A STUDY TO CORRELATE SERUM ZINC WITH INSULIN RESISTANCE IN NEWLY DIAGNOSED TYPE II DIABETES MELLITUS PATIENTS

Dissertation Submitted in partial fulfillment of the requirement for the award of the Degree of
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CERTIFICATE FROM THE DEAN

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INTRODUCTION

Diabetes is a set of metabolic illnesses marked by hyperglycemia caused by problems with insulin secretion, insulin action, or both. People living with diabetes are anticipated to increase from 463 million in 2019 to 700.2 million by 2045, according to figures provided by the International Diabetes Federation in their 9th edition of Diabetes Atlas(1). Its incidence is rapidly increasing in developing countries around the world. Type 2 diabetes mellitus (T2DM) has become a serious public health problem due to its rising prevalence. Diabetes has become more common as a result of population growth, urbanisation, obesity prevalence, a sedentary lifestyle, and patients with diabetes living longer. Diabetes is a very disabling condition that can result in blindness, amputations, renal disease, anaemia, cardiovascular, and brain issues, limiting one's functional capacity, autonomy, and quality of life**.**

Zinc is necessary for the synthesis, storage, and secretion of insulin in response to carbohydrate consumption, as well as for energy production. Insulin's structural integrity is also preserved. Reduced zinc levels in the blood reduce the ability of the pancreas' islet cells to make and secrete

insulin, which can contribute to the development of insulin resistance, which is linked to the development of type 2 diabetes(2).

The development of diabetes complications is influenced by oxidative stress. Zinc is a fundamental component of many antioxidants and has an antioxidant effect on its own. It reduces the generation of free radicals and protects against the damage caused by lipid peroxidation. These data suggest that zinc deficiency may play a role in the onset of diabetes complications.

HOMA-IR is one parameter that provides an index of insulin resistance in a collective sense. It is a reliable indicator of long-term hyperglycemia and the measurement of HOMA-IR helps in identifying the risk of developing diabetic complications(3).

With the above background, this study is designed to estimate serum zinc level and its correlation with insulin resistance in newly diagnosed Type II diabetes mellitus patients from Govt Rajaji hospital,Madurai. It is anticipated that our study will provide evidence regarding the role of zinc in the pathogenesis of Type II Diabetes mellitus.

AIMS AND OBJECTIVES

1.Estimate serum zinc level in Newly diagnosed type 2 diabetes mellitus.

2. Findout correlation between serum zinc concentration and Insulin resistance in patients with Newly diagnosed type II diabetes mellitus patients.

REVIEW OF LITERATURE

HISTORY OF DIABETES MELLITUS:

Diabetes mellitus has been known from ancient times, and disorders having cardinal traits of diabetes mellitus were recognised and recorded in an Egyptian papyrus discovered by Georg Ebers around 1550 B.C. Aretaeus of Cappodocia (81-138 AD) was the first to invent the name "DIABETES," which comes from the Greek word "diabetes," which literally means "to run through or syphon." ...no vital portion of the drink is absorbed by the body, while huge volumes of the flesh are dissolved into the urine, Aretaeus asserted emphatically(4).

The Hindu physicians Charaka, Susruta, and Vaghbata described polyuria and glycosuria. They named it madhumeha ("honey urine") in the 5th-6th century BC after the attraction of flies and ants to the urine of persons suffering from the disease. Dobson was the first to confirm the existence of extra sugar in urine and blood as an explanation for their sweetness in 1776.

a)The Glycogenic Action of the Liver:

Claude Bernard (1813-1878) discovered that the liver was accumulating a starchy substance that was water-insoluble, which he dubbed glycogen, which was transformed into sugar or glucose and discharged into the bloodstream after his amazing research. He believed that diabetes was caused by an overabundance of this substance.

b)The Discovery of Pancreatic Diabetes:

Bouchardat (1870) was the first to propose that the more severe form of Diabetes was caused by a pancreatic problem. The experiments of Oskar Minkowski (1858-1931) and Joseph von Mering (1849-1908) established that the pancreas' internal secretion was responsible for maintaining glucose homeostasis.

c)The Nobel Prize for Insulin Discovery:

Frederick Banting (1891-1941), a young surgeon, and John MacLeod (1876-1935), a professor of physiology, were given the Nobel Prize in 1923 for discovering insulin.

Diabetes was a death sentence in the pre-insulin era, so it was a significant scientific breakthrough. James Bertram Collip (1892-1965), a

talented chemist, developed a better extraction and purification procedure at the end of 1921, and the resulting compound was initially dubbed "Insletin" by the team and later dubbed "Insulin" by MacLeod.

Leonard was given insulin for the first time on January 11, 1922. Thompson is a 14-year-old child who is being treated in Toronto Hospital for diabetes. Thompson lived another 13 years after starting insulin treatment.

Insulin purification technologies evolved, and novel insulin formulations were produced, including Protamine–zinc insulin, long-acting insulin in the 1930s, neutral protamine Hagedorn in the 1940s, and the Lente series in the 1950s. In 1955, Nobel Laureate Fred Sanger (1918-2013) defined the structure of insulin, while Donald Steiner (1930-2014) and his colleagues found proinsulin in 1967. In the same year, William Kelly, Richard Lillehei (1927-1981), and colleagues at the University of Minnesota performed the first pancreas transplant in a human; in 1982, recombinant human insulin became available; and in the early 1990s, insulin pen delivery devices became popular, followed by the discoveries of short (1996) and long (2001) acting insulins.

DIABETES MELLITUS: (AN OVERVIEW)

Diabetes mellitus is a mixture of metabolic diseases characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Several distinct sorts of DM are caused by a complex interaction of genetics and environmental factors.

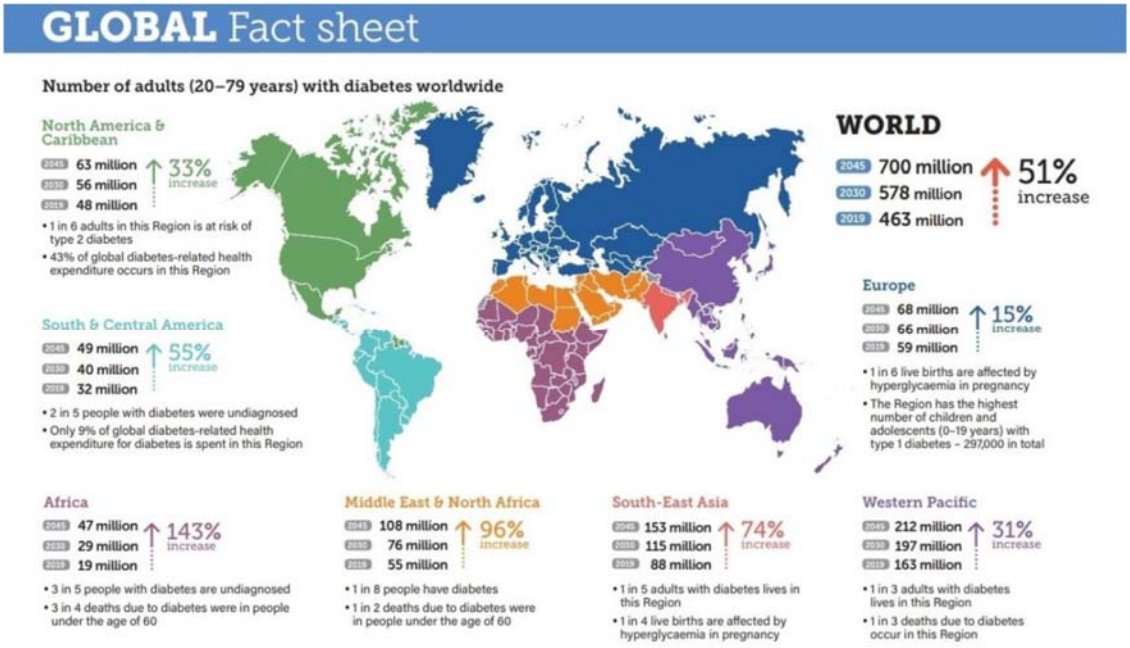
DIABETES MELLITUS EPIDEMIOLOGY:

Diabetes, particularly type 2 diabetes, is one of the most common chronic diseases worldwide. According to the International Diabetes Federation (IDF), 463 million persons aged 20 to 79 years have diabetes as of 2019.

This equates to 9.3 percent of the global population in this age bracket. By 2030, the overall population is expected to reach 578 million (10.2 percent) and 700 million (10.9 percent) by 2045. Type 1 diabetes affects an estimated 1.1 million children and adolescents under the age of 20.

China, India, and the United States of America had the highest numbers of adults with diabetes aged 20–79 years in 2019 and are expected to remain so in 2030. In 2019, one in every two persons , or 231.9 million of

the 463 million adults with diabetes (mostly type 2 diabetes, aged 20–79 years), is unaware that they have the disease.



DIABETES GLOBAL BURDEN MAP 2019,2030 & 2045

Diabetes prevalence rises with age, with persons over 65 years old having the highest estimated prevalence. In 2019, 135.6 million persons aged 65 to 99 are anticipated to have diabetes (19.3 percent). If current trends continue, the number of diabetics aged 65 and up (65–99) will reach 195.2 million in 2030 and 276.2 million in 2045.

Diabetes and its consequences are expected to claim the lives of 4.2 million people in 2019. Diabetes is predicted to cost the global health system

USD 760 billion per year. Spending is expected to reach USD 825 billion in 2030 and USD 845 billion in 2045(5).

INDIAN SCENARIO

India has the world's second-largest population of adults with diabetes (77 million). India accounts for one out of every six diabetic adults worldwide. With more than 1 million fatalities attributed to diabetes and related complications, India was the leading contributor to regional diabetes mortality(6).

DIABETES MELLITUS CLINICAL FEATURES:

Polyuria, polydipsia, weight loss, weariness, weakness, blurring of vision, recurrent superficial infections (vaginitis, fungal skin infections), and poor healing of skin sores after moderate trauma are all symptoms of hyperglycemia. Hyperglycemia (osmotic diuresis) and the patient's catabolic state (urinary loss of glucose and calories, muscle breakdown owing to protein degradation, and so on) are the most common metabolic abnormalities.Changes in the water content of the lens cause blurred vision, which resolves when the hyperglycemia is controlled.

CRITERIA FOR DIAGNOSIS OF DIABETES: (ADA GUIDELINE 2020)21

FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours.*

(OR)

Two-hour Plasma glucose ≥200 mg/dL (11.1 mmol/L) during OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

(OR)

HbA1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*

(OR)

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

* In the absence of unequivocal hyperglycemia, diagnosis requires two abnormal test results from the same sample or in two separate test samples(2).

ETIOLOGICAL CLASSIFICATION OF DIABETES MELLITUS:

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

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

III. Specific types of diabetes:

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

1. Hepatocyte nuclear transcription factor (HNF) 4α (MODY 1)
2. Glucokinase (MODY 2)
3. HNF-1α (MODY 3)
4. Insulin promoter factor-1, HNF-1β, NeuroD1, and others leading to other forms of MODY
5. Insulin, subunits of ATP-sensitive potassium channel leading to permanent neonatal diabetes
6. Mitochondrial DNA
7. Other pancreatic islet regulators/proteins such as KLF11, PAX4, BLK, GATA4, GATA6, SLC2A2 (GLUT2), RFX6, GLIS3

B. Transient neonatal diabetes

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

D. Genetic defects in insulin action, including type A insulin resistance, Leprechaunism, Rabson-Mendenhall syndrome, Lipodystrophy syndromes

E. Endocrinopathies—acromegaly, Cushing‘s syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma

F. Drug- or chemical-induced—glucocorticoids, vacor (a rodenticide), pentamidine, nicotinic acid, diazoxide, β-adrenergic agonists, thiazides, calcineurin and mTOR inhibitors, hydantoins, asparaginase, α-interferon, protease inhibitors, antipsychotics (atypicals and others), epinephrine

G. Infections—congenital rubella, cytomegalovirus, coxsackievirus

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

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

IV. Gestational diabetes mellitus (GDM)(7)

COMPLICATIONS OF DIABETES:

It is estimated that half of the patients with diabetes are unaware of their disease and are thus more prone to developing diabetic complications. Diabetes complications are common among patients with type 1 or type 2 diabetes but, at an equivalent time, are liable for significant morbidity and mortality. The chronic complications of diabetes results from vascular injury due to long-standing hyperglycemia are broadly divided into microvascular and macrovascular, with the former having a much higher prevalence than the latter(8).

Acute

1. Diabetic ketoacidosis

2. Hyperglycemic Hyperosmolar state

3. Hypoglycemia

4. Diabetic coma

Chronic

Microvascular

1.Diabetic nephropathy

2.Diabetic neuropathy

3.Diabetic retinopathy

Others

Dermatologic manifestations

 Infectious

 Ocular (Cataracts, Glaucoma)

 Charcot‘s arthropathy

 Periodontal disease

 Hearing loss

 Other comorbid conditions associated with diabetes (relationship to hyperglycemia is uncertain): depression, obstructive sleep apnea, fatty liver disease, hip fracture, osteoporosis (in type 1 diabetes), cognitive impairment or dementia, low testosterone in men.

