	N	-	N	Y	-	N	N	N	Y	9	T	N	-	N	-	170	88	30.45	104	140	90	140	155	56	244	66	80
56	4045589	M	65	SR	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	19	T	N	-	N	-	169	78	27.31	103	140	90	120	170	34	204	80	212

ANNEXURE III - MASTER CHART

Serial Number	In Patient Number	Demography			History																					Clinical examination						Investigations					
					T2 DM			Cerebrovascular events	Cerebrovascular events				Peripheral neuropathy	Diabetic retinopathy		Diabetic nephropathy	Diabetic foot	Autonomic neuropathy	HTN			PVD		Dislipidemia							BP (mm Hg)							
		Sex	Age (Years)	Occupation	History	Duration (Years)	Treatment		Angina pain	MI	IHD	Treatment		History	Treatment				History	Duration (Years)	Treatment	History	Treatment	History	Treatment	Height (Cms)	Weight (Kg)	BMI (Kg/m2)	WC (Cms)	Systolic	Diastolic	FBS (mg/dL)	Total TG (mg/dL)	Total HDL (mg/dL)	Total CHL (mg/dL)	Estimated LDL (mg/dL)	Serum Ferritin (ng/mL)
57	409548	M	52	LB	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	1	T	N	-	N	-	168	76	26.93	90	140	110	148	168	44	200	78	407
58	411261	M	69	RT	Y	16	I	N	N	N	N	-	N	Y	-	N	N	N	Y	6	T	N	-	N	-	164	79.5	29.56	102	160	90	104	188	34	218	90	58
59	411263	M	64	FR	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	8	T	Y	-	N	-	168	82	29.05	102	140	80	108	172	56	192	76	114
60	411810	M	55	SR	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	3	T	N	-	N	-	168	84	29.76	102	140	100	148	168	44	200	78	213
61	410767	M	60	BS	Y	15	I	N	N	N	N	-	N	Y	-	Y	N	N	Y	13	T	N	-	N	-	170	88	30.45	100	140	90	144	166	34	218	98	301
62	461759	M	50	BS	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	8	T	N	-	N	-	164	78	29.00	104	140	90	108	172	56	192	79	127
63	412091	F	78	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	4	T	N	-	N	-	170	84.5	29.24	88	140	96	104	146	38	154	60	7
64	412143	F	64	HW	Y	10	O	N	N	N	N	-	N	Y	-	N	N	N	N	-	-	N	-	N	-	166	80	29.03	90	110	80	108	150	38	176	60	136
65	409488	M	71	BS	Y	10	I	N	Y	N	N	-	N	Y	-	N	N	N	Y	7	-	N	-	N	-	168	84	29.76	102	140	90	136	190	34	210	86	89
66	409168	F	32	HW	Y	4	I	N	N	N	N	-	N	N	-	N	N	N	Y	5	T	N	-	N	-	160	68	26.56	84	140	90	190	154	52	160	36	301
67	419238	F	30	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	16	T	N	-	N	-	161	69	26.59	92	148	100	112	180	34	184	76	58
68	416103	F	64	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	10	T	N	-	N	-	168	76.5	27.10	94	140	100	96	170	52	230	88	1500
69	409328	F	76	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	4	T	N	-	N	-	168	80	28.34	104	160	90	106	188	39	190	110	325
70	409416	F	28	LB	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	9	T	N	-	N	-	165	68.5	25.16	89	150	80	78	251	44	202	105	220
71	409123	F	53	HW	Y	4	O	N	N	N	N	-	N	N	-	N	N	N	N	-	-	N	-	N	-	158	66	26.44	90	110	80	148	264	34	203	58	563
72	416025	M	58	LB	Y	10	O	N	Y	N	N	-	N	Y	-	N	N	N	Y	14	T	N	-	N	-	170	84	29.07	102	160	90	130	168	30	170	65	1500
73	416147	M	57	FR	Y	8	I	N	N	N	N	-	N	Y	-	N	N	N	Y	1	-	N	-	N	-	168	84	29.76	103	160	90	166	170	32	210	88	301
74	416164	M	65	FR	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	6	T	N	-	N	-	170	84	29.07	103	150	90	104	180	38	210	70	359
75	416221	M	35	BS	Y	2	O	N	N	N	N	-	N	N	-	N	N	N	Y	8	T	N	-	N	-	167	69.5	24.92	104	140	90	110	220	40	128	90	174
76	416486	F	75	HW	Y	15	I	N	N	N	N	-	N	Y	-	N	N	N	Y	18	T	N	-	N	-	166	69.5	25.22	88	140	80	190	179	23	190	68	19
77	416491	F	52	HW	Y	8	O	N	N	N	N	-	N	Y	-	N	N	N	Y	7	T	N	-	N	-	164	74	27.51	88	140	90	132	170	39	160	82	127
78	416605	M	58	BS	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	6	T	N	-	N	-	170	76	26.30	102	140	90	106	178	39	160	80	359
79	416630	M	48	BS	Y	2	O	N	N	N	N	-	N	N	-	N	N	N	Y	11	T	N	-	N	-	166	80	29.03	99	150	90	168	210	52	189	78	212
80	409781	F	64	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	16	T	N	-	N	-	168	76.5	27.10	94	140	100	96	170	52	230	88	1500
81	416682	F	52	HW	N	-	-	N	Y	N	N	-	N	N	-	N	N	N	Y	7	T	N	-	N	-	178	80	25.25	90	140	90	98	179	49	192	79	407
82	416795	M	70	RT	Y	25	O	N	N	N	N	-	N	Y	-	N	N	N	Y	6	-	N	-	N	-	168	74	26.22	100	140	90	138	210	36	178	80	136
83	416947	F	60	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	15	T	N	-	N	-	170	88	30.45	90	140	90	960	160	48	190	78	159
84	417027	F	60	HW	Y	10	O	Y	N	N	N	-	N	Y	-	N	N	N	N	-	-	N	-	N	-	166	80	29.03	88	110	80	110	142	46	160	58	127

