"A CROSS-SECTIONAL STUDY TO ESTIMATE SERUM ZINC AND HDL LEVELS IN TYPE 2 DIABETES MELLITUS PATIENTS".

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LIST OF ABBREVIATIONS USED

Zn	-	Zinc
T2DM	-	Type 2 Diabetes mellitus
DM	-	Diabetes mellitus
HDL	-	High density lipoprotein
LDL	-	Low density lipoprotein
IZiNCG	-	International Zinc Nutrition Consultative Group
WHO	-	World health organization
IDF	-	International Diabetes Federation
NCDs	-	NNON Communicable Diseases
CAD	-	Coronary Artery Disease
MT	-	Metallothionein
ZnT	-	Zinc Transporter
Zip	-	Zrt/ Irt-like protein
Slc family	-	Solute carrier family
MRE	-	Metal response element
MTF1	-	MRE – binding transcription factor 1
IDDM	-	Insulin dependent diabetes mellitus
GSK3	-	Glycogen synthase kinase 3
PKB	-	Protein kinase B
PI3 –kinase	-	Phosphoinositol 3 kinase
LDL-c	-	Low density lipoprotein-cholesterol

HDL-c	-	High density lipoprotein-cholesterol
VLDL	-	Very low density lipoprotein
apo-B	-	Apolipoprotein-B
CETP	-	Cholesterol ester transfer protein
FFA	-	Free fatty acid
TRLs	-	Triglyceride rich lipoproteins
LPL	-	Lipoprotein lipase
HL	-	Hepatic lipase
RCT	-	Reverse cholesterol transport
TG	-	Triglycerides
ABCA1	-	ATP-binding cassette transporter A1
SR-B1	-	Scavenger receptor – B1

ABSTRACT

Background and Objectives:

Trace minerals have been a subject of interest for a long time and reports citing as early as 1930s could be seen as a proof of document on the studies being conducted in the trace minerals aspect of biochemistry. There has been a rising interest as to how the trace minerals act or bring about their effect in the human health. Of particular interest among the trace minerals are the zinc and copper. The interaction of these minerals has been noted in many of the enzymatic reactions of our body. These micro minerals or the trace minerals as called are required for the optimum human health mainly because of their involvement in various metabolic reactions. Their interactions range from various catalytic, structural and regulatory functions during which these elements of importance react with the so called macro molecules i.e. enzymes, pro-hormones and biological membranes. The zinc has been found to amplify the effectiveness of insulin *in-vitro* and also there are many studies suggesting that zinc deficiency may aggravate the insulin resistance in the diabetics and zinc replenition could improve the insulin sensitivity. DM being one of the most important non communicable disease and also as the incidence is seen to be increasing not only in the developed countries but also in the developing countries like India and Africa the effective measures to prevent or minimize the incidence of occurrence or the complications arising out the diabetes have to be considered significantly. As zinc supplementation studies have shown to improve the glycemic control in diabetics the supplementation of zinc could be used for long time to prevent the complications arising out of the uncontrolled blood glucose levels over prolonged periods. Few other zinc supplementation studies have also shown to reduce the HDL-cholesterol concentrations in the diabetic patients. Thus we estimated serum zinc and HDL levels

in patients of type 2 DM and non-diabetic subjects and correlate levels of zinc & HDL in cases without any zinc supplementation to see the relation of zinc and HDL-cholesterol.

Materials and methods:

All cases of type 2 diabetes mellitus from 35 to 50 years admitted or attending Medicine unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. Based on calculations from the power of the previous study sample size were estimated 35 cases of type 2 diabetes mellitus. 35 controls were also selected depending on the age and sex match criteria.

10 ml of blood was collected from the patients and controls under aseptic precautionary measures using disposable syringe in heparinized tubes, centrifuged, serum separated and estimated for HDL-cholesterol and kept at -20^0C for the estimation of serum zinc which was analyzed within 30 days from the sample collection.

Methods of assay

Serum

Zinc – by flame Atomic absorption spectrophotometry (AAS).

HDL – Phosphotungstate/Magnesium precipitation method.

Results

The mean serum value of zinc and HDL-c in cases was 43.03±12.11µg/dl and 29.26 ± 2.42 mg/dl. Statistical analysis carried out by using unpaired student's t – test showed significantly reduced serum zinc and HDL-c in cases when compared to controls (p-value<0.001). The mean serum values of zinc and HDL-c in controls was

100.43±11.02 µg/dl and 40.11±4.15 mg/dl. There was significant decrease in the level of serum zinc in cases when compared to controls. Co-relation was done using Karl Pearson's co-relation coefficient method between the levels of zinc and HDL-c in the cases showed positive co-relation but it is not statistically significant.

Interpretation & Conclusion:

Our study revealed that there was positive co-relation between the serum levels of zinc HDL-cholesterol concentrations. However, the statistical co-relation was not evidently significant.

Thus supplementation of zinc supplements to the individuals with type 2 diabetes mellitus without proper blood glucose regulation could prove to be beneficial in regulating the blood glucose levels and thus prevent the complications arising out of chronic conditions in diabetes. Thus we would like to conclude that the study has shown decreased levels of zinc in diabetic cases, supplementation of which may be helpful & the effect of this supplementation on HDL need to be explored.

Key words: type 2 diabetes mellitus, HDL-cholesterol, zinc, DM, zinc transporters, metallothioneins.

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INTRODUCTION

Mineral nutrients are the chemical substances consistently present in the food we consume along with the other four basic components i.e. carbon, hydrogen, nitrogen and oxygen in the form of organic molecules required by living organisms and known as dietary minerals. The word dietary minerals are bimodal, as it defines the organic chemical compounds rather than actual inorganic minerals. The following minerals calcium, phosphorus, potassium, sulphur, sodium, chlorine, and magnesium are the seven minerals known as major minerals in order of their presence in the human body, whereas one more group i.e. iron, cobalt, copper, zinc, molybdenum, iodine, and selenium are the important "trace" or minor minerals, necessary for mammalian life.[1]

Since the last two and half decades the number of people working on trace elements has been on the rise and because of their various metabolic characteristics and functions, trace elements are considered to be an essential part for optimum human health. They take part in reaction with many of the important biomolecules such as enzymes, pro-hormones, pre-secretory granules and biological membrane and take part in a variety of catalytic, structural and regulatory functions.[2] Being an integral part of many enzymes zinc, becomes a trace element of importance, and plays a significant role of action in the maintenance of several tissue functions[3] to mention of importance the synthesis, storage and release of insulin.[4]

In the studies performed at molecular and cellular levels quite a several roles for zinc (Zn) are being demonstrated starting from the insulin production and including the consequent actions of insulin on metabolism. The substantiation to the

saying that decreased zinc concentration is connected with diabetes is proposed by scientific and epidemiological studies. Studies conducted for zinc in rodent models with diabetes have derived an appreciated connection for understanding at the molecular, cellular, clinical and epidemiological explanations in the background of inter-organ metabolism and the metabolic disturbances of diabetes.[5]

Zinc is documented as a competitor in paracrine inhibition of glucagon secretion in α-cells. Zinc accumulates in the islet cells and is related to insulin secretion. Islet cells act as an entity from within and the hormone secretion in the islets is profoundly influenced by paracrine and autocrine regulation.[6]

Without a proper demarcation point and an indistinct leading edge, diabetes has become to be a disease, concern of the world. Type 2 diabetes mellitus (T2DM) comprises of a group of dysfunctions characterized by hyperglycemia which is due to the grouping of resistance to insulin action, insufficient insulin secretion, and excessive or inappropriate glucagon secretion. Micro-vascular, macro-vascular and neuropathic complications is due to the reason that being type 2 diabetes mellitus is not under strict regulation.[7]

Individuals diagnosed to have Diabetes mellitus (DM) may have several forms of dyslipidemia. Lipid abnormalities should be assessed aggressively and treated as part of comprehensive diabetes care because of the additive cardiovascular risk of hyperglycemia and hyperlipidemia. Hypertriglyceridemia and reduced high-density lipoprotein (HDL) cholesterol levels is the most common pattern of dyslipidemia. DM does not itself increase levels of low-density lipoprotein(LDL), but the small dense particles found in type 2 DM are more atherogenic and also they are more easily glycated and susceptible to oxidation.[8]

Since the 1930s many studies have been conducted, when zinc was first demonstrated to be an integral element of the insulin crystalline structure, to shed light on the relationship between zinc and insulin action.[9]

Interestingly rodent models effectively have shown, oral or intraperitoneal administration of certain zinc complexes showed insulinomimetic effects in, including stimulating lipogenesis and attenuating hyperglycemia.[10,11] Increasing evidence has additionally, supported zinc in the role as an antioxidant that could protect insulin and cells from being attacked by free radicals. From animal studies with supportive evidence that zinc intake may have protective effects against type 2 diabetes, few studies in humans have been conducted to examine this relationship.[12]

In a cross-sectional analysis conducted in Indian population, the increased dietary zinc intake was found to be associated with a decreased prevalence of diabetes and metabolic syndrome.[13]

Studies done earlier suggest that there is decreased level of zinc in diabetics and also dyslipidemia. Hence this study will be done to estimate the levels of zinc and high density lipoproteins in type 2 DM patients to check for its altered or deranged metabolism in the patients on treatment with oral hypoglycemic drugs.

OBJECTIVES OF THE STUDY

1. To study the levels of following parameters in type 2 diabetes mellitus patients on oral hypoglycemic agents.

- Serum Zinc
- Serum HDL

2. To compare the values of the above parameters with that of controls.

REVIEW OF LITERATURE

It always remains a hypothetical kind of question and remains unclear which arises first: is it the occurrence of diabetes mellitus and increased blood glucose levels on the zinc homeostasis or is it the modifications in the zinc levels that lead to the alterations on the carbohydrate metabolism. Perhaps to be more precise, are the simultaneous, inter-independent events that lead to the changes of one on the other.[14]

The International Zinc Nutrition Consultative Group (IZiNCG) designed a technical review to provide an overview of current knowledge regarding zinc nutrition in relation to human health, summarizing the available information on assessing population zinc status, and to describe the range of programmatic options for controlling zinc deficiency that was published in the year 2004.Because of zinc's critical structural and functional roles in many enzymes adequate zinc nutrition is essential for human health whereby they are involved in gene expression, cell division growth and immunologic and reproductive functions. Dramatically the worldwide recognition for the importance of zinc nutrition in human health has expanded, and there has been more accumulation of experience on the design and implementation of zinc intervention programs, since the publication of that document. Moreover, during the workshop on zinc supplementation and child mortality and morbidity held by the World Health Organization (WHO) in September 2006, it was concluded that "in view of the results of all the trials examining the impact of zinc supplementation on mortality, morbidity and growth, a consensus was reached on the need to develop new feasible approaches to improve the intake of zinc and its bioavailability in young children, in order to achieve adequate population coverage".Hence, the IZiNCG Steering Committee concluded that this would be appropriate time to re-examine the

latest information on the strategies to control zinc deficiency and to reassess the state of knowledge concerning interventions to enhance the zinc nutrition. [15]

The prolonged duration of diabetes results in the outcome of long-term damage, abnormal functioning and failure of multiple organs, especially those of eyes, kidneys, nerves, heart, and blood vessels. Many non-physiological processes take part simultaneously and resulting outcome is the diabetes. These abnormalities vary from self-mediated break down of the beta-cells of the pancreas with simultaneous decrease in insulin, leading to abnormalities that result in resistance to insulin action. This forms the ground work for the abnormalities in the metabolism of the basic bio molecules i.e. carbohydrate, fat, and protein in diabetes with deficiency of action of insulin on the target tissues. This inadequate insulin action is due to the deficiency of insulin secretion and/or decrease in the tissue responses to insulin at many different steps in the complex pathways of hormone action. The abnormalities of insulin secretion together with the decrease in insulin action occur simultaneously in the same patient, and in most situations it remains unclear which incorrect function, i.e. if only one of the fore mentioned or whether together is the primary cause of the increased blood glucose levels.[16]

WHO projects that diabetes will be the 7th leading cause of death in 2030.[17] Diabetes epidemic more pronounced in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2002. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9million in India and this is further set to rise to 69.9million by the year 2025 (Fig. 1)[18]