TYPE 2 DIABETES MELLITUS: (OVERVIEW)

Type 2 diabetes, also known as noninsulin-dependent diabetes or adult-onset diabetes, is the most common type of diabetes, accounting for 90–95 percent of all cases.

Hyperglycemia develops in type 2 diabetes when the body's cells are unable to respond adequately to insulin, a condition known as insulin resistance. Insulin resistance causes the hormone to be inefficient, which leads to an increase in insulin production. Later, the pancreatic beta cells are unable to meet demand, resulting in insufficient insulin synthesis.

T2DM-ASSOCIATED GENES:

The genes that predispose to type 2 diabetes are still being identified, however recent genome-wide association studies have found an unusually large number of genes that carry a low risk of type 2 diabetes (>70 genes,

each with a relative risk of 1.06–1.5). A variant of the transcription factor 7–like 2 genes (TCF7L2) has been linked to type 2 diabetes in several populations, and other genes include peroxisome proliferator-activated receptor (PPRF), inward rectifying potassium channel (KCNJ11), zinc transporter, Insulin receptor substrate (IRS), and calpain 10. (CAPN10). Although the specific method by which these genes promote T2DM susceptibility is unknown, it is being researched(8).

It is believed that genetic loci identified so far account for 10% of genetic vulnerability. As a result, predicting type 2 diabetes using a combination of known genetic loci is now impossible.

Insulin resistance encompasses the suppressive effects of insulin on endogenous glucose production (in the liver), the stimulatory effects of insulin on peripheral glucose uptake and glycogen synthesis (mostly in skeletal muscle), and the inhibitory effects of insulin on adipose tissue lipolysis. It is mostly caused by genetic predisposition (defects in the insulin receptor's post-receptor signalling pathway) and obesity.

The inflammasome, a multiprotein cytoplasmic complex that leads to the release of the cytokine interleukin-1, is activated by excess FFAs and TGs in macrophages and beta cells as a result of obesity and insulin

sensitivity. Adipocytes also release IL-1 in response to high FFA. IL-, in turn, promotes insulin resistance by mediating the release of other pro-inflammatory cytokines that operate on the primary sites of insulin action.

DYSFUNCTION OF BETA CELLS:

In type 2 diabetes, beta-cell dysfunction is caused by these cells' inability to adjust to the long-term demands of peripheral insulin resistance and increasing insulin production. This hyperinsulinemic state compensates for peripheral resistance and can typically keep plasma glucose levels normal for years. Eventually, however, beta-cell compensation is insufficient, and hyperglycemia develops, along with a decrease of beta-cell mass in absolute terms. Beta-cell failure in type 2 diabetes is caused by a variety of biological processes, many of which overlap with those who have been linked to insulin resistance Excess nutrients, such as FFAs and glucose, can increase local release of pro-inflammatory cytokines, leading to -cell malfunction and, eventually, cell death(9).

Amyloid replacement of islets is a common finding in people who have had type 2 diabetes for a long time, and it can be found in more than 90% of diabetic islets tested. The beta cells secrete the islet amyloid

polypeptide (IAPP), also known as amylin, in combination with insulin, and its aberrant aggregation results in amyloid. IAPP also activates the inflammasome and stimulates IL-1 release, allowing the inflammatory insult to be sustained on surviving beta cells until late in the disease.

T2DM PATHOPHYSIOLOGY:

Because the pancreatic beta cells adjust by increasing insulin secretion, glucose tolerance stays near-normal in the early stages of T2DM despite insulin resistance. The pancreatic islets in certain people become unable to maintain the hyperinsulinemic state as insulin resistance and compensatory hyperinsulinemia advance. Impaired glucose tolerance (IGT), characterized by elevations in postprandial glucose, then develops Overt diabetes with fasting hyperglycemia is caused by a further decrease in insulin secretion as a result of -cell malfunction and an increase in hepatic glucose production(10).

ZINC'S HISTORY:

Zinc is the 24th most prevalent element in the Earth's crust, and it exists in five stable isotopes. Within the medical Lexicon attributed to the

Hindu monarch Madanapala (of the Taka dynasty) and written around the year 1374, zinc was clearly identified as a metal under the title of Yasada or Jasada. In India in the 13th century, impure zinc was smelted and extracted by reducing calamine with wool and other organic compounds. The alchemist Paracelsus is thought to have called the element after the German word Zinke. Andreas Sigismund Marggraf, a German chemist, is credited with discovering pure metallic zinc in 1746. By 1800, Luigi Galvani and Alessandro Volta had discovered zinc's electrochemical characteristics. Zinc carbonate and zinc gluconate (as nutritional supplements), zinc pyrithione (anti-dandruff shampoos), zinc chloride (in deodorants), zinc sulphide (in luminous paints), and zinc methyl or zinc diethyl (in organic laboratories) are all commonly used zinc compounds.

ZINC BIOCHEMICAL FUNCTIONS :

Zinc is an important chemical element that is required by plants, animals, and microbes. Zinc is found in approximately 100 enzymes, is stored and transported in metallothioneins, and is structural ions in transcription factors. In organisms, it is "usually the second most prevalent transition metal."

Zinc is found in the human body in amounts ranging from 2 to 4 grammes. The brain, muscle, bones, kidney, and liver contain the most zinc, with the prostate having the highest quantities. Zinc, which is essential for prostate gland function and reproductive organ growth, is abundant in sperm. In the prostate, zinc plays a variety of biological roles. It interacts with a wide variety of organic ligands and has a role in RNA and DNA metabolism, signal transduction, and organic phenomena. Apoptosis is also regulated by it. A 2006 study indicated that around 10% of human proteins (2800) are zinc-bound, with hundreds more transporting and trafficking zinc; this is nearly identical to the Silico study in Arabidopsis thaliana, which discovered 2367 zinc-related proteins.

Zinc is held in synaptic vesicles by glutaminergic neurons in the brain, and it may influence brain excitability.

It is important for synaptic plasticity and learning. It has been named as "the brain's dark horse" since it may also be a neurotoxic, implying that 7 zinc homeostasis is important for appropriate brain and central nervous system function. Zinc is a helpful catalytic agent in hydroxylation and other enzymatic activities because it is an effective Lewis acid. The metal also has a flexible coordination geometry, which allows proteins to perform biological reactions by rapidly shifting conformations. Carbonic anhydrase

and carboxypeptidase are two zinc-containing enzymes that are essential for the management of carbon dioxide (CO2) and the digestion of proteins, respectively.

Carbonic anhydrase turns CO2 to bicarbonate in vertebrate blood, and the same enzyme then converts bicarbonate back to CO2 for expiration through the lungs. This conversion would take a million times longer without this enzyme at a typical blood pH of 7, or would require a pH of 10 or higher. During protein digestion, carboxypeptidase cleaves peptide bonds.

The terminal peptide forms a coordinate covalent link with a C=O group connected to zinc, which gives the carbon a positive charge. This aids in the formation of a hydrophobic pocket near the zinc on the enzyme, which draws the non-polar portion of the protein to be digested. In zinc finger twists and clusters, zinc plays a strictly structural role.

Following are the important effects of Zinc on cellular homeostasis:

1) Stimulation of glucose uptake,

2) Lipogenesis in adipocytes,

3) Tyrosine phosphorylation of the insulin / IGF-1 receptor and insulin receptor substrate

4) Activation of epidermal growth factor receptor,

5) Inhibition of PTP,

6) Activation of mitogen activated kinases (MAPKs),C- jun Nterminal kinases, 7) Increase in glycogen synthesis

ZINC METABOLISM:

Zinc is bound and transported in plasma by albumin (60 percent, but poor affinity) and transferrin (10 percent). Because transferrin also carries iron, excess iron reduces zinc absorption, and vice versa. Copper undergoes a similar reaction. Regardless of zinc intake, the content of zinc in plasma remains essentially consistent. Zinc signalling is used by cells in the salivary gland, prostate, immune system, and intestine to communicate with other cells. Zinc could potentially be stored in microbes' metallothionein reserves, as well as in the intestines and livers of mammals. Intestinal cells that contain metallothionein can reduce zinc absorption by 15–40%. Inadequate or excessive zinc intake, on the other hand, might be hazardous; excess zinc inhibits copper absorption, as metallothionein absorbs both metals(11).

ZINC DEFICIENCY:

Hypozincemia is a condition in which the metabolic need for zinc is insufficient.

PREVALENCE:

Zinc insufficiency affects one-third of the world's population, with rates ranging from 4 to 73 percent depending on the country. In the poor world, zinc deficiency is the fifth most common cause of disease. One of the four quick-win solutions to significant global problems suggested in the Copenhagen Consensus by an international panel of prominent economists is providing micronutrients, especially zinc, to people. According to conservative estimates, 25% of the world's population is at risk of zinc deficiency.

CAUSES:

Hypozincemia is typically caused by a nutrient deficit, but it can also be caused by malabsorption, diarrhoea, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, cancer, and other chronic disorders. It can also happen as a result of bariatric surgery.

Zinc shortage is mainly caused by a lack of zinc in the diet, illness states that encourage zinc loss, or physiological conditions that require more zinc.

Zinc deficiency is common in populations who eat largely plant-based diets that are poor in bioavailable zinc. Zinc loss is accelerated by diseases or situations that affect intestinal absorption. Diarrhea, which is widespread in developing nations, is one factor that contributes to zinc losses in the faeces. Changes in intestinal tract permeability and absorbability, which are caused in part by viral, protozoal, and bacterium pathogens, can also contribute to faecal zinc losses. Periods of growth in babies and children, as well as in mothers, are physiological states that demand more zinc(12).

SIGNS AND SYMPTOMS:

Hair loss, skin sores, diarrhoea, and withering of body tissues are all signs of zinc deficiency. Acne, eyesight, taste, smell, and memory problems can all be caused by a zinc deficiency. White spots, bands, or lines on fingernails are one easily recognisable symptom of zinc insufficiency (leuconychia). A white spot on the skin indicates that the immune system has defeated a bacterial or other systemic infection. When a woman has a

minor zinc deficiency, she may have many parallel white bands or lines on her fingernails to mark her menstrual cycles.

IMPAIRMENT OF COGNITIVE AND MOTOR FUNCTION:

Zinc deficiency can also affect cognitive and motor performance in youngsters. Zinc insufficiency can affect a variety of organ systems, particularly when it happens during a period of rapid growth and development, when nutritional demands are high, such as during childhood.

Low maternal zinc levels have been linked to decreased attention and motor function during childhood. Zinc supplementation has been shown in certain trials to improve motor development in extremely low birth weight newborns and to increase robust and functional activity in infants and toddlers(13).

DIARRHOEA AND PNEUMONIA:

Zinc deficiency causes diarrhoea and pneumonia to occur more frequently and with greater intensity.

Zinc medication reduces the length of acute diarrhoea by 25% and the risk of treatment failure or death in persistent diarrhoea by 40%, according to studies. Zinc medication for ten days has been shown in studies to reduce the duration and intensity of diarrheal episodes, reduce stool output, and reduce the need for hospitalisation. Zinc can also helps to prevent diarrhoea for up to three months.

A zinc taste test that identifies a person's taste problems could be used to diagnose zinc insufficiency(12).

DYSMENORRHOEA:

A high dose of zinc, 30 mg 1-3 times a day, is effective in preventing dysmenorrhea.

PREGNANCY:

Zinc deficiency can harm both the mother and the foetus during pregnancy. According to an analysis of pregnancy outcomes in women with acrodermatitis enteropathica, two out of every seven pregnancies resulted in foetus malfunctions and one termination, indicating that the human foetus is vulnerable to the teratogenic effects of severe zinc deficiency. However,

after evaluating certain studies, it was discovered that zinc supplementation had no influence on infant survival throughout pregnancy(13).

ZINC'S EFFECT ON MYELOID AND LYMPHOID CELLS:

Zinc is required for the function of thymulin, a thymic hormone. Th1 cytokines are reduced in zinc deficient illness, but Th2 cytokines are unaffected. As a result, Th1 becomes the Th2 function. Zinc inhibits gene expression and the production of TNF, IL1, and IL-8 cytokines.

ZINC DEFICIENCY AND ITS TREATMENT:

Zinc supplementation has been demonstrated to lower diarrhoea rates and mortality in children under the age of five. Five management techniques can be employed to combat zinc deficiency:

1) Medications as a supplement

2) Food fortification by the use of zinc compounds in foods

3) Dietary diversification/modification

4) Genetic biofortification

5) Agronomic biofortification through zinc fertilization(14)

DIABETES' EFFECTS ON ZINC METABOLISM:

In diabetes, hypozincemia can be caused by hyperzincuria, reduced gastrointestinal Zn absorption, or both. It appears that hyperzincuria is caused more by hyperglycemia than by any special impact of endogenous or exogenous insulin on the renal tubule(15).