ANNEXURE III - MASTER CHART

Serial Number	In Patient Number	Demography			History																						Clinical examination						Investigations					
					T2 DM			Cerebrovascular events	Cerebrovascular events				Peripheral neuropathy	Diabetic retinopathy		Diabetic nephropathy	Diabetic foot	Autonomic neuropathy	HTN			PVD		Dislipidemia								BP (mm Hg)						
		Sex	Age (Years)	Occupation	History	Duration (Years)	Treatment		Angina pain	MI	IHD	Treatment		History	Treatment				History	Duration (Years)	Treatment	History	Treatment	History	Treatment	Height (Cms)	Weight (Kg)	BMI (Kg/m2)	WC (Cms)	Systolic	Diastolic	FBS (mg/dL)	Total TG (mg/dL)	Total HDL (mg/dL)	Total CHL (mg/dL)	Estimated LDL (mg/dL)	Serum Ferritin (ng/mL)
85	417128	M	80	RT	Y	25	O	N	Y	N	N	-	N	Y	-	N	N	N	Y	9	T	N	-	N	-	170	84	29.07	102	156	90	150	146	52	190	88	301
86	417218	M	68	FR	N	-	-	Y	N	N	N	-	N	N	-	N	N	N	Y	17	T	N	-	N	-	169	80	28.01	102	140	90	100	208	36	200	60	359
87	416510	F	68	HW	N	-	-	Y	N	N	N	-	N	N	-	N	N	N	Y	10	T	N	-	N	-	168	76	26.93	90	148	90	102	156	48	180	86	212
88	416510	F	62	HW	Y	15	O	N	Y	N	N	-	N	N	-	N	N	N	N	-	-	N	-	N	-	166	69	25.04	90	110	90	160	218	36	200	106	114
89	417481	F	76	HW	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	12	T	N	-	N	-	168	80	28.34	104	160	90	106	188	39	190	110	325
90	416398	M	65	RT	Y	10	O	N	N	N	N	-	N	N	-	N	N	N	N	-	-	N	-	N	-	176	90	29.05	102	110	70	168	190	36	290	148	58
91	417577	M	56	SR	Y	6	O	N	N	N	N	-	N	N	-	N	N	N	Y	19	T	N	-	N	-	170	88	30.45	102	146	92	118	220	36	280	108	45
92	416636	M	80	RT	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	10	T	N	-	N	-	169	77	26.96	100	140	94	120	166	52	190	60	365
93	417600	M	66	FR	Y	10	O	N	N	N	N	-	N	Y	-	N	N	N	Y	14	T	N	-	N	-	170	80	27.68	102	160	94	156	140	36	200	89	398
94	416467	M	66	RT	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	20	T	N	-	N	-	164	70	26.03	102	148	92	190	166	38	200	96	136
95	417690	M	54	SR	Y	6	I	N	N	N	N	-	N	N	-	N	N	N	Y	6	T	N	-	N	-	165	72.5	26.63	101	146	100	122	160	50	178	110	89
96	416131	M	50	SR	Y	4	O	N	N	N	N	-	N	N	-	N	N	N	Y	11	T	N	-	N	-	168	82	29.05	100	170	90	136	141	39	180	64	28
97	417361	M	77	BS	Y	10	I	N	N	N	N	-	N	Y	-	N	N	N	N	-	T	N	-	N	-	165	76	27.92	101	160	90	112	198	36	200	96	212
98	417409	M	49	SR	N	-	-	N	N	N	N	-	N	N	-	N	N	N	Y	24	T	N	-	N	-	164	78	29.00	104	160	90	104	166	38	180	86	178
99	416418	M	78	BS	Y	20	O	N	N	N	N	-	N	Y	-	N	N	N	Y	8	T	N	-	N	-	170	88	30.45	100	150	90	176	180	41	240	121	301
100	417421	F	80	HW	Y	20	I	N	Y	N	N	-	N	Y	-	N	N	N	Y	12	-	N	-	N	-	168	70	24.80	87	140	90	150	158	32	220	106	361

Annexures

Annexure III

ANNEXURE III – KEY TO MASTER CHART

ACC	– Accountant
BMI	– Body mass index
BP	– Blood pressure
BS	– Business
CHL	– Total Cholesterol
CL	– Clerk
Cms	– Centimeters
dL	– Deciliter
F	– Female
FBS	– Fasting blood sugar
FR	– Farmer
HDL	– High density lipoproteins
HTN	– Hypertension
HW	– House Wife
I	– Insulin
IHD	– Ischaemic heart disease
Kg	– Kilogram
LB	– Labourer
LDL	– Low density Lipoprotein
M	– Male
m	– Meter
mg	– Milligram
MI	– Myocardia infarction

mm Hg	– Millimeter of mercury
N	– No
ng	– Nano gram
NPDR	– Non proliferative diabetic retinopathy
O	– Oral
PVD	– Peripheral vascular disease
RT	– Retired
S	– Service
T	– Tablet
T2DM	– Type 2 diabetes mellitus
TG	– Triglycerides
WC	– Waist circumference
Y	– Yes