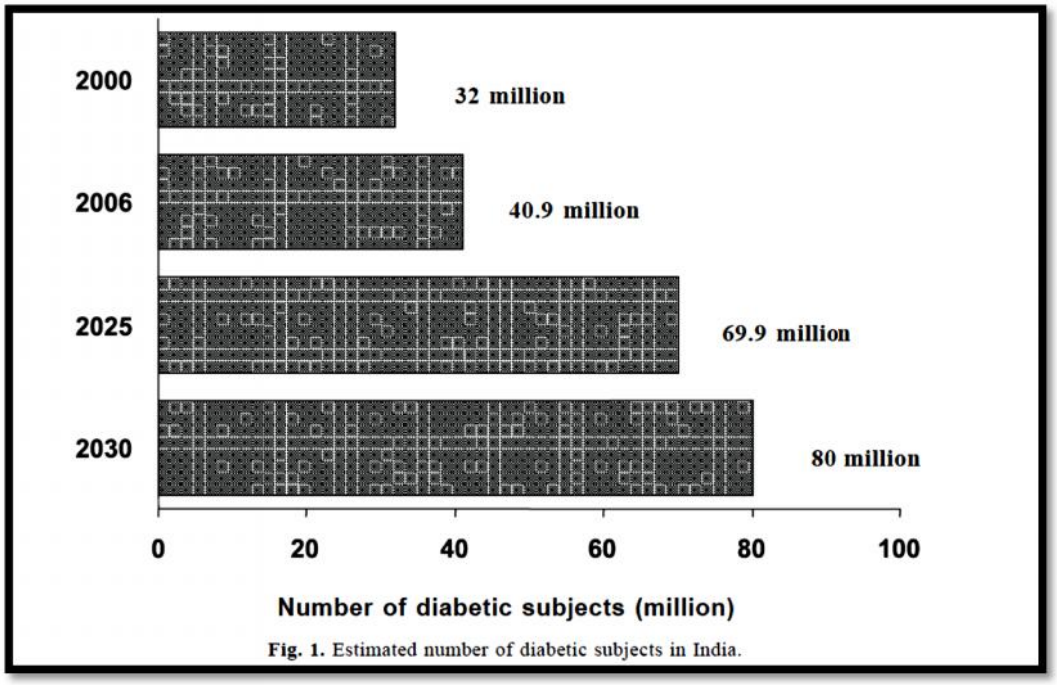


Fig. 1. Estimated number of diabetic subjects in India.

A total of about nearly 57 million deaths occurred in the world during 2008; of that about 36 million (63%) were due to Non-communicable diseases (NCDs), principally involving cardiovascular diseases, diabetes, cancer and chronic respiratory diseases. And of these NCDs 80% of deaths (29 million) occurred in low- and middle-income or the developing countries. NCDs most frequently stand as the cause of death in most developed countries like America, the Eastern Mediterranean, Europe, South-East Asia, and the Western Pacific. In the African Region, there are still more deaths from infectious diseases than NCDs. Even there, however, the prevalence of NCDs is rising rapidly and is projected to cause almost three-quarters as many deaths as communicable, maternal, perinatal, and nutritional diseases by 2020, and to exceed them as the most common causes of death by 2030. WHO projections show that NCDs will be responsible for a significantly increased total number of deaths in the next decade. NCD deaths are projected to increase by 15% globally between 2010 and 2020 (to 44 million deaths). The greatest increases will be in the WHO regions of Africa, South-East Asia and the Eastern Mediterranean, where they will increase by

over 20%. In contrast, in the European Region, WHO estimates there will be no increase. In the African Region, NCDs will cause around 3.9 million deaths by 2020. The regions that are projected to have the greatest total number of NCD deaths in 2020 are South- East Asia (10.4 million deaths) and the Western Pacific (12.3 million deaths).[19]

In people with type 2 diabetes mellitus, the risk of cardiovascular disease are higher, also with cardiovascular deaths representing on top in the list.Epidemiological studies have demonstrated that diabetes mellitus is an independent risk factor for cardiovascular disease and it is amplified by the other common risk factors such as smoking, hypertension and dyslipidemia.One of the most important risk factors for coronary artery disease (CAD) is hyperlipidemia the prevalence of which is more among the adults with type 2 diabetes mellitus with a four to six fold greater cardiovascular mortality than in the general population.[20] Though there is no well-defined role of zinc deficiency in atherosclerosis; but the studies conducted by epidemiologists in some population groups suggest that there is association of coronary artery disease with low serum concentrations of zinc. The effect of the mechanism for zinc on lipoprotein metabolism is not clear, a suggestion is made regarding requirement of zinc for enzymes involved in lipoprotein metabolism.[20] The onset and the pace of progression of atherosclerotic events are closely linked to lifestyle and nutrition.[21] The progression of accelerated pathology of atherosclerosis is associated with abnormal glucose tolerance, truncal obesity, increased lipid level and overall insulin resistance being the most important among the many risk factors.[22]

Animal studies performed with rodent models suggest that there is decreased incidence of both type 1 and type 2 diabetes with effective zinc supplementation. The

decrease of insulin resistance can be bought about in the activation of stress pathway as a cause of zinc deficiency in the individuals; also accumulating evidence suggests that patients with type 2 diabetic experience zinc malabsorption and increased excretion of urinary zinc.[23]

Although it is unclear about the effect on lipoprotein metabolism with zinc supplementation, studies have shown lipid-lowering effects of zinc in humans showing zinc supplementation in the form of tablets to decreases total and LDL cholesterol, whereas those of HDL cholesterol are increased in both normal and diabetic humans.[24,25] Other studies, however, found a very little significant co-relation with that of zinc supplementation and its effect on lipoprotein profiles mainly HDL-c which either is reduced or not changed.[23]

Source of Zinc

The food sources with high-protein content are a good source of zinc. The animal sources - beef, pork, and lamb contain more zinc than fish. Also in chicken red meat have more zinc than the white meat. Vegetables are the good sources of zinc – nuts, whole grains, legumes, and yeast. The zinc in the plant protein is not readily available as the animal protein and thus the fruits and vegetables are not good sources, animal proteins are better sources. As a result of this the complete vegetarian's diet and the low protein diet tend to be low in zinc. Zinc as an additive is seen in most multivitamin and mineral supplements. The zinc in these supplements may be present in the form of zinc gluconate, zinc sulfate, or zinc acetate although it is not clear which of its form has a better bio-availability. Also zinc is found in some over-the-counter medicines, such as cold lozenges, nasal sprays, and nasal gels.[26]

Distribution of Zinc

Zinc is the second most prevalent trace element essential to the body and is involved in association of structure and function of over 300 enzymes, thus representing on a whole all major biochemical categories and in turn essential for normal cell functioning and metabolism.[27]

The body distribution of Zn is such that the muscle and bone contain 85% of the whole body Zn, the skin and the liver 11% and the remaining in rest of other tissue. With respect to organ distribution prostate, retina, muscle, bone, liver, and kidney have the highest concentrations of Zn.[27,28]

Considering the intracellular distribution 30%–40% of zinc is located in the nucleus, 50% in the cytoplasm, organelles and specialized vesicles (for digestive enzymes or hormone storage) and the rest of the zinc is seen distributed in the cell membranes. Therefore, the mobilization of zinc from the tissues is restricted, i.e., under normal circumstances there is no free Zn. Depending on the source for the intake the value ranges from 107 to 231 μmol/day and the estimated human Zn requirement is 15 mg/day. In enzymes zinc has both catalytic and structural roles, while in zinc finger motifs, the role is more with formation of a scaffold that organizes protein sub-domains for purpose of interaction with either DNA or other proteins. The role of Zn is critical as seen with the functioning of a number of metalloproteins, inducing members of oxido-reductase, hydrolase, ligase, lyases family and with copper the function is of co-activating in phospholipase C and superoxide dismutase. In the biological medium there is no active participation of zinc ion (Zinc 2+) thus does not participate in redox reactions, which makes it structurally a stable ion. The ions of Zn are hydrophilic in nature and there is no crossing across

the cell membranes by passive diffusion; therefore, as a result the availability of zinc in the cells is dependent on its transporters.[29]

Also in maintaining the zinc homeostasis another cysteine-rich metal binding protein, metallothionein (MT), plays a significant role.[30,31] Significant amount of zinc excretion takes place mostly in feces and these amounts to about 12–15 mg/day and to lesser amounts about 0.5 mg/day in urine. Several reviews have shown essentiality of zinc.[27,32]

Metallothionein (MT)

The proteins belonging to this class are intracellular cysteine-rich, with a low molecular weight of around 6–7 kDa and are known as Metallothioneins (MTs). As of now four isoforms of MTs have been identified among those MT-I and MT-II are majorly distributed in most tissue. The regulation of the intracellular zinc in the body system is strictly bought about with the MT by compartmentalization through the activities of zinc transporters (ZnT) (Fig. 4). The binding affinity of the MTs to zinc is significantly high and thus aids to play a central role in stabilizing the intra-cellular zinc availability either through sequestration or release of metal directly. Several reviews have tried summarizing these physical, chemical, and biological properties of MT in various approaches.[33,34]

Requirement of Zinc with insulin secretion

Large amounts of zinc are stored up in the islets of Langerhans, β cells of the pancreas; the significant role of importance in relation of zinc with insulin in holding together the hexameric structure of insulin. In its hexameric structural form the insulin is a crystalline structure and it comprises of two metals of zinc ions that are attached to six individual units of insulin, which are kept as a stable non-reactive storage form in the secretory granules of the pancreas.[35] When the stimulus for release of insulin is brought about from glucose either in diet or other component breakdown it leads to co-secretion of part of the zinc ion pool of the β cell along with insulin. Once released the hexameric structure (Fig.2) of insulin becomes immediately unstable on secretion and then splits into its respective components i.e. active zinc monomeric units and the individual zinc ions, one of the reasons for the disassociation could be the sudden release without the pressure and change in pH (5.5 to 7.4) from intracellular pancreas to extracellular environment. A study done in vitro came to a suggestion that the zinc ions which in co-secretion along with insulin during the increased blood glucose levels might contribute to the actual death of the β cell by a paracrine mechanism; it has been hypothesized that such activity could link increased hyperinsulinism with β cell necrosis and thus ensuing type 2 diabetes. Thus the role of zinc becomes indispensable for insulin structure as it makes a requirement when insulin analog was prepared to ensure its proper activity and stabilization of the structure.[36]

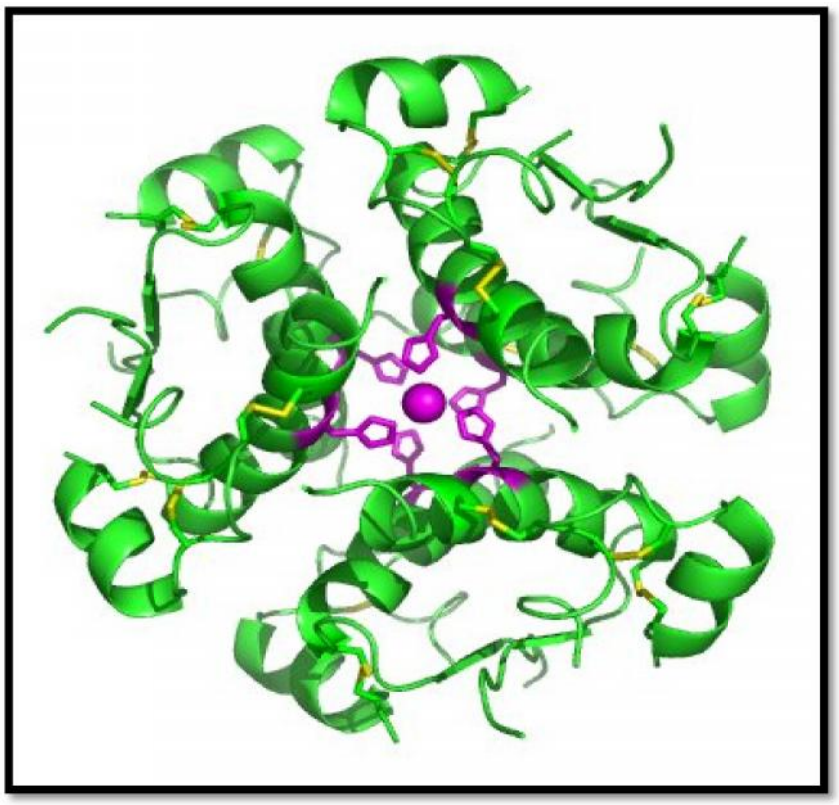


Fig. 2.Computer-generated image of six insulin molecules assembled in a hexamer, highlighting the three fold symmetry, the zinc ions holding it together, and the histidine residues involved in zinc binding. Insulin is stored in the body as a hexamer, while the active form is the monomer. [37]

The zinc transporters

So far more than 20 zinc transporters that have been identified.[38-40] The classificationof the zinc transporters is mainly done into two families(Fig. 3):

- Zinc transporter (ZnT: the vertebrate cation diffusion facilitator family of proteins or Slc30a family) and
- Zip (Zrt/Irt-like protein or Slc39a family)