Yazigi discovered that the absolute and creatinine corrected urine excretions of zinc were higher in diabetics than in matched controls in both Type I and Type II diabetics, and that there was a positive connection between Zn excretion and haemoglobin Alc concentrations.

Insulin improves hyperzincuria in Type 2 diabetes, whereas other treatments have little effect on zinc excretion(16).

Hyperglycemia is also thought to interfere with the active transport of Zinc back to the renal tubular cells. Insulin treatment lowers hyperzincuria but does not appear to totally alleviate it. McNair verified that hyperzincuria, but not glycosuria, is related to the degree of hyperglycemia.

Abnormal Zn tolerance tests in diabetic individuals, according to Kinlaw suggest reduced absorption.

Escobar also discovered a decrease in fractional Zn transport in the stomach and kidney, which they linked to increased metallothionein synthesis in diabetics. Metallothionein is a cation-binding protein found inside cells that appears to block Zn transport. This reduction in gastrointestinal absorption, along with hyperzincuria, could result in considerable intracellular Zn loss.

Toxicity :

Zinc toxicity can occur in two types:

1) Acute form

2) Chronic form

Acute form:

Nausea,

Vomiting,

Abdominal cramps,

Diarrhea, and headaches and loss of appetite . One study showed that severe nausea and vomiting within 30 minutes of ingesting 4 gram of Zinc gluconate (570 mg elemental Zinc)(17)

Chronic form:

Intake of 150-450 mg of Zinc per day have been associated with chronic effects like low copper status, affecting iron metabolism, reduced immune function, and reduced levels of high density lipoproteins. Afkhami-Ardekani reported that patients receiving Zinc sulphate 660 mg /day for 12 weeks had mild abdominal pain .

Patients who received Zinc sulphate (22 mg /day) and Zinc acetate (50 mg /day) for a period of 34 months showed that no significant adverse effects on renal and liver function tests(18).

EFFECTS OF ZINC ON DIABETES MELLITUS (PRIMARY DISEASE EFFECTS):

Quarterman demonstrated in 1966 that diet-induced zinc deficiency in rats reduced the pancreas' ability to release insulin in response to a glucose load, a hallmark of diabetes(19).

In zinc-deficient hamsters, Boquist colleagues demonstrated a decrease in glucose tolerance with no change in insulin production in response to an intravenous glucose load a few years later. They also found that with zinc shortage, the islet cell's granulation was reduced using light and electron microscopy. These findings are consistent with decreased islet cell insulin content in zinc shortage situations since zinc is required for the storage granulation of insulin within the beta cell and greater insulin production lowers beta-cell zinc concentration.

Because zinc is a required component of a number of antioxidant enzymes, including SOD, catalase, and peroxidase, abnormalities in zinc metabolism that result in insufficient zinc for these enzymes could contribute to the tissue damage seen in diabetes.

Rabinovitch et al investigated the link between cytokine-induced (interleukin 1b, tumour necrosis factor (TNF), and interferon-gamma) inflammation and mortality.

Destruction of pancreatic beta cells, formation of malondialdehyde (MDA), a lipid peroxidation end result, and nitrite, a nitric oxide end product These findings revealed that cytokines are hazardous to human beta cells in the pancreas islets by causing oxygen free radicals, lipid peroxidation, and aldehyde formation, with MDA being one of the cytotoxic mediators.

Other researchers have proposed that the Zn-metallothionein complex in the islet cell guards against free radicals created within the cell from any source, and immune-mediated cytokine-induced oxidative stress would likely represent considerable oxidative stress. The cell's ability to fight itself against this oxidative load decreases when intracellular Zn reserves get depleted. This is an essential way by which zinc deficiency affects diabetes progression.

The stimulation of metallothionein production within the islet cell in response to Streptozocin (STZ)-induced OH radical generation has been established by Zimny.

STZ-induced lipid peroxidation and reduced superoxide dismutase were demonstrated by Yang and Cherian (SOD). Roza discovered that the loss of beta-cell function was preceded by a drop in pancreatic SOD and catalase antioxidant activity, implying that beta-cells are more vulnerable to free radical attack and cell destruction in genetically diabetic rats. All of these findings point to Zn having a protective role(20).

There is no good evidence for oxidative stress as a major factor in the development of insulin insufficiency or damage in the islet cell, however there is substantial evidence of increased insulin secretion, at least early in the disease's progression. Because Zn exits the cell with insulin, increased insulin secretion induces Zn depletion in the cell. The cell can produce more insulin, but not more Zn, and the Zn co-secreted by hyperzincuria is more likely to be ejected and not available for absorption into the cellular pool. Because of the gradual reduction of intracellular zinc, less insulin is released for a given glucose level, making the islet cell more vulnerable to injury.

This is consistent with the clinical picture of islet cell death following persistent hyperglycemia and inability of the islet cell to produce adequate insulin to regulate glucose. This suggests that Zn deficiency may have an impact on the progression of Type 2 diabetes.

EFFECTS OF ZINC ON DIABETES MELLITUS: (SECONDARY COMPLICATIONS)

Hyperglycemia has been identified as a major factor in the development of microvascular problems such as retinopathy, nephropathy, neuropathy, and small artery occlusions, as well as birth defects such as foetal deformities and macrosomia in recent years. While hyperglycemia is a substantial component, it does not appear to be the primary factor in the development of these problems(21).

As mice were given STZ to make them diabetic, there was a significant increase in foetal loss and deformity when compared to non-diabetic animals. There were fewer foetal abnormalities in transgenic mice for the human copper-zinc superoxide dismutase (Cu-Zn SOD) than in controls lacking the gene, indicating that SOD lowers diabetes embryopathy, likely by lowering oxygen-free radicals. Minami found that when Zn shortage was generated either by increased renal excretion or by dietary-induced deficiency, the progression of diabetic nephropathy in STZ diabetic rats accelerated.

In humans, Faure found that Zn supplementation had a protective effect on the development of diabetic retinopathy, which was linked to an

increase in SOD. This suggested that decreased lipid peroxidation of retinal polyunsaturated fatty acids could be contributing to the observed decrease in retinopathy.

INSULIN RESISTANCE

Insulin resistance is identified as an impaired biologic response to insulin stimulation of target tissues, primarily the liver, muscle, and adipose tissue. Insulin resistance impairs glucose disposal, resulting in a compensatory increase in beta-cell insulin production and hyperinsulinemia. The metabolic consequences of insulin resistance can result in hyperglycemia, hypertension, dyslipidemia, visceral adiposity, hyperuricemia, elevated inflammatory markers, endothelial dysfunction, and a prothrombic state. Progression of insulin resistance can lead to metabolic syndrome, nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes mellitus(22).

Insulin resistance is primarily an acquired condition related to excess body fat, though genetic causes are identified as well. The clinical definition of insulin resistance remains elusive as there is not a generally accepted test for insulin resistance. Clinically, insulin resistance is recognized via the

metabolic consequences associated with insulin resistance as described in metabolic syndrome and insulin resistance syndrome.

The gold standard for measurement of insulin resistance is the hyperinsulinemic-euglycemic glucose clamp technique. This is a research technique with limited clinical applicability; however, there are a number of clinically useful surrogate measures of insulin resistance, including HOMA-IR, HOMA2, QUICKI, serum triglyceride, and triglyceride/HDL ratio(23). In addition, several measures assess insulin resistance based on serum glucose and insulin response to a glucose challenge(24).

The predominate consequence of insulin resistance is type 2 diabetes (T2DM). Insulin resistance is thought to precede the development of T2DM by 10 to 15 years. The development of insulin resistance typically results in a compensatory increase in endogenous insulin production. Elevated levels of endogenous insulin, an anabolic hormone, is associated with insulin resistance and results in weight gain which, in turn, exacerbates insulin resistance. This vicious cycle continues until pancreatic beta-cell activity can no longer adequately meet the insulin demand created by insulin resistance, resulting in hyperglycemia. With continued mismatch between insulin demand and insulin production, glycemic levels rise to levels consistent with T2DM.

Resistance to exogenous insulin has also been described. An arbitrary but clinically useful benchmark considers patients requiring greater than 1 unit/kilogram/day of exogenous insulin to maintain glycemic status(15) . Patients requiring greater than 200 units of exogenous insulin per day are considered severely insulin resistant.

In addition to T2DM, the spectrum of disease associated with insulin resistance includes obesity, cardiovascular disease, nonalcoholic fatty liver disease, metabolic syndrome, and polycystic ovary syndrome(PCOS)(25). These are all of great consequence in the United States with a tremendous burden being placed on the healthcare system to treat the direct and indirect conditions associated with insulin resistance.

Lifestyle modification should be the primary focus for the treatment of insulin resistance. Nutritional intervention with calorie reduction and avoidance of carbohydrates that stimulate excessive insulin demand are a cornerstone to treatment. Physical activity helps to increase energy expenditure and improve muscle insulin sensitivity. Medications also can improve insulin response and reduce insulin demand.

ETIOLOGY OF INSULIN RESISTANCE

Insulin resistance etiology can be divided into acquired, hereditary, and mixed. The great majority of people with insulin resistance fall into the acquired categories.

Acquired

- Excess dysfunctional adipose tissue
- Aging
- Physical inactivity
- Nutritional imbalance
- Medications (glucocorticoids, anti-adrenergic, protease inhibitors, atypical antipsychotics, and some exogenous insulin)
- Increased sodium diets
- Glucose toxicity
- Lipotoxicity from excess circulating free fatty acids

In addition to the heritable components of the above etiologies of insulin resistance, there are a number of unrelated genetic syndromes with associated insulin resistance(26).

Genetic

- Myotonic Dystrophy
- Ataxia-telangiectasia
- Alstom syndrome
- Rabson-Mendenhall syndrome
- Werner syndrome
- Lipodystrophy(27)
- PCOS
- Type-A insulin resistance: Characterized by severe insulin resistance (abnormal glucose homeostasis, ovarian virialization, and acanthosis nigricans) in the absence of anti-insulin antibodies; typically occurs before middle age
- Type-B insulin resistance: Characterized by the development of anti-insulin antibodies (typically in middle age) with resultant abnormal glucose homeostasis, ovarian hyperandrogenism, and acanthosis nigricans(28)

PATHOPHYSIOLOGY

The three primary sites of insulin resistance are the muscle, liver, and adipose tissue. Insulin resistance is postulated to begin in muscle tissue with immune-mediated inflammatory change and excess free fatty acids, causing ectopic lipid deposition. Muscle accounts for up to 70% of glucose disposal. With impaired muscle uptake, excess glucose returns to the liver increasing de novo lipogenesis and circulating free fatty acids, further contributing to ectopic fat deposition and insulin resistance

Adipose Tissue

By use of the hyperinsulinemic-euglycemic clamp technique, researchers determined that lipolysis is most sensitive to insulin. Failure of insulin to suppress lipolysis in insulin-resistant adipose tissue, especially visceral adipose tissue, increases circulating free fatty acids. Higher levels of circulating FFAs directly affect both liver and muscle metabolism, further exacerbating insulin resistance.

Muscle Tissue

After intake of a caloric load and conversion to glucose, muscle is the primary site for glucose disposal, accounting for up to 70% of tissue glucose uptake. With excess calorie loads, glucose uptake by muscle exceeds capacity, and excess glucose returns to the liver where it triggers de novo lipogenesis . Increased de novo lipogenesis increases triglyceride and FFA production, causing ectopic fat deposition into the liver, muscle, and adipose tissue. As a result, insulin resistance increases as well as the production of inflammatory markers. Additional factors influencing insulin resistance in muscle tissue include physical inactivity and genetic risk.

Hepatic Tissue (29)

Insulin resistance in muscle results in increased delivery of glucose substrate to the liver, which triggers DNL, with associated inflammation, and ectopic lipid deposition. Insulin resistance in adipose tissue results in increased lipolysis in adipocytes, resulting in increased circulating FFA and further exacerbating steatosis and insulin resistance in muscle tissue. In the presence of caloric intake, insulin reduces hepatic glucose production via inhibition of glycogenolysis, limiting the postprandial rise in glucose. With insulin resistance, this feedback mechanism is impaired, and hepatic glucose

production continues to rise, even as postprandial glucose rises. Glucotoxicity, associated with elevated glucose levels, further contributes to insulin resistance(30).

EVALUATION OF INSULIN RESISTANCE

The gold standard for measurement of insulin resistance is the hyperinsulinemic-euglycemic glucose clamp technique. This is a research technique in which a fasted, non-diabetic patient is placed on a high rate constant infusion of insulin to suppress hepatic glucose production; the blood glucose is frequently monitored while a concomitant 20% dextrose solution is given at varying rates to clamp the blood glucose in the euglycemic range. The amount of glucose required to reach a steady state reflects exogenous glucose disposal required to compensate for the hyperinsulinemia. Insulin resistance calculation is based on whole-body glucose disposal and body size(31).