Thus those of the transporters that belong to the classes of the ZnT members actively bring about the outwardly directed movement of the zinc to the extracellular space or intracellular compartments from the zinc present in the cytoplasm of cell, whereas those belonging to Zips move zinc in a direction opposite to that of ZnT i.e. from extracellular space to intracellular compartments or the cytoplasm. The simultaneous action of both of these types of zinc transporters is required essentially to the maintenance of zinc equilibrium in the cytoplasm (Fig. 4).[41]

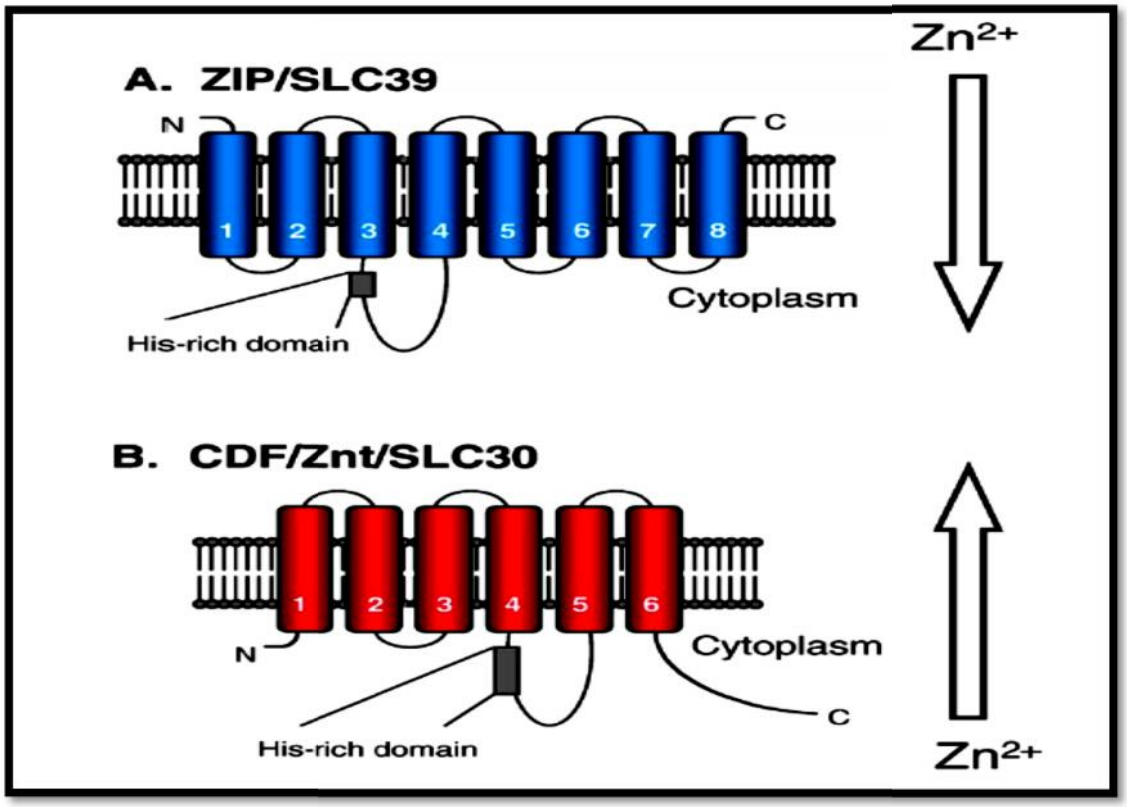


Fig. 3.The predicted membrane topologies of the ZIP/SLC39 and CDF/Zinct/SLC30 families of metal ion transporters. The transmembrane domains are numbered 1, 2,7.[41]

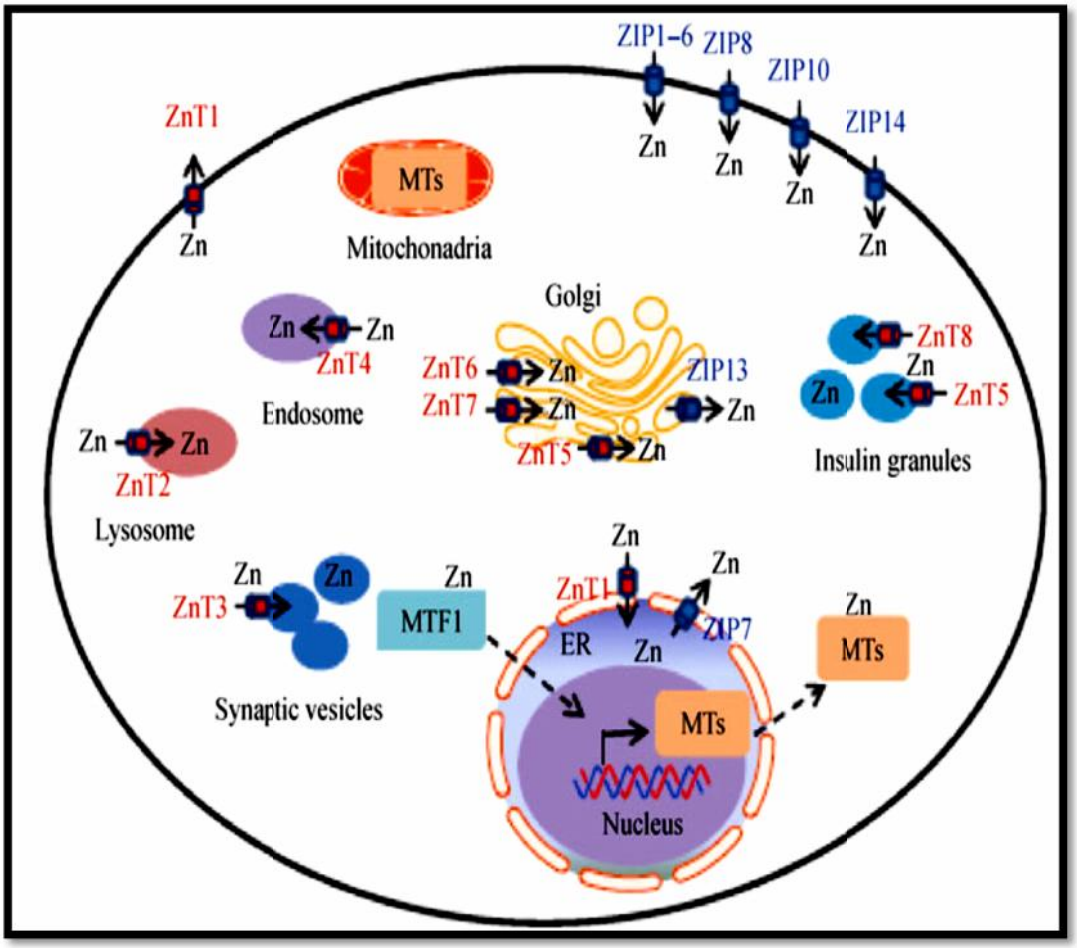


Fig. 4 Subcellular localization of Zinc transporters and MTs. Localization and potential functions of Zinc transporters from the Slc39/ZIP (blue) and Slc30/ZincT (red) families, MT, and metal response element (MRE)-binding transcription factor 1 (MTF1) within the cell. Arrows show the predicted direction of Zinc mobilization. ER, endoplasmic reticulum. The figure was made based on the previous report.[42]

Zinc deficiency and insulin resistance and type 2 diabetes

Another study performed by Park et al. using weanling male Sprague-Dawley rats to research the effect of absence or reduced zinc on glucose tolerance by mandatory intra-gastric feeding by supplementing zinc to prevent the reduced food intake and altered eating patterns demonstrated pattern of producing of glucose tolerance in rats fed on a zinc-deficient diet. Further their study indicated that in the rats on a zinc deficient diet, the tolerance to the glucose levels is brought up not due to change in the blood insulin and glucagon levels but it is possibly due to the opposition in the distant tissue for the insulin action.[43] Further, studies performed by other associates also validated that the stimulation of insulin resistance in the animals fed with diet devoid of zinc.[44,45]

Diabetes mellitus is one of the most common metabolic diseases, which affect zinc homeostasis in many ways differently.[46]It is found that in patients with good glycemic control there is increased concentration of plasma zinc levels than those without a good glycemic control.[47] The zinc thus plays an important role in the glucose utilization by muscle and fat cells[48] Also it is required as a co-factor for the proper functioning of many intracellular enzymes that may be involved in the metabolism of protein, lipid and glucose.[49] Zinc may be involved in the regulation of insulin receptor-initiated signal transudation mechanism and insulin receptor synthesis.[50] The synthesis, storage, and secretions of insulin by pancreatic tissue requires the active participation of the zinc metal for the conformation and integrity of insulin in its hexameric crystalline form.[51] Zinc is an active integral component of many of the antioxidant enzymes. Much of the resulting complications of diabetes mellitus could be the result of an increase in the concentration of intracellular oxidant

and free radicals, the association of which could be linked with decrease in the intracellular zinc concentration and the antioxidant enzymes dependent on zinc.[52]

The relationship of zinc with the endocrine system is very closely associated and it is essential for the normal growth, reproductive function, immune function, and glucose metabolism. Studies have now demonstrated many chronic illnesses have now been recognized to be associated with zinc deficiency. Diabetes mellitus is one of the diseases, which affect zinc homeostasis in different ways. Thus the dependency between these three is complex with no clear mechanism.[46]

Although clinical signs of deficiency are not quite obvious many observations are being related to the deficiency of zinc. Many of the consumable products like cereals, meat, seafood and dairy products have zinc as constituent. Though the normal daily intake of zinc is considered to be harmless there is no clear cut demarcation between the margin for safe and unsafe intake and is relatively very narrow.[53] As there is no specific storage form of zinc, the need for a constant supply persists and zinc availability to cells is particularly well regulated, inspite of being poorly understood.[54]

In accordance, with respect to metabolic diseases like insulin resistance, metabolic syndrome and diabetes, zinc is considered to play a significant role mainly because 1) the stabilization brought about by zinc in the storage of insulin in pancreatic β cells in its inert hexameric form and 2) its role in effectively bringing about the antioxidation, whereas in insulin resistance and diabetes the oxidative stress is considered to be a main component in its initiation and progression.[55]

The frequency of severe zinc deficiency is not very commonly seen but not much concern has been shown about the decreased zinc levels in diabetic patients because of increased loss of zinc due to typical polyuria being one of its main symptoms. Whereas a few of the studies have reported the presence of zinc deficiency in type 2 diabetes mellitus individuals, other studies failed to point out significant differences when compared against the healthy subjects. In the insulin dependent diabetes (IDDM), with the occurrence of zinc deficiency the pancreatic damage is expected to flare up the condition. A recent study report has as well described the occurrence of reduced levels of zinc in obese, insulin resistant subjects. Concentrations of plasma zinc were found to be lower in type 2 diabetics but that was not associated with glycemic status or duration of diabetes or parameters of the metabolic syndrome. Interestingly it was reported that due to the fact that there are elevated levels of copper in diabetics it could be put up that copper is in fact linked to metabolic syndrome and diabetes. The increased risk for coronary artery disease (cardio vascular disease) and mortality due to these has been related to reduced zinc levels in diabetics.[55]

Mechanisms of action

There have been several methods trying to improve and explain mechanism of action of insulin by zinc. It appears that the inhibition of the important glycogen-regulating enzyme GSK3 (glycogen synthase kinase-3) could lead direct to insulin-like action of zinc.[54] The stimulation of the post-receptor proteins like serine/ threonine kinase Akt also called protein kinase B (PKB) and phosphatidylinositol 3-kinase (PI3-kinase) include the other mechanisms of action. The levels of cytokines such as IL-1b as well as NKB can also be reduced by zinc. Also there is possible

indirect effect possibly brought about by the cysteine-rich protein-metallothionein synthesis.[56]Finally the intracellular concentrations of zinc are suggested to be important for its action rather than the plasma- levels.[54]

Zinc is an outstanding antioxidant: its action is straight forwardly site specific competing for iron and copper; the attachment of zinc to SH groups in proteins prevents the proteins from their oxidation. Because of the property to control the expression of metallothionein at cellular level Zn is involved in regulation of cellular redox reactions. The elevation of hepatic antioxidant enzymes has shown to correct the oxidative stress by dietary Zn as demonstrated.[55]

In a prospective study performed with a group of 8300 women with high zinc intake they showed to have slightly reduced the risk for type 2 diabetes and comparatively this effect was seen to be limited to the zinc-deficient subgroup in the study.[57] There was however reporting of aggravation of glucose intolerance in zinc-deficient diabetic patients in another study.[58] In type 2 diabetic patients with the presence of micro-albuminuria zinc levels lowered the homocysteine levels while vitamin B12 and folic acid levels were seen to be increasing. There was no change in the levels of leptin seen in obese, non-diabetic subjects with the improvement of sensitivity of insulin.[59]Whereasin obese women there was no preventive effect seen against diabetes development.[60]While in healthy elderly subjects oxidative stress was reduced by zinc.[61]

Alteration of zinc in diabetes

There is increasing evidence to point the evidence that diabetes could alter zinc homeostasis in many different ways, but the most important thing being the

hyperglycemia, rather than could be due to the primary lesion associated with diabetes, leading to the increased urinary excretion and decrease in the concentration of total body zinc levels. The amount of excretion of zinc via urine is comparatively much increased in the diabetic patients.[62,63] Zinc supplementation is likely to be of benefit in the treatment of diabetes as it causes a significantly improved level of retention of zinc in the body tissues. However, it remains unclear whether the significantly low levels of plasma zinc concentrations which is noticed typically in the diabetic patients and lab animals whether it is dependent on the type of the diabetes, duration of time of diabetes, and the age of onset of diabetes.[63-66]

Altered lipid levels in DM

There is a frequent association between the dyslipidemia and percentages of increased concentration of glycated hemoglobin. Generally, there is no much incidence of hyperlipidemia in patient associated with type 1 DM, if they are under regular treatment with good glycemic control. But in patient with type-2 DM there is usually much of the incidence of dyslipidemia even if they are undergoing regular treatment with relatively good glycemic control. The scenario of dyslipidemia in type 2 diabetics is more of like those including elevated plasma triglycerides, elevated Low Density Lipoprotein-Cholesterol (LDL-C) and decreased High Density Lipoprotein-Cholesterol (HDL-C). Dyslipidemia stands alone to be a significant risk factor for the causation of coronary artery disease, one of the leading causes of mortality in patients with diabetes mellitus. This dyslipidemia remains largely undiagnosed and also remains under treated in case of the high risk populations.[67]

In diabetes the improper management may be a factor contributing to the increased incidence of heart disease among persons with type 2 diabetes especially.[68]

The association of lipid abnormalities with type 2 diabetes is typically defined by an increased concentration of triglycerides and small dense LDL and a low concentration of HDL-c. There is no much of alterations in the plasma LDL-c levels are generally and remains normal. The contribution to this atherogenic dyslipidemia is believed to be due to increased insulin resistance in the diabetics and thus in turn increasing the hepatic production of very low density lipoprotein(VLDL) and other apolipoprotein(apo) B containing lipoprotein particles, due to these alterations there is an increased flux of free fatty acid to the liver.[69,70] As a result there may also be altered or reduced suppression effect of insulin secretion of apolipoprotein B, which could be at the regulation or degradation level of the apo B, or halting the activity of microsomal triglycerides transfer protein. Through the action of cholesterol-ester transfer protein (CETP), the transfer of triglycerides takes place from VLDL to HDL, thus leading to the formation of triglycerides-dense HDL particles, which are degenerated by the hepatic lipase and then are rapidly cleared from plasma. Also the formation of small dense LDL particles mechanism is similar like cholesterol ester protein-mediated transfer of triglycerides from VLDL to LDL. These could be other mechanisms which could lead to the impaired clearance of lipid and lipoproteins.[71]

The impaired lipid levels could be related intrinsically to the alteration in the normal physiology due to insulin resistance or insufficient insulin action and parallel metabolic disturbances. One of the most important reasons for the elevated triglycerides concentration could be the increased turnover of VLDL in the liver, which is stimulated by an increased movement of glucose and free fatty acids (FFA) to the liver. In addition to this, also there may be reduced breakdown of the triglyceride rich lipoproteins (TRLs), including VLDL and chylomicrons, due to the reduced activity of lipoprotein lipase (LPL). Insulin controls the activity of LPL in

adipose and its activity may be brought down by insulin deficiency for longer periods or decrease in the action of insulin. In addition to altered action of the lipoproteins, changes in size and shape of lipoproteins have also been observed.[72,73]The increased concentration of triglycerides(TG) also has significant alterations on HDL metabolism, since triglycerides metabolism is directly correlated to HDL levels. If the concentration of triglycerides level increases, the quantity of HDL particles comes down proportionally. This could be due to increased breakdown rate of HDL. The enzymes LPL, HL (hepatic lipase) and cholesterol ester transfer protein (CETP) are the main modulators of HDL-c. These modulators may be affected in type 2 diabetes. The rate of cholesteryl ester transfer, mediated by CETP, between HDL and the apolipoprotein-B containing lipoproteins is enhanced during hypertriglyceridemia due to an elevated concentration of apolipoprotein-B containing lipoproteins. In diabetic dyslipidemia the HDL particles are often smaller and denser. This is partly due to the expanded VLDL pool and to CETP over-activity resulting in an increased neutral lipid exchange. This subsequently leads to triglycerides enrichment of both HDL and LDL. The smaller and denser HDL particles have altered physiological functions. The reverse cholesterol transfer capacity may be diminished and the antioxidant potential may be reduced.[74]

In order to elucidate the co-relation between HDL and risk of atherosclerosis, a better understanding of the key factors which are concerned with the regulation in determining the plasma concentration of HDL particles as a whole and the flux of lipids through the HDL and reverse cholesterol transport pathways need to be better understood.[75]

Previously done animal studies suggest the strong direct association of HDL and apoA-I lipoprotein with the potential to cause atherogenesis. The metabolism of HDL is comparatively much more complicated when compared with that of the other types of major lipoprotein fractions. Components of HDL are mostly organized after their secretion. Inter change of apoprotein with or their transfer takes place with the other lipoproteins and are actively restructured within the plasma component and their clearance takes place independently in part from one another. Although the most commonly conceived concept of reverse cholesterol transport (RCT) from movement of macrophages to liver and then to the biliary excretion is the mechanism most popularly used to explain the ability of HDL to reduce the chances of atherosclerosis, there are also other mechanism of HDL been demonstrated in the lab studies that could support and contribute to anti-atherogenic effects of HDL.[76] The apoA-I is secreted predominantly by liver and intestine as lipid-poor apoA-I and nascent phopholipid-rich cholesterol-poor HDL particles (Figure 1). Nascent apoA-I/HDL acquire additional phospholipids and cholesterol via cellular efflux as well as by transfer of surface components of triglyceride rich lipoproteins (TRL; chylomicrons and VLDL) during lipoprotein lipase (LPL) mediated intravascular lipolysis of TRL.[75]

In one of the other reviews, they have attempted to summarize the other components of HDL metabolism, strongly pointing to three major themes identified. The first of the theme is about the concept that there is one sided movement of flux for cholesterol from the extra hepatic tissues to liver in the RCT.[75] Also HDL increases the substantial external movement of cholesterol from the liver tissue, which serves as a most important source of lipidation for newly secreted nascent HDL particles. HDL could perhaps be viewed on a whole as a universal plasma acceptor lipoprotein or as component directing the external movement of cholesterol from the

cells, thus playing an important role in the regulation of the increased cholesterol component from the cells for the maintenance of cellular cholesterol homeostasis. Although the externally directed movement of cholesterol from the macrophages summarizes to only a very small fraction of overall cellular cholesterol movement, but it is the most important when concerned to atherosclerosis, thus pointing that it be more aptly termed as macrophage RCT. The second major theme tries to highlight the ultimate metabolic fate of HDL in that it has a critical role in remodeling the intravascular part of HDL by lipid transfer factors, lipases, cell-surface receptors, and non-HDL lipoproteins. Thus this change in HDL composition due to mechanism of increased intravascular restructuring leads to more rapid HDL clearance, compared to the production, and one of the major determinant of decrease concentration of plasma HDL-c. The last and third major theme is the increasing appreciation that insulin resistance seems to be co-related with the increased incidence of reduced HDL-c in humans.[77,78]In spite of the fact that there is no proper establishment of prevalence of the metabolic syndrome and insulin resistance among those with low HDL-c in the general population yet. Therefore understanding the method of HDL lowering in the insulin resistant states which remain improperly charted out could provide importance in understanding the co-relation between the occurrence of reduced HDL and atherosclerosis. There are many other atherogenic factors that could be seen with the metabolic syndrome, and there is difficulty in this setting to know whether reduced HDL is simply one of the co morbid risk marker or an important causal factor of atherosclerosis. To conjure, the reduced HDL aptly represents both a risk marker as well as a causal factor for atherosclerotic disease in the metabolic syndrome.[75]

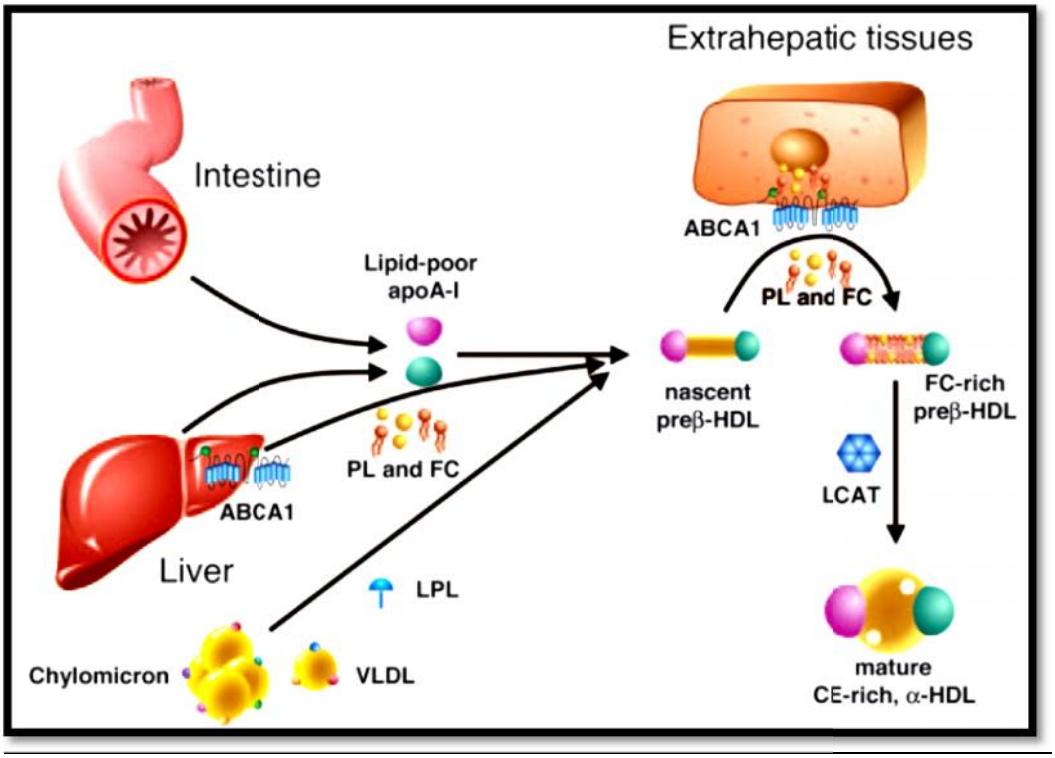


Fig. 3 Secretion, lipid acquisition, and maturation of HDL particles. The process of HDL maturation begins with the secretion of lipid-poor apoA-I by liver and intestine, followed by acquisition of cholesterol and phospholipids via ABCA-1 mediated efflux from the liver and the transfer of cholesterol, phospholipids, and apolipoproteins from chylomicrons and VLDL during LPL mediated lipolysis to form nascent pre-HDL particles. Lipid-poor apoA-I and pre-HDL particles acquire additional cholesterol and phospholipids from cells in extrahepatic tissues via ABCA1-mediated efflux, progressively generating particles that are more cholesterol enriched. The enzyme LCAT, carried on HDL particles, then esterifies the free cholesterol molecules to form cholesteryl ester, which migrate to the core of the HDL particle to form mature - migrating HDL particles. These mature HDL particles can acquire additional lipid from certain cells via efflux mediated by ABCG1 and SR-BI (not shown).[75]