The complexity of the glucose clamp method limits its clinical usefulness. As a result, multiple surrogate markers for insulin resistance have been developed and tested. HOMA-IR and HOMA2, based on fasting glucose and fasting insulin levels, are widely utilized measures of insulin resistance in clinical research. Other measures based on fasting insulin

include the Glucose to Insulin Ratio (GIR) and the Quantitative Insulin Sensitivity Index (QUICKI). The McAuley Index utilizes fasting insulin and triglycerides. Post-glucose challenge tests, done after an overnight fast, measure insulin and glucose response to a 75 g glucose load. Methods include the Matsuda Index and Insulin Sensitivity Index (ISI).

Other surrogate markers involve triglycerides alone or in relation to HDL cholesterol. Patients with prediabetes and triglyceride greater than or equal to 150 g/dL were more likely to have insulin resistance(32).

Measures of insulin resistance have not been integrated into clinical guidelines. As a result, the presence of insulin resistance is generally inferred from the clinical presentation. The Metabolic Syndrome (MetS) and Insulin Resistance Syndrome (IRS) are considered clinical indicators of insulin resistance.

Multiple criteria for metabolic syndrome exist. In 2009, a joint scientific statement harmonizing criteria for MetS was released. MetS is identified by the presence of 3 or more of the following diagnostic cut points:

- A waist circumference of 32” to 40” based on gender and race
- Elevated triglycerides greater than or equal to 150 mg/dL

- Reduced HDL less than 40 mg/ dL in men, less than 50 mg/ dL in women
- Elevated blood pressure greater than or equal to 130 mmHg systolic and/or greater than or equal to 85 mmHg diastolic
- Elevated fasting glucose greater than or equal to 100 mg/ dL

The American College of Endocrinology identify specific physiologic abnormalities which increase the risk of Insulin Resistance Syndrome as follows:

- Impaired glucose tolerance or impaired fasting glucose
- Abnormal uric acid metabolism
- Dyslipidemia (increased triglycerides, decreased HDL-C, or small, dense LDL)
- Hemodynamic changes such as elevated blood pressure
- Prothrombic factors (PAI-1, fibrinogen)
- Markers of inflammation (CRP, WBC, etc.)
- Endothelial dysfunction

Other factors include the following:

- Body mass index (BMI) greater than or equal to 25 kg/m2
- Diagnosis of CVD, PCOS, NAFLD, or acanthosis nigricans
- A family history of T2DM, hypertension, or CVD
- Sedentary lifestyle
- Non-Caucasian ethnicity
- Age greater than 40 years

MATERIALS AND METHODS

SOURCE OF DATA:

40 Newly diagnosed Type II diabetes mellitus patients (out patients) - in Government Rajaji Hospital Madurai during the study period of 6 months from March 2021 to August 2021.

SAMPLE SIZE - 40

DESIGN OF THE STUDY:

Hospital based cross sectional observational study

STUDY DURATION: 6 months (Mar 2021 – Aug 2021)

INCUSION CRITERIA:

All newly diagnosed Type 2 diabetes mellitus patients above 30 years of age who attended Medicine outpatient clinic irrespective of sex and socioeconomic status.

EXCLUSION CRITERIA:

1. Patients receiving zinc supplementation or taking drugs which modify metabolism of zinc
2. Patients taking oral hypoglycemic drugs or on insulin therapy
3. Pregnant woman
4. Persons suffering from chronic diseases were excluded from the study
5. .Patients with diseases such as acrodermatitis enteropathica, chronic diarrhea, malabsorption, liver failure,malignant tumours eg; carcinoma stomach.
6. Patients with acute complications of diabetes eg; DKA, HHS.

DATA COLLECTION AND METHODS:

After selection of cases, thorough history taking and clinical examination was performed and following investigations were done using the method mentioned below:

Total bilirubin was estimated using DMSO method

Aspartate aminotransferase was measured using NADH, kinetic UV method, IFCC

Alkaline phosphatase by p- Nitrophenylphosphate, kinetic method DGKC

Complete blood count using automated hematology analyzer

Renal Function Test: Blood Urea was measured using the Modified Berthelot method. Serum creatinine was measured by the alkaline Picrate method. eGFR was calculated using the CKD- EPI formula.

Urine routine examination: It was done especially for sugar and albumin using UroColor 2 Urine Test Strips.

Fasting lipid profile: It was measured in the Seimon‘s auto-analyzer.

ESTIMATION OF BLOOD GLUCOSE:

Method:

GOD/POD method (Glucose oxidase and Peroxidase Method)

Principle:

Glucose was oxidized to glucuronic acid and hydrogen peroxide. Hydrogen peroxide further reacts with phenol and 4-amino antipyrine by the catalytic action of peroxidase to form a red-colored quinone imine dye complex. The intensity of the colored complex was directly proportional to the amount of glucose present. Sample Material: Serum, plasma. Glucose was reported to be stable in the sample for 7days when stored at 2-8°C.

ESTIMATION OF SERUM ZINC:

Serum zinc is estimated by the Colorimetric method using a Semiauto analyzer which works on the principle that zinc in an alkaline medium reacts with 2-(5- nitro-2-pyridylazo)-5-{N-n-propyl N-(3-sulfopropyl) amino} phenol, disodium salt, dehydrate [NITRO PAPS] to form a purple-colored complex. The intensity of the complex formed is directly proportional to the amount of zinc present in the sample(33).

Zinc + Nitro-PAPS (in alkaline medium) ⟶ Purple colored complex

Contents:

L1-buffer reagent - 20mL

L2-colour reagent - 5mL

S-zinc standard (200 microgram/dl) - 2ml

Composition:

Borate buffer 290 mM; pH 8.2; Salicilaldoxime 10 mM; Dimethylglyoxime 1.0 mM; NITRO-PAPS 0.08mM; Surfactants and Preservatives.

ZINC KIT CONTAINING ZINC STANDARD, BUFFER REAGENT, COLOUR REAGENT.

Working reagent:

The contents were poured in 1 bottle of L2 (color reagent) into 1 bottle of L1 (Buffer Reagent). This working reagent was stable for at least 2 weeks when stored at 2-8 ℃(34).

Procedure:

Wavelength/filter: 570nm (Hg 578 nm)/Yellow

Temperature : room temperature

Light path : 1cm

Pipette into clean dry test tubes labeled as Blank (B), Standard (S), and Test (T)

The solutions were mixed well and incubated at room temperature (25 degree C) for 5 minutes. The absorbance of standard (Abs.S) and test sample (Abs.T) against the blank, within 20 minutes was measured.

Calculation:

Zinc in microgram/dl= (Abs.T /Abs.S) x 200

Normal Reference Values: 70-105 microgram/dL

FASTING INSULIN MEASUREMENTS:

Method: Radioimmunoassay; enzyme-linked immunosorbent assay (ELISA)

Specifics for collection and panels are as follows:

Specimen type: Blood serum

Container: Vacutainer, red top

Collection method: Venipuncture

Specimen volume: 1 mL

An 8-hour fasting specimen required

Fasting insulin normal reference value – 2 to 20mIU/l

INSULIN RESISTANCE SCORE (HOMA-IR) : **fasting plasma glucose (mg/dl) times fasting serum insulin (mU/l) divided by 405.**

STATISTICAL METHODS USED:

The collected data was analyzed by SPSS software. Pearson correlation coefficient and the P value were calculated to find the correlation and the statistical significance of the study respectively.

P< 0.05- Significant.

P> 0.05 – not significant

P < 0.0001 – highly significant

OBSERVATION AND RESULTS

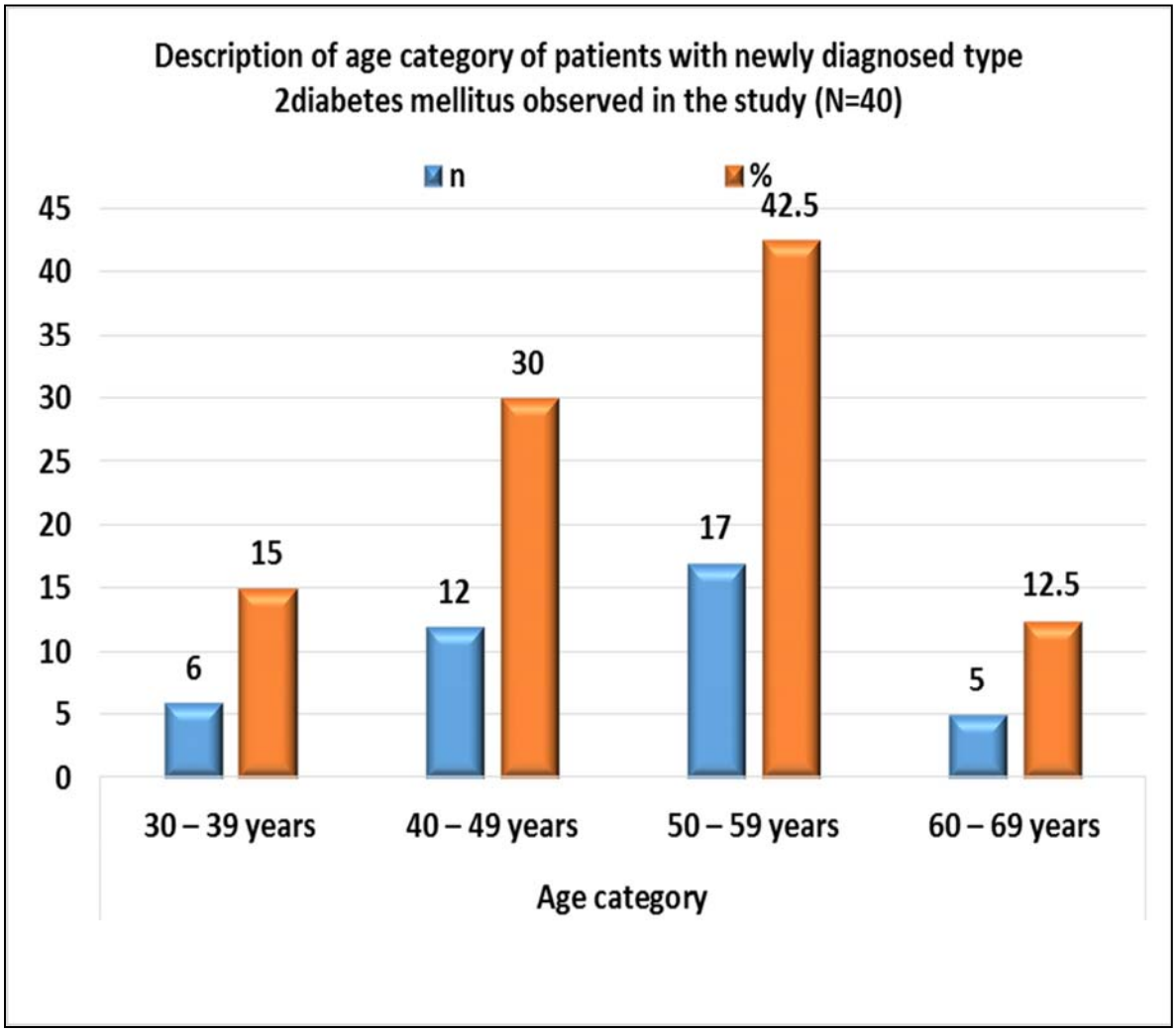
Statistical analysis:

The data were entered in MS office excel sheet and analyzed using SPSS version 16. Continuous data with normal distribution was expressed as mean with standard deviation. Categorical data were expressed as frequency with %. One Way ANOVA with Bonferroni post hoc test was used to compare the variance between three groups. Unpaired 't' test was used to compare the mean values between the two groups. Pearson's correlation test was performed to measure the direction and degree of association between various parameters. $P<0.05$ was considered statistically significant.

Table 1. Description of age category of patients with newly diagnosed type 2diabetes mellitus observed in the study

S.No	Age category	n	%
1	30 – 39 years	6	15
2	40 – 49 years	12	30
3	50 – 59 years	17	42.5
4	60 – 69 years	5	12.5

Data are expressed as n with %. Total N = 40.