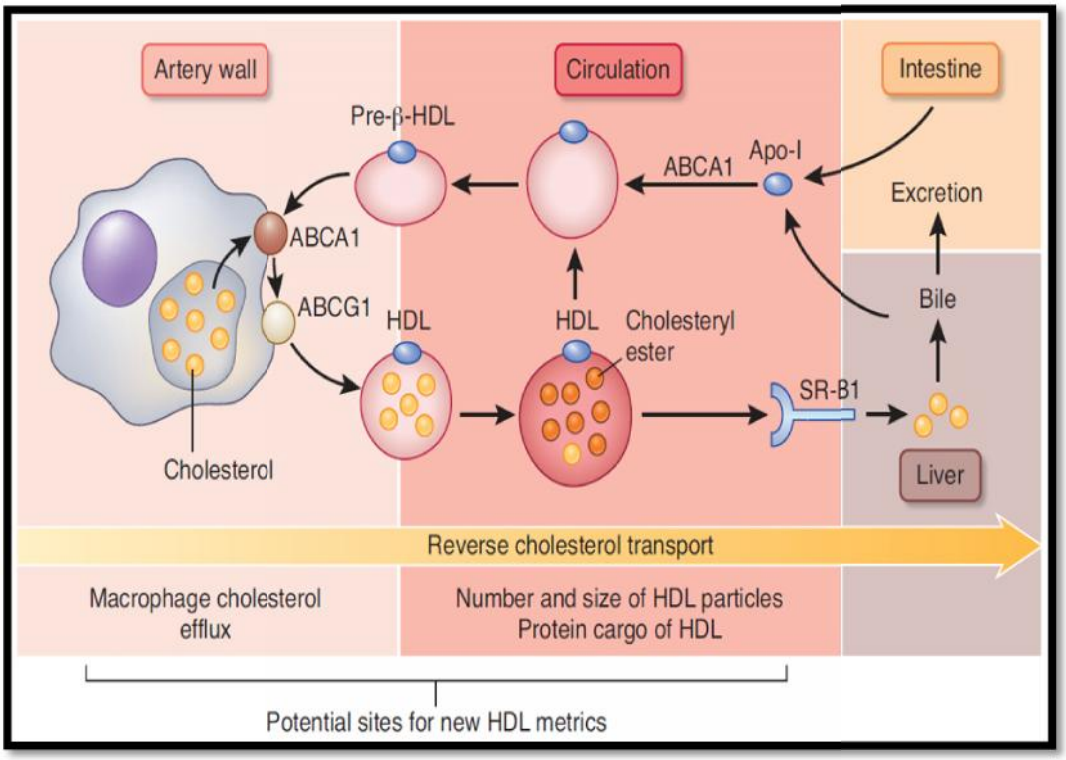


Fig. 4 Reverse cholesterol transport mechanism of HDL-cholesterol. Potential sites for new HDL metrics are indicated.[79]

The important sites of HDL degradation are kidney, liver, and steroidogenic tissues. The clearance of HDL may take place either by the process of selective removal of cholesterol (mainly) and other lipids from the particle, without involving the uptake of the whole particle, and this process is termed as selective cholesterol uptake, or by another mechanism wherein uptake and degradation of the whole particle by endocytosis, including apoA-I, a process referred to as holoparticle HDL uptake. In addition to reverse cholesterol transport, HDL may take part in various other processes such as inhibition of endothelial inflammation, promotion of endothelial nitric oxide and prostacyclin production, and the sequestration and transport of amyloidogenic proteins, oxidized lipids, and lipids derived from exogenous pathogens. These fore mentioned functions of HDL may affect the

processes and bring about atherogenesis. Further, more studies involving HDL metabolism, RCT, and other HDL functions will increase the likelihood in our understanding of finding therapies and proving their efficacy in reducing the chances of atherogenesis in humans.[75]

The assessments in the laboratory for zinc status are classified into 2 groups: analysis with the involvement of zinc in body tissues or fluids and the second one for the purpose of testing the functions of enzymes which are zinc-dependent. In the studies where the zinc status of large samples i.e. involving larger population groups the most often used approach to assess zinc status, the choice of analysis measurement is serum zinc levels. The accepted reference range for normal serum zinc level is 70-120 µg/dl (10.7-18.4 mmol/L), and the level of 70 µg/dl (10.7 mmol/L) was used as cut off value as an indicator of zinc deficiency.[80]

A recently performed meta-analysis on study of the efficacy of the supplementation of zinc on the level of serum lipoproteins found no general effect, and it was further hypothesized that it may occur as a result of the disturbed copper metabolism. In accordance when supplemented zinc in healthy men a 100 mg of zinc intake per day decreased the HDL concentrations to a considerable extent. Also, in case of young women who were also asked to ingest the same dose of 100 mg zinc per day showed a reduction in the concentrations of HDL cholesterol.[81]

MATERIALS AND METHODS

SOURCE OF THE DATA

The study group consist of 35 cases of type 2 DM patients and the control consists of 35 equally age and sex matched individuals.

STUDY DESIGN:

Cross sectional study.

STUDY PERIOD

Period of one year from January 2012 to December 2012.

SAMPLE SIZE

The sample size was calculated by the following formula.

$$n = \frac{2 \times (z_{\alpha} + z^{\beta})^2 (S_1^2 + S_2^2)^2}{(x_1 . x_2)^2}$$

(S1, S2, x1, x2, zα, zβ) [82].

STUDY POPULATION.

The study sample consists of 35 clinically and diagnostically confirmed cases of type 2 DM on oral hypoglycemic drugs admitted or attending medicine unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

CRITERIA FOR SELECTION OF THE STUDY GROUP

Inclusion Criteria

1. Clinically and diagnostically confirmed cases of type 2 diabetes mellitus on oral hypoglycaemic agents.
2. Criteria for the Diagnosis of Diabetes Mellitus. [83]

    - Symptoms of diabetes plus random blood glucose concentration ≥ 11.1 mmol/L (200 mg/dL)
    - Fasting plasma glucose > 7.0 mmol/L (126 mg/dL)
    - Two- hour plasma glucose ≥ 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test.

Exclusion Criteria

1. Type 1 diabetes mellitus.
2. Type 2 DM on insulin.
3. Type 2 DM on hemo dialysis.
4. Type 2 DM on multivitamin / zinc supplementation/anti-lipidemics
5. Smokers
6. Chronic alcoholics.

APPROVAL FROM THE AUTHORITIES:

Permission to conduct the study was obtained from all the concerned authorities viz.

1. Institutional ethics committee on human subjects research of Jawaharlal Nehru medical college, Belgaum.
2. Head of Department, Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum

OBTAINING INFORMED CONSENT

Informed consent was taken from all the participants in the study. (Annexure I)

SCHEDULING:

This study was carried out for a period of one year. It was undertaken during January 2012 to December 2012.

PATIENT INFORMATION

A structured proforma was used to collect socio demographic and clinical information about the study participants. (Annexure II)

COLLECTION OF SAMPLE

Patient preparation:

10 ml of fasting venous blood samples was collected from the patients and the controls under aseptic precautionary measures using sterile disposable syringes in plain tubes. The whole blood will be centrifuged and serum separated and kept at 0° C

to 4 °C for analysis of HDL and at -20°C for analysis zinc to be done within 30 days from the day of collection.

Methods of assay

Serum

Zinc – by flame atomic absorption spectrophotometry.[84]

HDL – Phosphotungstate/Magnesium method.[85]

PHOTOGRAPHS

Photo No-1: Digital spectrophotometer

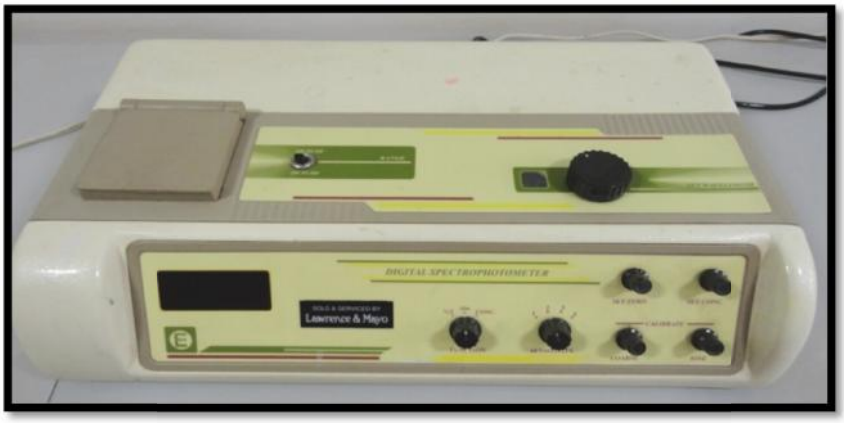


Photo No-2: Atomic absorption spectrophotometer

Photo No-3: HDL-cholesterol kit

RESULTS

The present study comprises of 35 clinically diagnosed cases of Type 2 Diabetes Mellitus on oral hypoglycemic and 30 normal healthy age and sex matched controls. The age group ranges from 35 to 50 years. There was significant decrease in the level of zinc and HDL-c in cases when compared to controls (p-value<0.001). There is positive co-relation between the levels of zinc and HDL-c in the cases but it is not statistically significant.

STATISTICAL TESTS USED

The data was tabulated and statistical analysis was carried out by using unpaired student's t – test and data correlation coefficient was plotted by Karl Pearson's correlation. The mean difference is significant at p value $\leq$ 0.05.

SERUM ZINC

The mean level of serum zinc in controls was 100.43 ±11.02µg/dl, whereas in cases it was 43.03±12.11µg/dl. The level was significantly decreased (P< 0.001) in all the cases of DM in comparison to the controls.

SERUM HDL

The mean HDL level in controls was 40.11±4.15mg/dl. The level in HDL cases was 29.26±2.42mg/dl. The level was significantly decreased (p< 0.001) in all the cases of DM when compared to controls.

Correlation between Zinc and HDL values in cases by Karl Pearson's correlation coefficient method

Deriving a co-relation from the data derived between the values of serum zinc and serum HDL-cholesterol using Karl Pearson's co-relation coefficient method we found that there was a positive co-relation between zinc and HDL-cholesterol but correlation was not statistically significant with ar-value of 0.1384 and p value being 0.4280 in the diabetic cases.

TABLES

Table 1: Comparison of control and cases with zinc and HDL values by t test

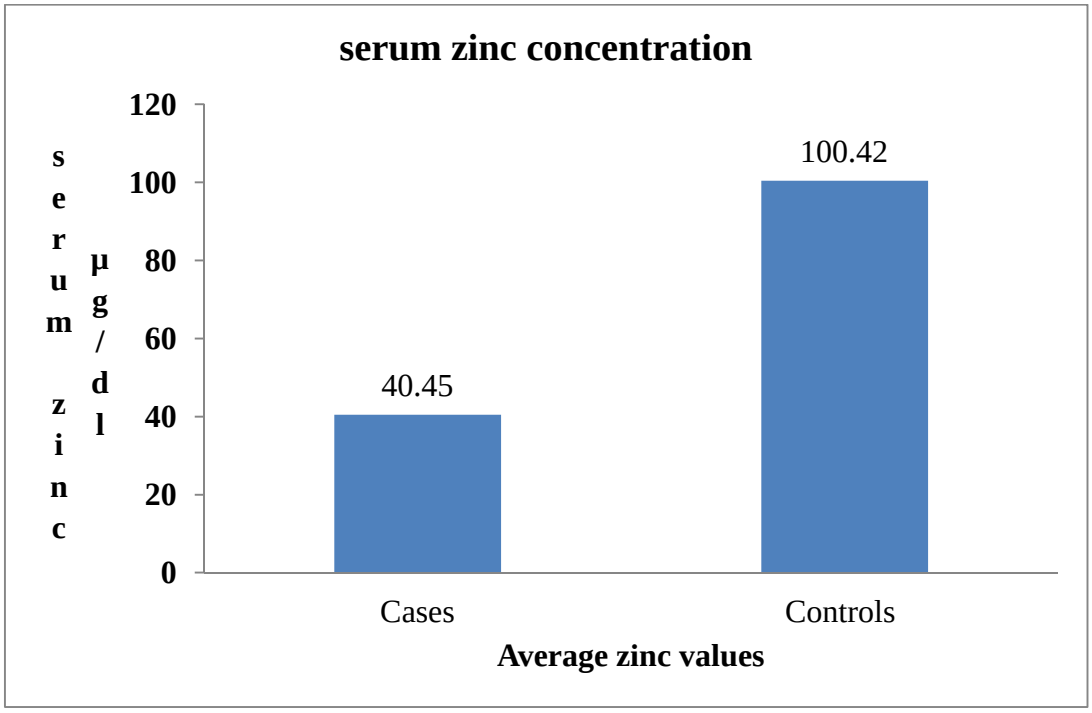
Variable	Group	Mean	SD	t-value	p-value
Zinc values	Control (n=35)	100.43	±11.02	20.7325	<0.0001
	Cases (n=35)	43.03	±12.11		
HDL values	Control (n=35)	40.11	±4.15	13.3742	<0.0001
	Cases (n=35)	29.26	±2.42		

Table 2: Correlation between Zinc and HDL values in control and cases by Karl Pearson's correlation coefficient method

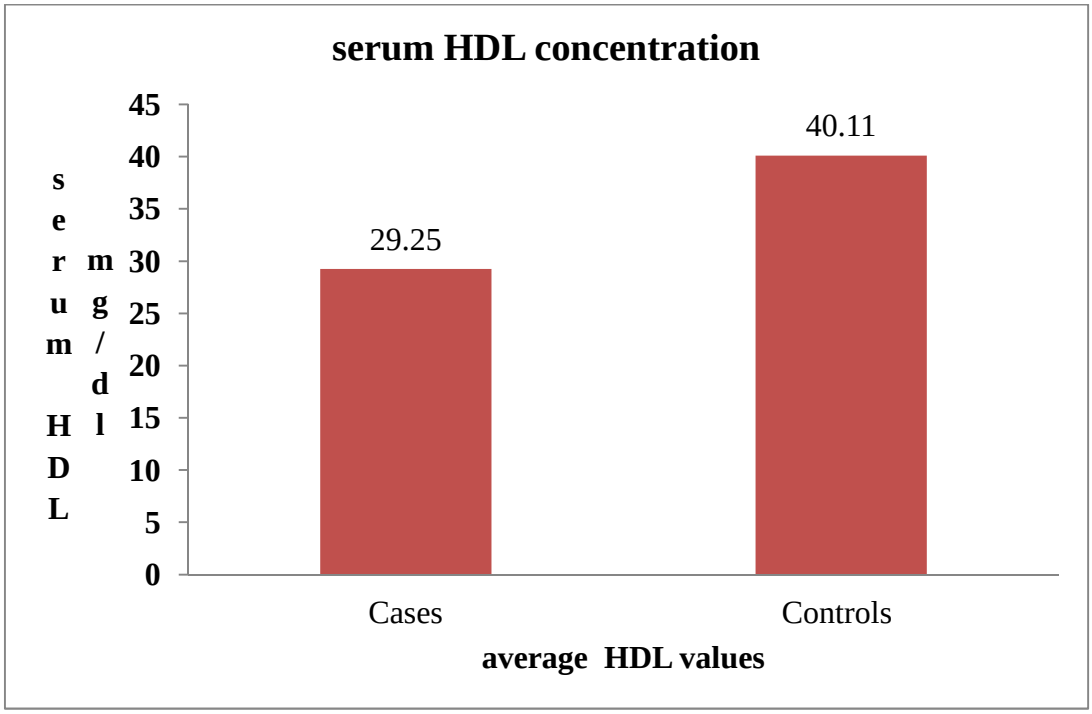
Correlation between	r- value	t-value	p-value
Zinc vs HDL in controls	0.1153	0.6665	0.5097
Zinc vs HDL in cases	0.1384	0.8025	0.4280

p<0.05 is taken as statistically significant

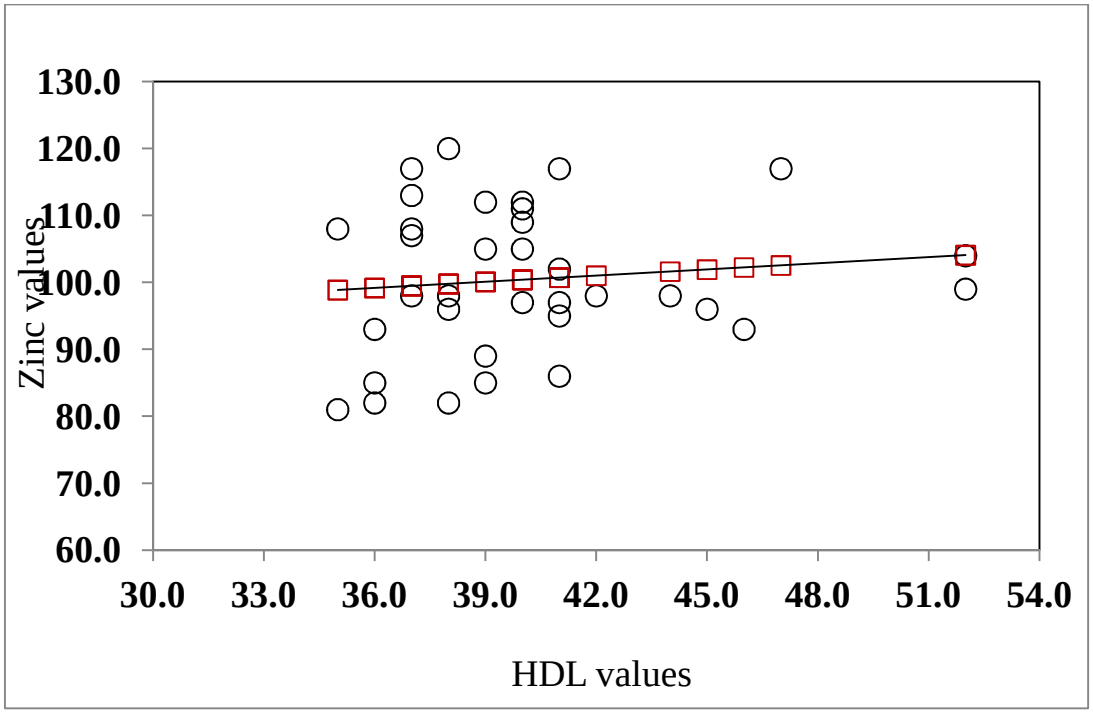
GRAPHS



Graph 1: Mean serum zinc levels between the diabetes(cases) and the control groups

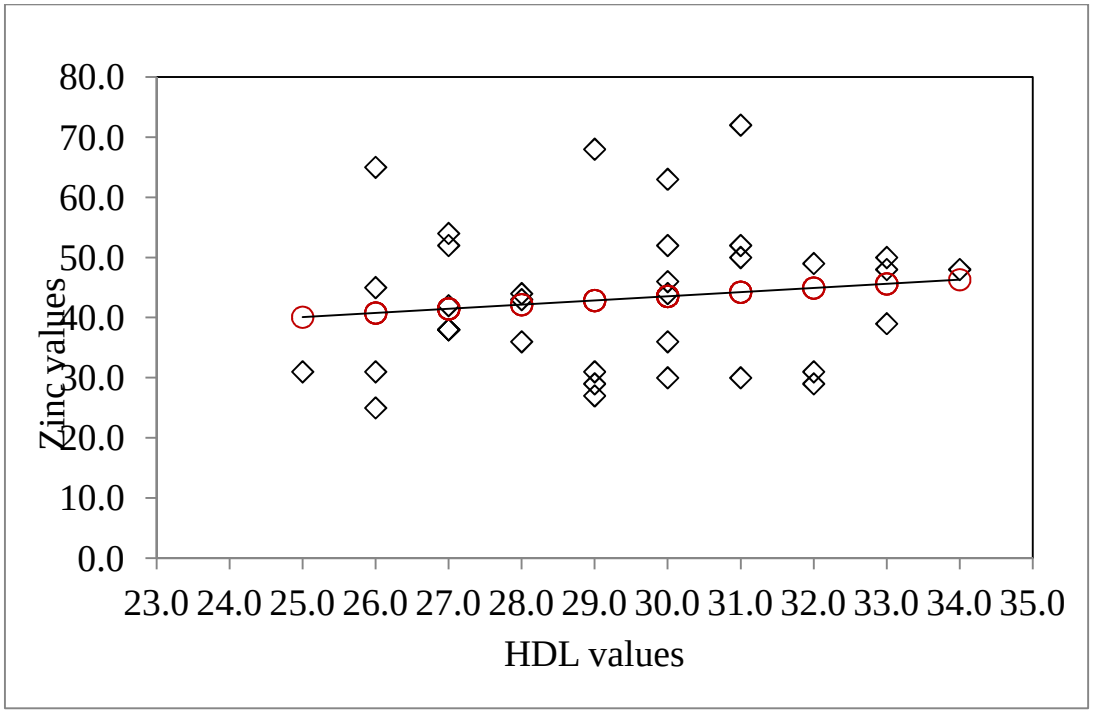


Graph 2: Mean serum HDL levels between the diabetes(cases) and the control groups



Graph 3: Relationship between Zinc and HDL values in control group.

Regression equation, Zinc = 0.3062(HDL) + 88.146



Graph 4:Relationship between Zinc and HDL values in cases group.Regression equation, Zinc = 0.6933(HDL) + 22.745

DISCUSSION

Diabetes mellitus is being watched upon as a multifactorial disease with multiple elements of causation, but nevertheless, the prominence of zinc dependency seems to be poorly understood regardless of the causal mechanisms leading to diabetes. The associations of zinc in numerous manners of the progress and advancement of diabetes mellitus stresses the diabetogenic effect of altered zinc homeostasis and offers new opportunities of diagnosis, prevention and therapy.[56] The prospective properties of mineral nutrients, considering the trace metals especially zinc, have received partial consideration. Zinc is involved in many processes in the body system ranging from the action of various enzymes, maintenance of cell membranes, regulation of gene expression, and cell signaling.[86] In a recent review of the literature they summarized the evidence and identified a tendency of plasma HDL cholesterol concentrations to be decreased following zinc supplementation.[87]

In the present study mean level of serum Zn are decreased significantly in cases compared to controls. Our findings of the significantly decreased serum zinc levels in cases when compared to controls are in accordance with the study organized by that of Al-Maroof et. al.[80] Kinlaw et. al.[88] and Pai et. al.[89] which also state that most of the patients with type 2 diabetes mellitus show zinc deficiency. Also the serum HDL levels of the controls when compared to cases are reduced significantly. The incidence of reduced HDL-cholesterol level in the patients with diabetes mellitus when compared to the non-diabetic individuals is significant. Also in accordance with the findings of our studies with reduced HDL-c in type 2 DM a number of studies on HDL levels in type 2 diabetic patients also have showed low HDL levels were common findings in comparison to non-diabetic control groups as reported by

Rainwater et.al.[90], Seyoum et. al.[91], Agrawal et. al.[92] and Saaddine et. al.[93] Thus, both the sexes i.e. men and women with the presence of diabetes had a greater frequency of hyper trigyceridemia and low HDL cholesterol levels.[94] A comparable design of changed plasma lipid profiles was observed in the UK Prospective Diabetes Study (UKPDS). In this study, the levels total cholesterol of those with presence of diabetes mellitus and control individuals did not differ. On the other hand, when we see women with type 2 diabetes mellitus they had markedly elevated levels of LDL cholesterol than compared to women who did not have diabetes. The plasma levels of triglyceride in the patients with type 2 diabetes mellitus were also substantially increased, whereas HDL-c levels were noticeably decreased in both men and women with diabetes mellitus compared with the non-diabetic controls.[95] Reduced Zn concentrations in the plasma were also associated with increased risk for coronary artery disease and mortality in diabetics as reported by R.B. Singh et.al.[13]

The close relationship between zinc and insulin action was first documented by Scott in early 1930s, when zinc was found to be an integral component of crystalline insulin. Thus over the period of years, several studies have been conducted to demonstrate the effectiveness of Zn in diabetes mellitus that zinc ions play an important roles in the biosynthesis, storage, and action of insulin. Fascinatingly, some of the zinc complexes, per se, showed insulinomimetic effects, including attenuating hyperglycemia and increasing lipogenesis, when these complexes were orally or intraperitoneally administrated to mice. Studies on mechanisms underlying the effects of zinc on insulin signaling are limited. But current evidence suggested that enhancement of tyrosine kinase phosphorylation in insulin signal transduction and improved binding of insulin to its receptor may be involved. Another line of evidence indicated that zinc could act as an antioxidant as well. Zinc may protect insulin and

cells from being attacked by free radicals by playing a structural role of antioxidant enzymes, such as copper, zinc, and superoxide dismutase (CuZnSOD) by competing with redox-active transitional metals, such as iron or by stimulating expression of metallothionein a free radical scavenger.[57]

CONCLUSION

This study was conducted in the Department of Biochemistry, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on 35 diagnosed type 2 diabetes mellitus cases with equal age and sex matched healthy controls.

The findings of the study suggest that there was a significant difference in the levels of serum zinc and HDL-cholesterol concentration in cases when compared to the controls. Data from the study thus demonstrates that though there been a positive co-relation between the levels of serum zinc and HDL levels the co-relation is not so significant.

Animal studies have demonstrated the anti-diabetic potential of Zn compounds thus from our study we would like to highlight to the significance of the regulation of blood glucose level in the diabetic patients which could be brought about by effective zinc supplementation and thus prevent the complications arising out of the impaired blood glucose level. The dyslipidemia being one of the most common manifestations in a DM and that studies suggesting a hypothetical query if the zinc supplementation could improve or reduce the HDL-c concentration. The effect of this zinc supplementation on HDL in DM patients needs to be explored.