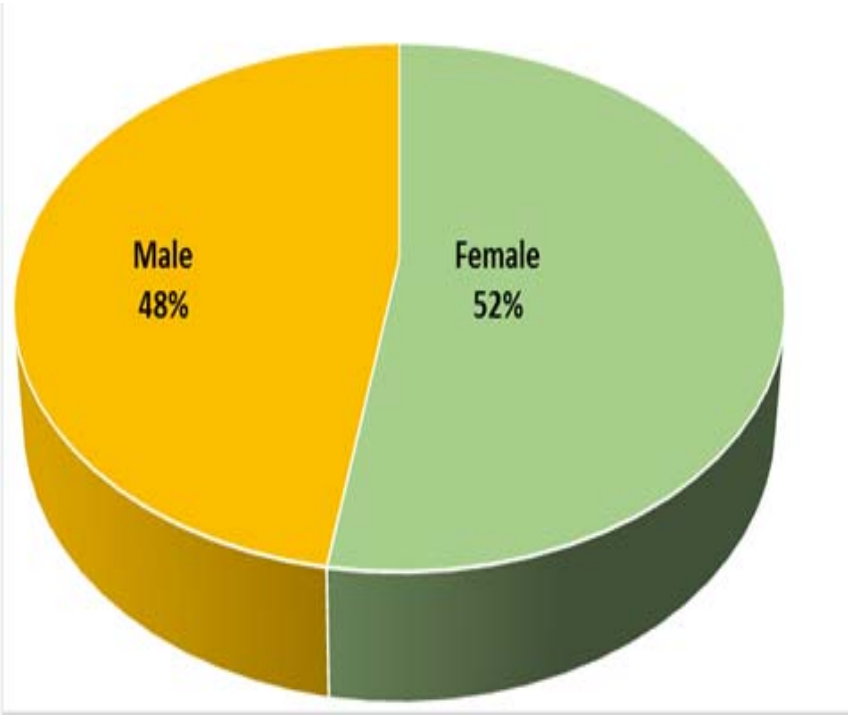


From the above age distribution table, it is evident that in our study population Type II diabetes mellitus patients are mostly occurring in patients in the age group of 50 to 59 .

Table 2. Description of gender category of patients with newly diagnosed type II diabetes mellitus observed in the study

S.No	Gender category	N	%
1	Female	21	52.5
2	Male	19	47.5

Data are expressed as n with %. Total N = 40.

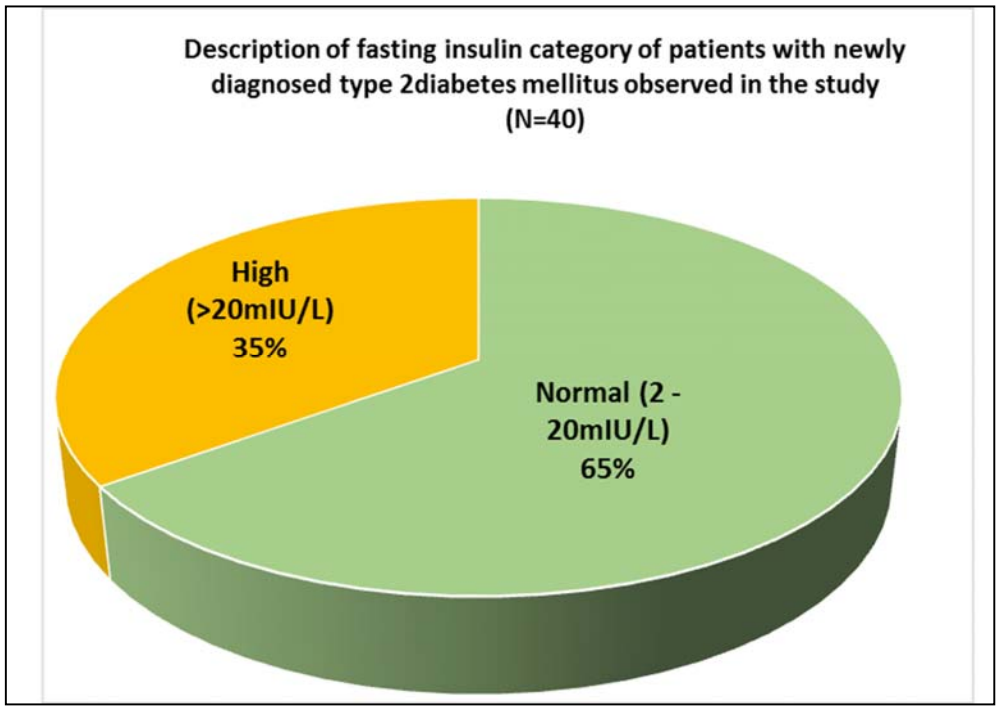


Among the gender distribution, 52% of diabetics are females whereas remaining 48% of diabetics are male in this study .

Table 3. Description of fasting insulin category of patients with newly diagnosed type 2diabetes mellitus observed in the study

S.No	Fasting insulin category	N	%
1	Normal (2 -20mIU/L)	26	65
2	High (>20mIU/L)	14	35

Data are expressed as n with %. Total N = 40.

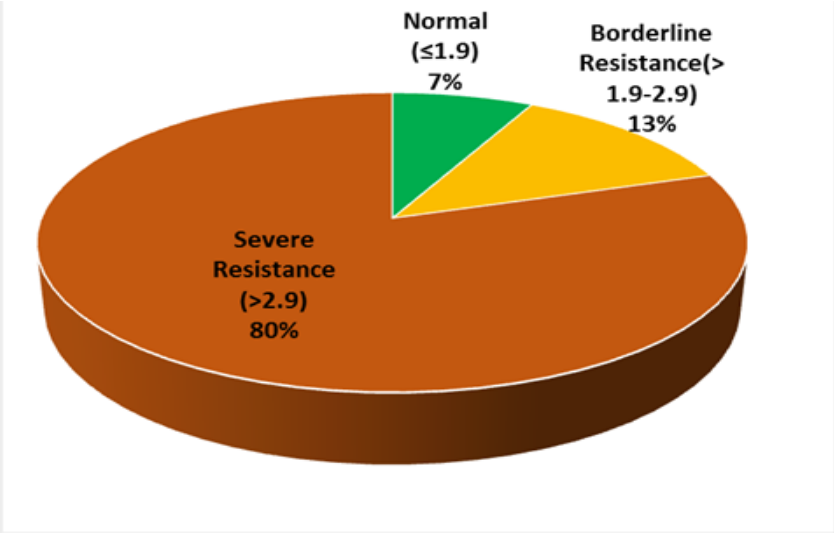


Among 40 patients participated in our study, 65% of patients had normal fasting insulin and 35% of patients had high fasting insulin .

Table 4. Description of insulin resistance in patients with newly diagnosed type 2diabetes mellitus observed in the study.

S.No	HOMA-IR Category	n	%
1	Normal (≤1.9)	3	7.5
2	Borderline Resistance(>1.9-2.9)	5	12.5
3	Severe Resistance (>2.9)	32	80

Data are expressed as n with %. Total N = 40.



Description of Insulin resistance in newly diagnosed type II diabetes mellitus observed in this study.(n=40)

The above pie chart and frequency distribution table shows that 80% of newly diagnosed type II diabetes mellitus patients had severe insulin resistance(HOMA-IR >2.9), whereas only 13% of Patients had borderline insulin resistance(1.9 -2.9).

Table 5. Description of Zinc level category of patients with newly diagnosed type 2diabetes mellitus observed in the study.

S.No	Zinc level Category	N	%
1	Low (<70mcg/dL)	23	57.5
2	Normal (≥70 mcg/dL)	17	42.5

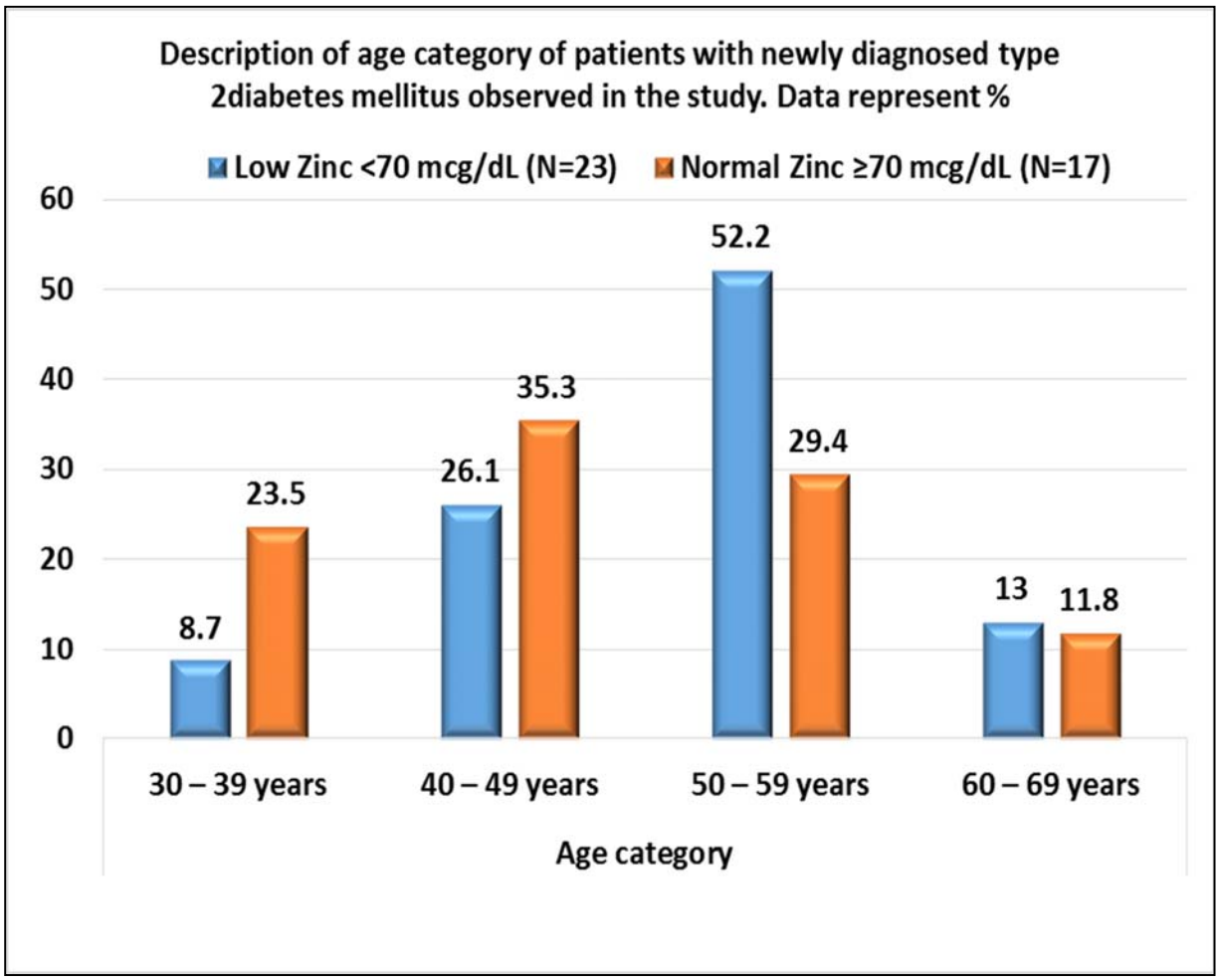
Data are expressed as n with %. Total N = 40.

The above table shows that 57.5% of newly diagnosed type II diabetes mellitus patients had low serum zinc(<70 mcg/dl) and 42.5% of Patients had normal serum zinc(>70mcg/dl) .

Table 6. Comparison of age category of newly diagnosed type 2 diabetes mellitus patient with respect to zinc level category.

S.No	Age category	Low Zinc <70 mcg/dL (N=23)		Normal Zinc ≥70 mcg/dL (N=17)		Chi square value	df	P value
		N	%	N	%			
1	30 – 39 years	2	8.7	4	23.5	2.9	3	0.405 (NS)
2	40 – 49 years	6	26.1	6	35.3			
3	50 – 59 years	12	52.2	5	29.4			
4	60 – 69 years	3	13	2	11.8			

Data are expressed as n with %. Fisher's exact test was used to compare the frequency between the groups. NS = Not significant.