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BIBLIOGRAPHY

1. Wikipedia [Internet] updated 20September 2013 [cited 20 September 2013]. Available from: http://en.wikipedia.org/wiki/Dietary_mineral

2. Zargar AH, Shah NA, Masoodi SR, Laway BA, Dar FA, Khan AR, et. al. Copper, Zinc and magnesium levels in fibrocalculous pancreatic diabetes Postgrad Med J. 1998;74(877):665-8

3. Winterberg B, Bocchichchio M, Horsdorf TH, Lahi H, Lison AE, Zimkley H. Zinc in treatment of diabetic patients. Trace Elem Med 1989;6:173-7.

4. Roth HP, Kirchgessner M. Zinc and insulin metabolism. Biol Trace Elem Res 1981;3:13-32.

5. Taylor CG. Zinc, the pancreas, and diabetes: insights from rodent studies and future directions. Biometals 2005;18(4):305-12.
6. Egefjord L, Petersen AB, Rungby J. Zinc, alpha cells and glucagon secretion. Curr Diabetes Rev 2010;6(1):52-7.
7. Khardori R, Griffing GT, BessenHA,Brenner BE, IsleyWL,Ligaray KP et. al. reference.medscape.com /medline. [homepage on the Internet]. 2013 [cited 2013 May 21]. Available from: http://emedicine.medscape.com/article /117853
8. Powers AC. Diabetes mellitus. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL et al. editors. Harrison's, principles of internal medicine. 17th ed. New York: McGraw Hill; 2008;2:2291

9. Scott DA: Crystalline insulin. Biochem J 1934;28:1592–1602.
10. Adachi Y, Yoshida J, Kodera Y, Kiss T, Jakusch T, Enyedy EAet. al. Oral administration of a zinc complex improves type 2 diabetes and metabolic syndromes. BiochemBiophys Res Commun 2006;351:165–70.
11. Fugono J, Fujimoto K, Yasui H, Kawabe K, Yoshikawa Y, Kojima Y et. al.Metallokinetic study of zinc in the blood of normal rats given insulinomimeticzinc(II) complexes and improvement of diabetes mellitus in type 2 diabetic GK rats by their oral administration. DrugMetabPharmacokinet 2002;17:340–47.
12. Faure P, Lafond JL, Coudray C, Rossini E,Halimi S, Favier A, et. al. Zinc prevents the structural and functional properties of free radical treated-insulin. BiochimBiophysActa 1994;1209:260–64.
13. Singh RB, Niaz MA, Rastogi SS, Bajaj S, Gaoli Z, Shoumin Z: Current zinc intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations of North India. J Am CollNutr 1998;17:564–70.
14. Chausmer AB. Zinc, insulin and diabetes. J Am College of Nutr 1998;17(2):109-15.
15. Hess SY, Lonnerdal B, Hotz C, Rivera JA, Brown KH.Recent advances in knowledge of zinc nutrition and human health. Food and Nutrition Bulletin 2009;30(1):S5-11.
16. Position statement. In: Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2008;31:S55-60.

17. World Health Organization. Media center. [Internet]. 2013[updated March 2013; cited 16 June 2013]. Available from: [http://www.who.int/mediacentre/factsheets/fs312/en/index/html]
18. V. Mohan, S. Sandeep, R. Deepa, B. Shah, C. Varghese. Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res, March 2007;125(3):217-30.
19. Burden: mortality, morbidity and risk factors. Chapter 1. In: Global status report on non-communicable diseases 2010. Geneva, Switzerland. WHO Press; 2011.p. 9-31.
20. Samatha P, Siva Prabodh V, Chowdary NVS, Shekha R. Glycated Hemoglobin and Serum Lipid Profile Associations in Type 2 Diabetes MellitusPatients. 2012; 17(12):1-4
21. Taubes G. Nutrition. The soft science of dietary fat. Science. 2001;291:2536-45.
22. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. 2009;5(3):150-59.
23. Shen H, MacDonald R, Bruemmer D, Stromberg A, Daugherty A, Li X, Toborek M et.al. Zinc Deficiency Alters Lipid Metabolism in LDL Receptor–Deficient Mice Treated with Rosiglitazone. J. Nutr. 2007;137:2339–2345.
24. Samman S, Roberts DC. The effect of zinc supplements on lipoproteins and copper status. Atherosclerosis. 1988;70:247–52.
25. Partida-Hernandez G, Arreola F, Fenton B, Cabeza M, Roman-Ramos R, Revilla-Monsalve MC. Effect of zinc replacement on lipids and lipoproteins in type 2-diabetic patients. Biomed Pharmacother. 2006;60:161–8.
26. MedlinePlus the National Institutes of Health's Web site. [Internet]. 2011 [updated February 2011; cited 12 June 2013]; Available from:URL:http://www.nlm.nih.gov/medlineplus/ency/article/002416.html

27. Prasad AS. Zinc: an overview. Nutrition 1995;11(1):93–99
28. Tapiero H, Tew KD. Trace elements in human physiology and pathology: zinc and metallothioneins. Biomed Pharmacother 2003;57(9):399–411
29. Kambe T, Yamaguchi-Iwai Y, Sasaki R, Nagao M. Overview of mammalian zinc transporters. Cell Mol Life Sci 2004;61(1):49– 68
30. Cai L, Klein JB, Kang YJ. Metallothionein inhibits peroxynitriteinduced DNA and lipoprotein damage. J BiolChem 2000;275(50):38957–60
31. Cai L. Metallothionein and cardiomyopathy. In: Zatta P. Metallothioneins in Biochemistry and Pathology. New Jersey: World Scientific, 2008:227–69
32. Prasad AS. Discovery of human zinc deficiency: 50 years later. J Trace Elem Med Biol 2012;26(2–3):66–69
33. Cai L. Metallothionein as an adaptive protein prevents diabetes and its toxicity. Nonlinearity BiolToxicol Med 2004;2(2):89–103
34. Cai L. Diabetic cardiomyopathy and its prevention by metallothionein: experimental evidence, possible mechanisms and clinical implications. Curr Med Chem 2007;14(20):2193–203
35. Goldman J, Carpenter FH. Zinc binding, circular dichroism, and equilibrium sedimentation studies on insulin (bovine) and several of its derivatives. Biochemistry 1974;13(22):4566–74
36. Bakaysa DL, Radziuk J, Havel HA, Brader ML, Li S, Dodd SWet. al. Physicochemical basis for the rapid time-action of Lys B28 Pro B29-insulin: dissociation of a protein-ligand complex. Protein Sci 1996;5(12):2521–31

37. Protein Data Bank 1ai0; Chang X, Jorgensen AM, Bardrum P, Led JJ. "Solution structures of the R6 human insulin hexamer". Biochemistry 1997;36 (31):9409–22.
38. Wang X, Zhou B. Dietary zinc absorption: A play of Zips and ZnTs in the gut. IUBMB Life 2010;62(3):176–182
39. Fukada T, Kambe T. Molecular and genetic features of zinc transporters in physiology and pathogenesis. Metallomics 2011;3(7):662–674
40. Kambe T. An overview of a wide range of functions of ZnT and Zip zinc transporters in the secretory pathway. Biosci Biotechnol Biochem 2011;75(6):1036–43
41. Eide DJ. Zinc transporters and the cellular trafficking of zinc. Biochimicaet Biophysica Acta 1763. 2006;711–22
42. Fukada T, Yamasaki S, Nishida K, Murakami M, Hirano T. Zinc homeostasis and signaling in health and diseases: Zinc signaling. J BiolInorg Chem 2011; 16(7):1123–34
43. Park JH, Grandjean CJ, Hart MH, Erdman SH, Pour P, Vanderhoof JA. Effect of pure zinc deficiency on glucose tolerance and insulin and glucagon levels. Am J Physiol 1986; 251(3 Pt 1): E273–78
44. Faure P, Roussel AM, Martinie M, Osman M, Favier A, Halimi S. Insulin sensitivity in zinc-depleted rats: assessment with the euglycaemic hyperinsulinic clamp technique. Diabete Metab 1991;17(3):325–31
45. Jou MY, Philipps AF, Lonnerdal B. Maternal zinc deficiency in rats affects growth and glucose metabolism in the offspring by inducing insulin resistance postnatally. J Nutr 2010;140(9):1621–1627

46. Prasad AS. Zinc deficiency has been known of for 40 years but ignored by global health organizations. BMJ 2003;326:409-10.
47. Krus-Jarres JD, Rukagauer M. Trace elements in diabetes mellitus. Peculiarities and clinical validity of determinations in blood cells. J. Trace Elements Med. Biol. 2000;14:21-27.
48. Song MK, Rosenthal MJ, Naliboff BD, Phanumas L, Kang KW. Effects of bovine prostate power on zinc, glucose and insulin metabolism in old patients with non-insulin dependent diabetes mellitus. Metabolism 1998;47:39-43.
49. Salgueiro MJ, Krebs N, Zubillaga MB, Weill R, Postaire E, Lysionek AE, et al. Zinc and diabetes. Biol Trace Elem Res 2001;81:215-28
50. Tang X, Shay NF. Zinc has an insulin-like effect on glucose transport mediated by phosphoinositol-3-kinase and Akt in 3T3-L1 fibroblasts and adipocytes. J Nutr 2001;131:1414-20.
51. Jindal RM, Taylor RP, Gray DW, Esmeraldo R, Morri PJ. A new method for quantification's of islets by measurement of zinc content. Diabetes 1992;41:1056-62.
52. Szaleczky E, Prechl J, Pusztai P, Rosta A, Feher J, Somogyi A. Antioxidant status of patients with well controlled type 1 diabetes. Med SciMonit 1997;3:163-66.
53. Maret W, Sandstead HH: Zinc requirements and the risks and benefits of zinc supplementation. J Trace Elem Med Biol 2006,20:3-18.
54. Lowe NM, Fekete K, Decsi T: Methods of assessment of zinc status in humans: a systematic review. Am J ClinNutr 2009;89:2040S-51.

55. Wiernsperger N, Rapin J R. Trace elements in glucometabolic disorders: an update. Diabetology& Metabolic Syndrome 2010;2:70
56. Jansen J, Karges W, Rink L: Zinc and diabetes–clinical links and molecular mechanisms. J NutrBiochem 2009,20:399-417.
57. Sun Q, van Dam RM, Willett WC, Hu FB: Prospective study of zinc intake and risk of type 2 diabetes in women. Diabetes Care 2009,32:629-34.
58. Raz I, Karsai D, Katz M: The influence of zinc supplementation on glucose homeostasis in NIDDM. Diabetes Res 1989;11:73-9.
59. Marreiro DN, Geloneze B, Tambascia MA, Lerario AC, Halpern A, Cozzolino SM: Effect of zinc supplementation on serum leptin levels and insulin resistance of obese women. Biol Trace Elem Res 2006;112:109-18.
60. Beletate V, El Dib RP, Atallah AN. Zinc supplementation for the prevention of type 2 diabetes mellitus. Cochrane Database Syst Rev. 2007 Jan 24;(1):1-16.
61. Mariani E, Mangialasche F, Feliziani FT, Cecchetti R, Malavolta M, Bastiani P et. al. Effects of zinc supplementation on antioxidant enzyme activities in healthy old subjects. ExpGerontol 2008;43:445-51
62. el-Yazigi A, Hannan N, Raines DA. Effect of diabetic state and related disorders on the urinary excretion of magnesium and zinc in patients. Diabetes Res 1993;22(2):67–75
63. Golik A, Cohen N, Ramot Y, Maor J, Moses R, Weissgarten J, Leonov Y, Modai D. Type II diabetes mellitus, congestive heart failure, and zinc metabolism. Biol Trace Elem Res 1993;39(2–3):171–75

64. Anderson RA, Roussel AM, Zouari N, Mahjoub S, Matheau JM, Kerkeni A. Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. J Am CollNutr 2001;20(3):212–18
65. Anetor JI, Senjobi A, Ajose OA, Agbedana EO. Decreased serum magnesium and zinc levels: atherogenic implications in type-2 diabetes mellitus in Nigerians. Nutr Health 2002;16(4):291–300
66. Roussel AM, Kerkeni A, Zouari N, Mahjoub S, Matheau JM, Anderson RA. Antioxidant effects of zinc supplementation in Tunisians with type 2 diabetes mellitus. J Am CollNutr 2003;22(4):316–21
67. Masram SW, Ghangle S, Bimanpalli MV. Study of Lipid Profile and Glycated Hemoglobin in Diabetes Mellitus. Indian Medical Gazette 2012;146(7):257-65
68. NayakBS, Ali P, Seepersad R, Seepersad D, Cooke N, GeawanS et. al. Type 2 diabetes, high triglycerides, LDL and low HDL are risk factors for cardiovascular disease in patients 45 years and older in Trinidad. Ijrpbsonline. 2013;4(1):165-69.
69. Krauss RM, Siri PW. Dyslipidemia in type 2 diabetes. Med Clin N Am 2004;88:897–909
70. Laws A, Hoen HM, Selby JV, Saad MF, Haffner SM, Howard BV. Differences in insulin suppression of free fatty acid levels by gender and glucose tolerance status: relation to plasma triglyceride and apolipoprotein B concentrations: Insulin Resistance Atherosclerosis Study (IRAS) Investigators. ArteriosclerThrombVascBiol 1997;17:64–71