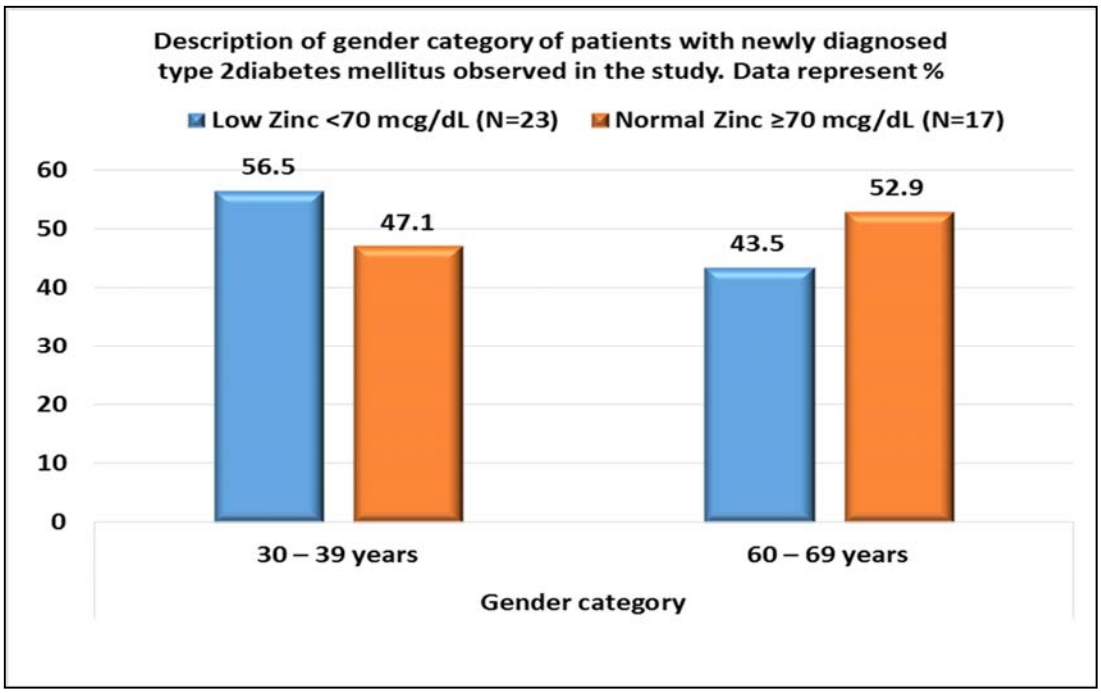


The above frequency distribution table shows 52.2% of type2 diabetes mellitus patients with low zinc level are in the age group of 50-59 years wherease type2 diabetes mellitus patients with normal zinc level are highest in the age group of 40-49 years(35.3%).however this association is not statistically significant with chi-square value of 2.9 and p value of 0.405.

Table 7. Comparison of gender category of newly diagnosed type 2 diabetes mellitus patient with respect to zinc level category.

S.No	Gender category	Low Zinc <70 mcg/dL (N=23)		Normal Zinc ≥70 mcg/dL (N=17)		Chi square value	Df	P value
		N	%	N	%			
1	Female	13	56.5	8	47.1	0.351	1	0.751 (NS)
2	Male	10	43.5	9	52.9			

Data are expressed as n with %. Fisher's exact test was used to compare the frequency between the groups. NS = Not significant.



Above bar diagram shows low zinc levels are higher in female patients (56.5%) whereas 52.9% of patients with normal zinc levels are males.however this association is not statistically significant with chi-square value of 0.351 and p value of 0.751.

Table 8. Comparison of various parameters of newly diagnosed type 2 diabetes mellitus patient with respect to zinc level category.

S.No	Parameter	Low Zinc <70 mcg/dL (N=23)		Normal Zinc ≥70 mcg/dL (N=17)		T value	df	P value
		Mean	SD	Mean	SD			
1	Age in years	51.8	8.9	48.4	9.6	1.16	38	0.251 (NS)
2	BMI	30.5	3.7	29.6	4.9	0.684	38	0.498 (NS)
3	FBS (mg/dL)	144.5	17.5	150.8	18.9	1.07	38	0.288 (NS)
4	PPBS (mg/dL)	236	27	230	24.4	0.678	38	0.502 (NS)
5	Blood Urea (mg/dL)	27.1	6.3	25.7	6.4	0.69	38	0.494 (NS)
6	Sr.creatinine (mg/dL)	0.96	0.18	0.9	0.18	1.08	38	0.283 (NS)
7	Hb (g/dL)	13.1	0.84	13.4	0.66	0.19	38	0.235 (NS)

Data are expressed as mean with SD. Unpaired ‘t’ test was used to compare the means between the group. NS = Not significant.

Table 9. Comparison of liver function test parameters of newly diagnosed type 2 diabetes mellitus patient with respect to zinc level category.

S.No	Parameter	Low Zinc <70 mcg/dL (N=23)		Normal Zinc ≥70 mcg/dL (N=17)		T value	df	P value
		Mean	SD	Mean	SD			
1	Sr. Bilirubin (mg/dL)	0.55	0.13	0.59	0.11	1.05	38	0.298 (NS)
2	SGOT (IU/L)	32.3	6.9	30.7	8.01	0.669	38	0.507 (NS)
3	SGPT (IU/L)	35.1	8.2	35.9	11.06	0.281	38	0.781 (NS)
4	ALP (IU/L)	64.3	14.9	67.4	13.6	0.677	38	0.502 (NS)

The above table shows there is no significant association between serum bilirubin,liver enzymes and serum zinc level in type II diabetes .

Data are expressed as mean with SD. Unpaired 't' test was used to compare the means between the group. NS = Not significant

Table 10. Comparison of lipid profile parameters of newly diagnosed type 2 diabetes mellitus patient with respect to zinc level category.

S.No	Parameter	Low Zinc <70 mcg/dL (N=23)		Normal Zinc ≥70 mcg/dL (N=17)		T value	df	P value
		Mean	SD	Mean	SD			
1	LDL (mg/dL)	141.6	25	133	24	0.987	38	0.33 (NS)
2	TGL (mg/dL)	141	25	141	26.7	0.04	38	0.969 (NS)
3	HDL (mg/dL)	41.4	7.2	42.7	4.4	0.643	38	0.524 (NS)
4	Cholesterol (mg/dL)	211	25	202	30	1.03	38	0.307 (NS)
5	VLDL (mg/dL)	16.3	8.5	14.8	5.27	0.628	38	0.534 (NS)

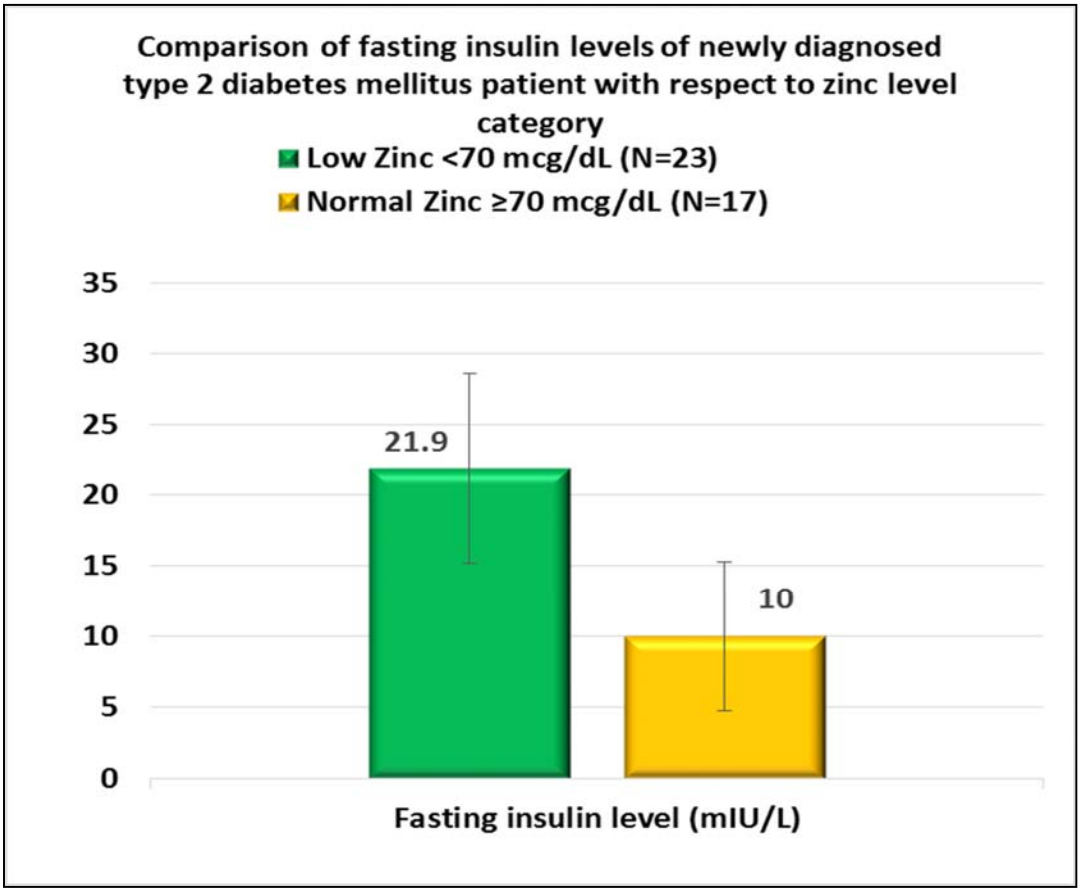
Data are expressed as mean with SD. Unpaired 't' test was used to compare the means between the group. NS = Not significant

The above table shows there is no significant association between serum lipid profile and serum zinc level in type II diabetes

Table 11. Comparison of fasting insulin levels of newly diagnosed type 2 diabetes mellitus patient with respect to zinc level category.

S.No	Parameter	Low Zinc <70 mcg/dL (N=23)		Normal Zinc ≥70 mcg/dL (N=17)		T value	df	P value
		Mean	SD	Mean	SD			
1	Fasting insulin level (mIU/L)	21.9	6.7	10	5.26	6.05	38	<0.0001*

Data are expressed as mean with SD. Unpaired ‘t’ test was used to compare the means between the group. *indicates $p<0.05$ and considered statistically significant.



The above table shows mean fasting insulin level in typeII diabetes with low zinc level is 21.9mIU/l with SD of 6.7mIU/l. mean fasting insulin level in typeII diabetes with normal zinc level is 10mIU/l with SD of 5.26mIU/l

This association is statistically significant with p value of 0.0001.

Table 12. Comparison of HOMA-IR value of newly diagnosed type 2 diabetes mellitus patient with respect to zinc level category.

S.No	Parameter	Low Zinc <70 mcg/dL (N=23)		Normal Zinc ≥70 mcg/dL (N=17)		T value	df	P value
		Mean	SD	Mean	SD			
1	HOMA-IR value	8.19	2.8	3.7	2.1	5.41	38	<0.0001*

Data are expressed as mean with SD. Unpaired 't' test was used to compare the means between the group. *indicates $p<0.05$ and considered statistically significant.

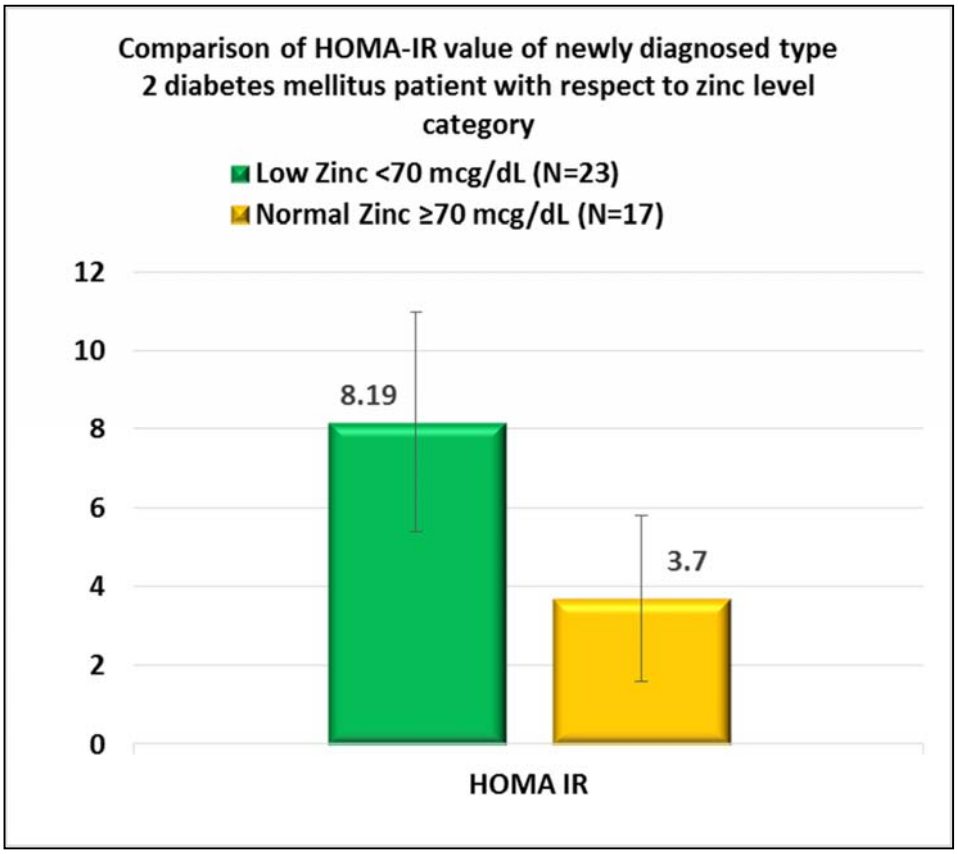


Table 13. The above table shows mean HOMA-IR value in typeII diabetes with low zinc level is 8.19 with SD of 2.8. mean HOMA-IR value in typeII diabetes with normal zinc level is 3.7 with SD of 2.1

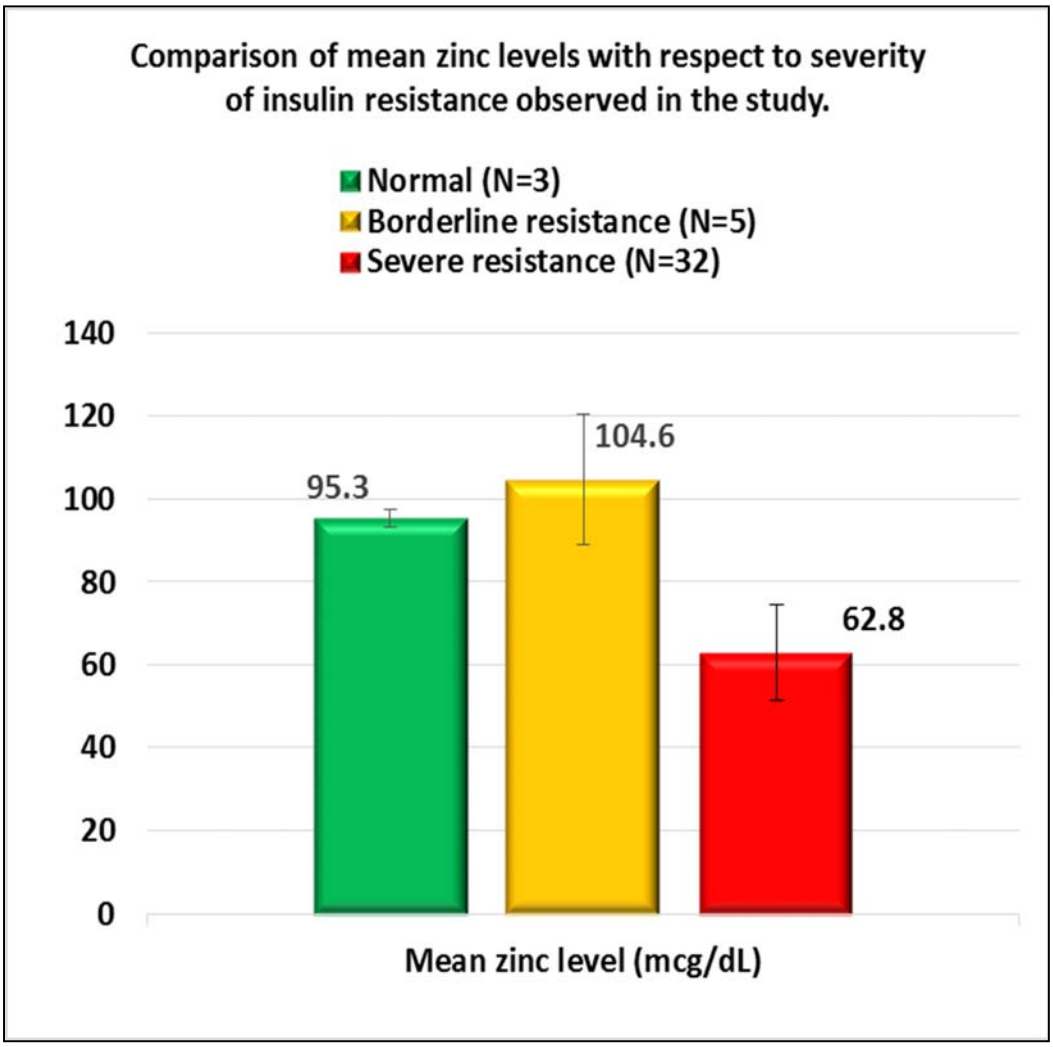
This association is statistically significant with p value of 0.0001.

Comparison of mean zinc levels with respect to severity of insulin resistance observed in the study.

S. No	Parameter	Severity of insulin resistance						F value	df	P value
		Normal (N=3)		Borderline resistance (N=5)		Severe resistance (N=32)				
		Mean	SD	Mean	SD	Mean	SD			
1	Mean zinc level (mcg/dL)	95.3	2.08	104.6	15.8	62.8	11.7	33.3	2, 37	<0.0001*

Post hoc analysis					
S.No	Group vs Group	P value	S.No	Group vs Group	P value
1	Normal Vs Borderline	0.886 (NS)	3	Borderline Vs severe	<0.0001*
2	Normal Vs Severe	<0.0001*			

Data are expressed as n with %. One way ANOVA with Bonferroni post hoc test was used to compare the variances between the groups. *indicates $p<0.05$ and considered statistically significant.



The above table and bar diagramme shows there is significant association between mean zinc level and severity of insulin resistance. Mean zinc level in severe insulin resistance is lower with value of 62.8 microgram/dl and SD of 11.7 mic gram/dl

Table 14. Correlation of zinc levels with respect to various factors observed in the study

S.No	Correlation of zinc levels with	Pearson's r	P value	Inference
1	Age in years	-0.255	0.113 (NS)	No correlation
2	BMI	-0.141	0.388(NS)	No correlation
3	FBS mg/dL	-0.014	0.931(NS)	No correlation
4	PPBS mg/dL	-0.234	0.145(NS)	No correlation
5	Blood Urea (mg/dL)	0.014	0.931(NS)	No correlation
6	Sr.creatinine (mg/dL)	-0.237	0.141(NS)	No correlation
7	Hb (g/dL)	0.208	0.198(NS)	No correlation
8	Sr. Bilirubin (mg/dL)	0.079	0.627(NS)	No correlation
9	SGOT (IU/L)	-0.181	0.264(NS)	No correlation
10	SGPT (IU/L)	-0.048	0.767(NS)	No correlation
11	ALP (IU/L)	0.088	0.589(NS)	No correlation
12	LDL (mg/dL)	-0.252	0.117(NS)	No correlation
13	TGL (mg/dL)	0.123	0.449(NS)	No correlation
14	HDL (mg/dL)	0.088	0.591(NS)	No correlation
15	Cholesterol (mg/dL)	-0.201	0.215(NS)	No correlation
16	VLDL (mg/dL)	-0.059	0.718(NS)	No correlation
17	Fasting insulin level (mIU/L)	-0.788	<0.0001*	Significant negative correlation of strong strength
18	HOMA-IR value	-0.757	<0.0001*	Significant negative correlation of strong strength

Total N = 40. Correlation was performed using Pearson's correlation test. Degree and direction of association was represented using 'r' value. *indicates $p<0.05$ and considered statistically significant. The above table shows there is significant negative correlation between fasting insulin level, HOMA-IR value and serum zinc level.

DISCUSSION

Our study was mainly conducted to access the correlation of serum zinc level and Insulin resistance in newly diagnosed TypeII diabetes mellitus patients.

Our study population consisted of 40 newly diagnosed TypeII diabetes mellitus patients. FBS,PPBS levels were measured in all those 40 patients along with routinely performed tests like complete blood count,serum bilirubin,SGOT,SGPT,RFT, FLP.

Analysis was done to study the correlation between the levels of serum zinc and insulin resistance in newly diagnosed typeII diabetes mellitus patients. The Following observations were made from our study(33).

Age distribution:

Out of 40 study population, most of the patients were in the 50 – 59 years age group (42.5%). This showed that typeII diabetes mellitus is most commonly seen in 50-59 years of age in our study population.

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Sex distribution:

Out of 40 study group population, 21 patients were females (52%) and the remaining 19patients (48%) were males.

Fasting insulin level:

Among the 40 study population, 26 patients had fasting insulin levels less than 20mIU/L which accounts for 65% of the patients. 16 patients (35%) had fasing insulin levels more than 20mIU/l .This shows that majority of our study population had normal fasting insulin levels.

HOMA-IR:

In our study population 32 patients (80%) had severe insulin resistance and 5 patients (12.5%) patients had borderline insulin resistance.

Serum zinc:

In this study population 52.2% of type2 diabetes mellitus patients with low zinc level are in the age group of 50-59 years.but type2 diabetes mellitus patients with normal zinc level are highest in the age group of 40-49 years(35.3%).

CORRELATION BETWEEN SERUM ZINC AND FASTING BLOOD GLUCOSE:

In this study, we found that there is no significant association between seum zinc level and fasting blood glucose in newly diagnosed type to diabetes maellitus patients.

CORRELATION BETWEEN SERUM ZINC AND FASTING INSULIN LEVEL:

In this study, we found that serum zinc levels are negatively correlated with serum fasting insulin level with patients with newly diagnosed typeII diabetes mellitus and the p value <0.0001 which was significant statistically. This is comparable with the outcome of study done by Bang-In Ahn & Moon Jong Kim et al(35).

CORRELATION BETWEEN SERUM ZINC AND INSULIN RESISTANCE:

In this study, we found that serum zinc levels are negatively correlated with serum insulin resistance with patients with newly diagnosed typeII diabetes mellitus and the p value <0.0001 which was significant statistically. This is comparable with the outcome of study done by Bang-In Ahn & Moon Jong Kim.

LIMITATIONS OF THE STUDY

- Size of the study population is small.
- It is a single centered study.
- This is the cross-sectional design, we could not determine the cause-effect relationship between serum zinc concentrations and insulin resistance.
- However, previous studies have demonstrated that lower zinc intake precedes insulin resistance or diabetes risk . An additional longitudinal study will contribute to verifying the cause-effect relationship between serum zinc concentrations and insulin resistance.
- We used HOMA-IR as an index of the insulin resistance. Although the gold standard of evaluating the insulin resistance is the euglycemic hyperinsulinemic clamp test that measures IR both directly and accurately, it is an invasive and time-consuming procedure. HOMA-IR is a less invasive and inexpensive method to measure insulin resistance, and it has been widely validated and applied for quantifying insulin resistance(22).
- All the subjects were from the same centre studies for the subjects with different ethnic backgrounds can be compared to our results.

CONCLUSION

In conclusion, serum zinc concentration is inversely associated with insulin resistance in newly diagnosed type-II diabetes mellitus patients.

Further prospective studies on the relationship of serum zinc concentrations with insulin resistance and metabolic risk factors should be performed in a large cohort of various ethnic groups.

Furthermore, zinc-based intervention study in patients with insulin resistance will be helpful for the clinical application of zinc supplementation.

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PROFORMA

NAME:

AGE:

SEX:

O.P NO:

OCCUPATION & ADDRESS:

DURATION OF DIABETES MELLITUS:

SYMPTOMS

Polyuria	Oliguria	Visual disturbances
Polydypsia	Puffiness of Face	Weight loss/gain
Polyphagia	Swelling of legs	High coloured urine
Giddiness	Anorexia	Headache/ LOC/ Seizures Altered sensorium
Chest Pain	Vomiting/Hiccup	Sensory disturbances
Palpitation	Easy fatiguability	Poor wound healing
Leg ulcers	Dyspnea	Altered bowel habit
Limb weakness	Fever	Dysuria Abdominal Pain

PAST HISTORY •

* Hypertension * Renal disorders *CAD * CVA

*Seizure disorder * Pulmonary TB *Surgical illness

* Blood transfusion *Hypoglycemic attacks *Thyroid Disorder

* Liver disorders * Drug intake- Ethambutol ,Penicillamine, Iron, Dietary fibres, Sodium valproate.

PERSONAL HISTORY

• Smoking

• Alcoholism

• Drug abuse

• STD

• Prolonged Starvation

• Exercise

ANTHROPOMETRY

Ht: cms

Wt: kgs

BMI:

Hydration Status

FUNDUS

•Features of Thyroid Disorders:

•Features of Cushing's Syndrome:

• Pulse:

• Blood Pressure:

SYSTEMIC EXAMINATION:

Cardiovascular system:

Respiratory system:

Abdomen:

Central nervous system:

INVESTIGATIONS:

- 1.FBS,PPBS
- 2. Fasting insulin level
- 3. Serum Zinc
- 4. Complete blood count
- 5. Liver function test
- 6. Fasting lipid profile
- 7. Serum urea,creatinine

ABBREVIATIONS

ADA : American Diabetes Association

AGE : Advanced Glycosylation End products

BMI : Body Mass Index

CAD : Coronary Artery Disease

Cu : Copper

CVA : Cerebro Vascular Accident

DCCT : Diabetes Control and Complication Trial DAG : Diacyl Glycerol

DM : Diabetes Mellitus

ECM : Extra Celllular Matrix

FBS : Fasting Blood Sugar

GLUT : Glucose Uptake and Transport

HBA1C : Glycosylated Haemoglobin

HNF : Hepatocyte nuclear transcription factor

IL : Interleukin

MDA : Malondialdehyde

MODY : Maturity onset diabetes of Young

PAD : Peripheral Artery Disease

RDA : Recommended daily allowance

SOD : Superoxide dismutase

STZ : Streptozocin

TNF : Tumor necrosis factor

Zn : Zinc

CONSENT FORM

ஆராய்ச்சி ஒப்புதல் படிவம்:

பெயர்:

வயது:

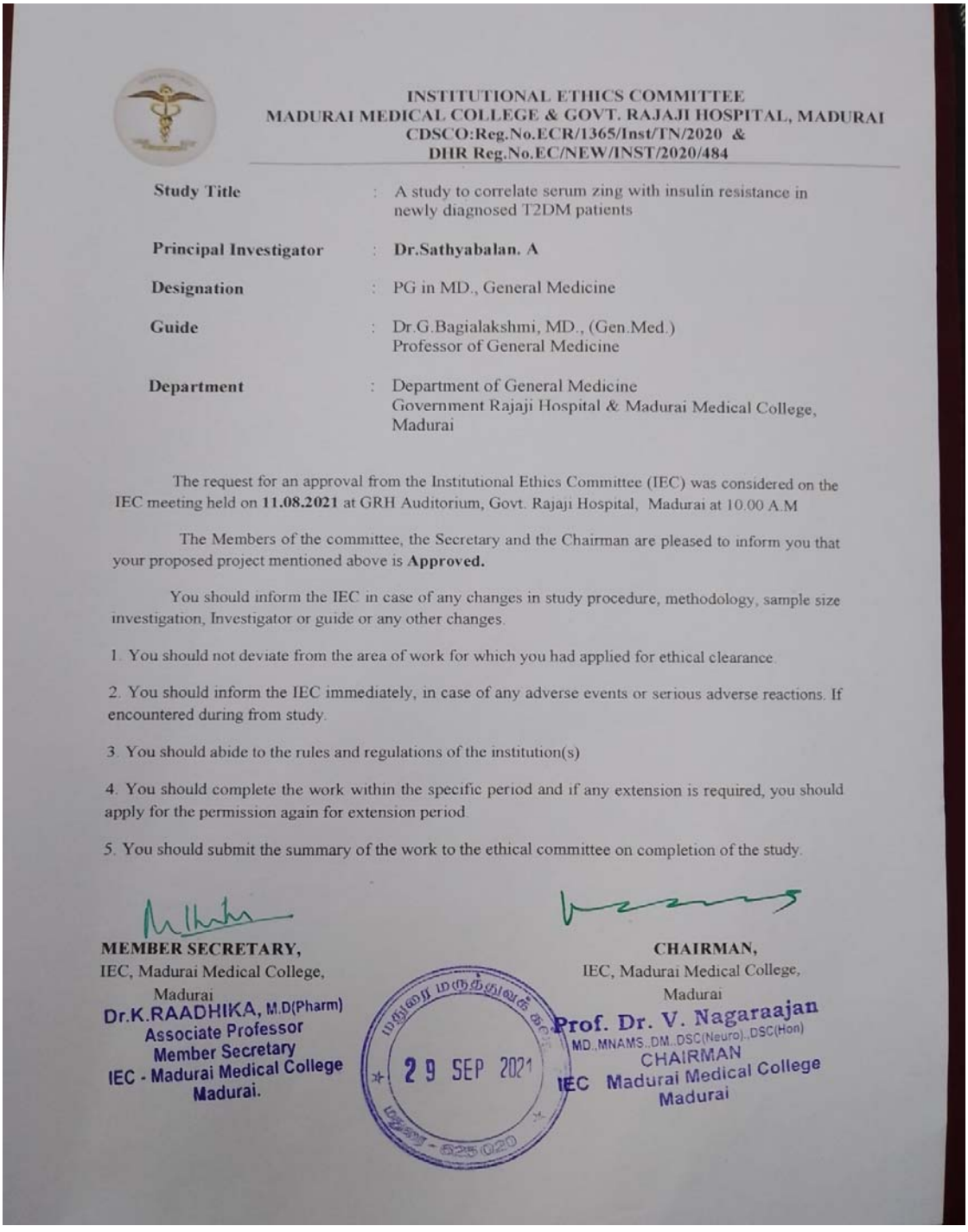
தேதி:

நோயாளிஎண்:

ஆராய்ச்சிசேர்க்கைஎண்:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு விளக்கபட்டது. எனக்கு விளக்கபட்ட விஷயங்களை நான் புரிந்துகொண்டு எனது முழுமனதுடன் சம்மதிக்கிறேன். இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்குபெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியில் இருந்து எந்த நேரமும் பின்வாங்கலாம் என்றும் அதனால் எந்த பாதிப்பும் எனக்கு ஏற்படாது என்பதையும் புரிந்துகொண்டேன். நான் என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் பங்குகொள்ள சம்மதிக்கிறேன்.

ETHICAL APPROVAL LETTER



INSTITUTIONAL ETHICS COMMITTEE
MADURAI MEDICAL COLLEGE & GOVT. RAJAJI HOSPITAL, MADURAI
CDSCO:Reg.No.ECR/1365/Inst/TN/2020 &
DHR Reg.No.EC/NEW/INST/2020/484

Study Title	:	A study to correlate serum zing with insulin resistance in newly diagnosed T2DM patients
Principal Investigator	:	**Dr.Sathyabalan. A**
Designation	:	PG in MD., General Medicine
Guide	:	Dr.G.Bagialakshmi, MD., (Gen.Med.) Professor of General Medicine
Department	:	Department of General Medicine Government Rajaji Hospital & Madurai Medical College, Madurai

The request for an approval from the Institutional Ethics Committee (IEC) was considered on the IEC meeting held on **11.08.2021** at GRH Auditorium, Govt. Rajaji Hospital, Madurai at 10.00 A.M

The Members of the committee, the Secretary and the Chairman are pleased to inform you that your proposed project mentioned above is **Approved.**

You should inform the IEC in case of any changes in study procedure, methodology, sample size investigation, Investigator or guide or any other changes.

1. You should not deviate from the area of work for which you had applied for ethical clearance.

2. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions. If encountered during from study.

3. You should abide to the rules and regulations of the institution(s)

4. You should complete the work within the specific period and if any extension is required, you should apply for the permission again for extension period.

5. You should submit the summary of the work to the ethical committee on completion of the study.

MEMBER SECRETARY,
IEC, Madurai Medical College,
Madurai
Dr.K.RAADHIKA, M.D(Pharm)
Associate Professor
Member Secretary
IEC - Madurai Medical College
Madurai.

29 SEP 2021

CHAIRMAN,
IEC, Madurai Medical College,
Madurai
Prof. Dr. V. Nagaraajan
MD.,MNAMS.,DM.,DSC(Neuro).,DSC(Hon)
CHAIRMAN
IEC Madurai Medical College
Madurai

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Sources included in the report

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W	URL: https://www.aafp.org/afp/2016/0115/p103.html Fetched: 2019-09-26T13:47:14.2030000	1

ANTI PLAGIARISM CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY TO CORRELATE SERUM ZINC WITH INSULIN RESISTANCE IN NEWLY DIAGNOSED TYPE II DIABETES MELLITUS PATIENTS"** of the candidate **Dr.SATHYABALAN A** with **Registration Number 201911117** for the award of M.D degree in the branch of GENERAL MEDICINE. I personally verified the urkund.com report for the purpose of plagiarism check. I found that the uploaded thesis file containing from introduction to conclusion pages and result shows 9% percentage of plagiarism in the dissertation.

Place : Madurai
Date :

Prof.Dr.G.BAGIALAKSHMI.,M.D.,
Professor of Medicine,
Department of General Medicine,
Government RajajiHospital,
Madurai Medical College,
Madurai.

MASTER CHART

SL. NO.	AGE yrs	SEX	BMI	FBS mg/dl	PPBS mg/dl	FASTING INSULIN Miu	HOMA-IR	B.UREA mg/dl	S.CREAT mg/dl	LDL mg/dl	TGL mg/dl	HDL mg/dl	CHOL mg/dl	VLDL mg/dl	HB%	S.BILIRUBIN mg/dl	SGOT	SGPT	ALP	S.ZINC mic g/dl
1	45	M	27	130	210	24	7.7	19	0.8	120	140	40	188	22	13.8	0.5	20	28	52	54
2	39	M	29.6	113	198	11	3.06	17	0.9	130	125	48	203	13	14.2	0.6	32	29	44	64
3	54	F	34	145	223	23	8.23	25	1.1	158	194	34	231	32	12.5	0.8	40	51	74	51
4	34	M	32	130	185	11.5	3.69	20	0.7	154	142	41	223	11	14.6	0.7	32	37	84	71
5	55	M	35	145	211	18.2	6.51	36	1.2	171	148	45	245	22	13.6	0.6	30	30	54	64
6	43	F	32.4	132	256	34	11.08	23	1.1	182	164	39	254	27	11.9	0.4	38	37	61	56
7	54	F	32.3	156	226	19.8	11.04	30	1.2	174	150	26	230	15	12.6	0.7	41	47	77	51
8	51	F	36	128	216	19.3	6.09	21	1.2	155	148	45	229	21	12.7	0.8	28	26	51	65
9	32	M	30.5	133	213	11.8	3.87	32	0.7	128	137	41	196	11	13	0.4	19	24	71	69
10	57	F	25.5	142	255	29	10.16	31	0.9	169	164	40	242	26	12.3	0.8	31	28	92	57
11	58	F	36	188	297	26	12.06	34	0.8	180	174	34	249	14	11.6	0.7	28	28	59	51
12	65	M	32	144	259	18.4	6.54	21	0.8	123	142	43	194	7	13.5	0.6	39	44	58	59
13	57	M	31.9	159	207	24	9.42	22	0.9	177	155	51	259	9	11.9	0.6	41	47	77	71
14	66	F	39	188	269	13.8	6.4	25	1.1	122	134	49	157	15	12.6	0.7	18	17	49	78
15	56	F	28	139	212	7	2.4	22	0.8	108	132	41	175	17	13.9	0.8	29	33	79	91
16	69	M	31.5	127	214	17	8.98	34	0.7	149	179	36	221	4	12.7	0.4	31	45	87	69
17	45	M	25.6	131	220	9.6	3.1	34	1.2	96	140	41	165	15	13.9	0.5	17	19	65	87
18	37	M	24.5	149	211	4	1.47	22	0.7	112	119	43	178	9	13.2	0.7	24	47	84	97
19	55	M	34	157	277	29	11.24	29	1.2	110	139	39	176	22	14.2	0.6	29	31	79	59
20	47	F	39	187	287	10.9	5.03	22	1	115	140	44	187	10	13.1	0.5	37	39	63	77
21	57	F	33	145	213	7.2	2.57	21	1.1	141	198	41	221	17	12.9	0.6	40	42	79	102
22	54	F	29.2	145	228	7.5	2.68	25	0.8	132	155	44	206	14	13.1	0.7	40	55	57	91

23	41	F	33.4	177	256	31	13.54	29	0.9	139	112	53	214	11	11.7	0.5	37	28	81	55
24	61	M	24	145	245	14.9	5.33	21	0.9	159	149	44	232	16	12.9	0.5	22	26	55	79
25	46	M	28	129	237	9.2	2.93	22	0.7	177	114	39	239	15	13.2	0.7	32	33	49	87
26	33	M	22.5	139	235	7.9	2.7	29	0.9	145	177	51	231	11	14.2	0.6	39	29	66	110
27	56	M	32.9	158	224	4	1.56	33	1.1	131	166	39	203	9	13.6	0.4	32	41	67	96
28	39	F	30.7	141	219	6.3	2.19	37	0.7	104	114	41	168	30	13.1	0.5	24	29	49	129
29	59	M	22.9	143	224	29	10.2	19	0.7	102	141	51	181	27	12.9	0.4	36	36	44	49.6
30	49	F	31.5	164	258	29	11.7	22	0.9	145	114	49	216	11	14.6	0.5	27	32	55	51
31	54	F	27.9	155	212	16.2	6.2	20	0.7	133	155	36	200	16	14.1	0.5	31	32	59	67
32	44	M	32.5	177	251	3.9	1.7	24	0.8	147	166	45	225	18	13.7	0.6	24	29	77	93
33	59	M	29	166	241	9.1	3.72	29	0.9	129	112	34	185	7	12.9	0.5	18	23	51	51
34	64	F	31.3	129	211	24	7.64	34	1.1	144	166	34	211	11	13.5	0.5	39	39	67	56
35	51	F	23.4	133	239	15	4.92	28	1.2	98	123	49	171	6	13.6	0.4	34	39	77	69
36	43	F	29.2	141	290	22	7.65	32	1.1	109	93	53	181	32	14.1	0.6	36	39	43	43
37	52	M	25.5	133	219	24	7.88	39	1.2	166	107	49	236	6	12.4	0.5	39	44	57	51
38	48	F	24.9	136	226	17.3	5.8	40	1.2	122	102	39	181	15	13.7	0.5	33	33	56	71
39	43	F	25.6	166	246	11	4.5	19	0.7	134	101	34	188	21	14.2	0.5	39	55	91	78
40	44	F	32.6	144	223	24	8.5	21	0.9	144	133	36	206	12	12.9	0.4	41	47	87	51