71. Bitzur R, Cohen H, Kamari Y, Shaish A, Harats. Triglycerides and HDL Cholesterol. Diabetes Care, 2009;32(2);373-77.
72. De Man FH, Cabezas M, VanBarlingen HH, Erkelens DW, De Bruin TW. Triglyceride rich lipoproteins in non-insulin dependent diabetes mellitus: post prandial metabolism and relationto premature atherosclerosis. Eur J Clin Invest 1996;26:89-108.
73. Jeppesen J, Hollenbeck CB, Zhou MY, Coulston C, Jones C, Chen YD, and Reaven GM. Relation between insulin resistance, hyperinsulinemia, postheparin plasma lipoprotein lipase activity and postprandial lipemia. ArteriosclerThromb Vase Biol 1995;15:320-24.
74. Berk-Planken IL. Introduction In: Statin therapy in diabetic dyslipidemia. Efficacy and mechanisms. Enschede, Netherlands. Print partners. Chapter 1 2003; pg. 13-14
75. Lewis GF, Daniel J. Rader New Insights into the Regulation of HDL Metabolism and Reverse Cholesterol Transport. Circ Res. 2005;96:1221-32
76. Assmann G, Gotto AM Jr. HDL cholesterol and protective factors in atherosclerosis. Circulation. 2004;109:1118 –14
77. Despres JP, Lemieux I, Dagenais GR, Cantin B, Lamarche B. HDLcholesterol as a marker of coronary heart disease risk: the Quebec cardiovascular study. Atherosclerosis. 2000;153:263–72
78. Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB et. al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. N Engl J Med. 1999;341:410–18.

79. Heinecke JW. The not-so-simple HDL story: A new era for quantifying HDL and cardiovascular risk? Nature Medicine 2012;18:1346–47.
80. Al-Maroof RA, Al-Sharbatti SS. Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type 2 diabetics. Saudi Med J 2006;27(3):344-50
81. Plum LM, Rink L, Haase H. The essential toxin: impact of zinc on human health. Int J Environ Res Public Health 2010 Apr;7(4):1342-65.
82. Masood N, Baloch GH, Ghori RA, Memon IA, Memon MA, Memon MS. Serum zinc and magnesium in type-2 diabetic patients. J Coll Physicians Surg Pak 2009 Aug;19(8):483-6.
83. Powers AC. Diabetes mellitus. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL et al. editors. Harrison's, principles of internal medicine. 17th ed. New York: McGraw Hill; 2008. vol 2 p. 2277.
84. Haswell SJ. Atomic Absorption Spectrometry; Theory, Design and Applications. Amsterdam: Elsevier; 1991.
85. Gowenlock AH, McMurray, McLauchalan DM. Varley's Practical Clinical Biochemistry. 6th ed. London: Heinmann Medical books; 1998.
86. Kanoni S, Nettleton JA, Hivert MF, Ye Z, van Rooij FJ, Shungin D, et al. Total zinc intake may modify the glucose-raising effect of a zinc transporter (SLC30A8) variant: a 14-cohort meta-analysis. Diabetes 2011;60:2407–16.
87. Foster M, Petocz P, Samman S. Effects of zinc on plasma lipoprotein cholesterol concentrations in humans: a meta-analysis of randomized controlled trials. Atherosclerosis 2010;210:344–52.

88. King JC, Cousins RJ. Zinc. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, editors. Modern Nutrition in Health and Disease. 10th ed. Philadelphia: Lippincott Williams and Wilkins; 2006. p. 271–85.
89. Hughes S, Samman S. The effect of zinc supplementation in humans on plasma lipids, antioxidant status and thrombogenesis. J AmCollNutr 2006;25:285–91.
90. Rainwater DL, MacCluer JW, Stern MP, Vandeberg JL, Haffner SM. Effects of NIDDM on lipoprotein (a) concentration and apoprotein (a) size. Diabetes 1994;43:942-46.
91. Seyoum B, Abdulkadir J, Berhanu P,Feleke Y, Mengistu Z, Worku Y et. al. Analysis of serum lipids and lipoproteins in Ethiopian diabetic patients. Ethiop Med J 2003;41:1–8.
92. Agrawal RP, Sharma P, Pal M, Kochar A, Kochar DK. Magnitude of dyslipedemia and its association with micro and macro vascular complications in type 2 diabetes: a hospital based study from Bikaner (Northwest India). Diabetes Res ClinPract 2006;73:211–14.
93. Saaddine JB, Engelgau MM, Beckles GL, Gregg EW, Thompson TJ,Narayan KM. A diabetes report card for the United States: quality of care in the 1990s. Ann Intern Med 2002;136:565.
94. Kinlaw WB, Levine AS, Morley JE, Silvis SE, McClain CJ. Abnormal zinc metabolism in type II diabetes mellitus. Am J Med 1983;75(2):273–7.
95. U.K. Prospective Diabetes Study 27. Plasma lipids and lipoproteins at diagnosis of NIDDM by age and sex. Diabetes Care 1997;20:1683–87

ANNEXURE I

INFORMED CONSENT

Mr/Mrs/Ms…..…… you are invited to participate in "A cross-sectional study to estimate serum zinc and HDL levels in type 2 diabetes mellitus patients". Participation in this study is completely voluntary. All the patients with type 2 diabetes mellitus and equal number of healthy volunteers will be enrolled in this study at Jawaharlal Nehru Medical College, Belgaum under the supervision of

PURPOSE OF THE STUDY

Studies done earlier suggest that there is increased excretion of zinc in diabetics and also low serum levels of zinc. Hence this study will be done to estimate the levels of zinc and high density lipoproteins in type 2 DM patients to check for its altered or deranged metabolism in the patients on oral hypoglycaemic drugs.

PROCEDURE

For type 2 diabetes mellitus patients (Cases) and healthy subjects (Controls), 10 ml of venous blood was collected under aseptic precautionary measures using sterile disposable syringe in plain vacutainers and labelled centrifuged immediately to separate serum.

RISKS

Since the blood is drawn under aseptic precautionary measures by trained persons there is no scope for any risks. Further only small volume of blood is collected which will be spontaneously replenished in the body. However there may be minor risks associated with having blood drawn that may include bruising, redness, discomfort or bleeding at the puncture site.

BENEFITS

No direct benefit is guaranteed to you from participating in our study. You can make use of blood levels of studied parameters if desired.

OPTIONS

If you decide not to participate in this study, the hospital will provide you the usual standard care and treatment.

NEW INFORMATION

Does not apply to this research.

PRIVACY AND CONFIDENTIALITY

All information collected about you during the course of the study will be kept confidential to the extent permitted by law. You will be identified in this research record by the code numbers. Information which identifies you personally will not be revealed without your written permission. However your records may be revealed to the sponsor of the study. Information from this study may be published but your identity will be confidential in any publication.

INSTITUTIONAL POLICY

In the event that you are physically injured as a result of participating in this research emergency care will be available. There is no commitment to provide any compensation for research related injury. The Jawaharlal Nehru Medical College will provide, within the limitations of the laws of the state of Karnataka, facilities and medical attention to subjects who suffered any harm as the result of your participation in this study. In the event you believe that you have suffered any how as a result of your participation in this study you may contact research guide

COST FOR PARTICIPATION

You will not be charged for the test to be carried out on your blood sample.

FINANCIAL INCENTIVE FOR PARTICIPATION

You will not receive any remuneration for participating in this study.

VOLUNTARY PARTICIPATION/WITHDRAWAL

If you decide not to participate in this study, it will not affect the quality of the medical care you receive at this institution.

You may withdraw from the study anytime. The researchers might use the information learned from the study in scientific journal articles or in presentations.

In case you have any questions regarding your rights as a study participant, you may please contact Chairman of Institutional Ethics Committee of Human Subjects Research,

EMERGENCY PROVISION

If you have any questions as a participant in our study, you can contact the study investigator or contact the research guide

CONSENT TO PARTICIPATE IN A RESEARCH TRIAL

I voluntarily agree to take part in this study. If I choose to take part in the study, I may withdraw at anytime. I am not giving any of my legal right by signing this form. My signature below indicates that I have read, or had read to me, this entire consent form including the risks and benefits. I may ask questions at any time.

_____ _____

Signature of participant Date

_____

Participants Name (Printed):

_____ _____

Name and Signature of witness-1 Date

_____ _____

Name and Signature of witness-2 Date

_____ _____

Signature of researchers or Date

Person obtaining consent

ANNEXURE II

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING THE DATA

"A cross-sectional study to estimate serum zinc and HDL levels in type 2 diabetes mellitus patients"

Name : Sex :

Age : I.P. No. :

DOA :

Address :

Presenting complaints

Past history

Family History

Personal history

Sleep

Appetite

Bowel and bladder

General Physical Examination

Vitals

Pulse rate :Blood pressure :

Pallor : Nail clubbing :

Cyanosis : Respiratory rate :

Icterus : Temperature :

Lymphadenopathy : Pedal oedema :

Per abdomen/local examination

Inspection :

Palpation :

Percussion :

Auscultation :

Respiratory system

Inspection :

Palpation :

Percussion :

Auscultation :

CVS

Pulse :

Blood pressure :

CVS – Central

Inspection :

Palpation :

Percussion :

Auscultation :

Central nervous system

Higher Function Tests:

Cranial Nerves examination:

Motor System:

Sensory System:

Cerebellar Functions:

Skull & Spine:

Investigations:

Serum

- Serum zinc
- Serum HDL

Final Diagnosis

ANNEXURE III

MASTER CHART – CONTROLS

Sl. No.	Controls	HDL values	Zinc values	Sex	Age
1	CON 01	37	113	F	43
2	CON 02	41	95	M	42
3	CON 03	35	108	M	50
4	CON 04	40	109	F	45
5	CON 05	38	98	F	41
6	CON 06	45	96	M	49
7	CON 07	39	112	M	38
8	CON 08	37	117	F	37
9	CON 09	37	107	M	40
10	CON 10	42	98	F	45
11	CON 11	38	82	F	44
12	CON 12	39	89	M	35
13	CON 13	40	111	M	40
14	CON 14	52	104	F	42
15	CON 15	41	97	F	50
16	CON 16	37	108	M	53
17	CON 17	40	112	M	50
18	CON 18	38	96	F	45

19	CON 19	36	85	F	43
20	CON 20	35	81	M	47
21	CON 21	46	93	M	44
22	CON 22	41	102	M	50
23	CON 23	40	105	F	46
24	CON 24	52	99	F	48
25	CON 25	41	117	F	45
26	CON 26	38	120	F	42
27	CON 27	44	98	F	40
28	CON 28	41	86	M	43
29	CON 29	39	105	M	35
30	CON 30	47	117	M	46
31	CON 31	36	82	M	45
32	CON 32	37	98	M	50
33	CON 33	36	93	F	42
34	CON 34	39	85	F	40
35	CON 35	40	97	M	41

MASTER CHART – CASES

Sl. No.	Cases	HDL values	Zinc values	Sex	Age
1	DM 01	29	29	F	38
2	DM 02	26	31	M	45
3	DM 03	31	30	M	35
4	DM 04	28	36	F	42
5	DM 05	33	48	F	41
6	DM 06	30	52	M	37
7	DM 07	26	45	M	43
8	DM 08	30	36	F	49
9	DM 09	32	49	M	40
10	DM 10	27	38	F	43
11	DM 11	27	42	F	45
12	DM 12	32	29	M	48
13	DM 13	30	63	M	42
14	DM 14	26	25	F	47
15	DM 15	31	52	F	50
16	DM 16	30	46	M	45
17	DM 17	29	31	M	50
18	DM 18	28	43	F	42

19	DM 19	27	54	F	44
20	DM 20	31	72	M	47
21	DM 21	27	38	M	43
22	DM 22	33	50	M	50
23	DM 23	26	65	F	44
24	DM 24	25	31	F	50
25	DM 25	28	44	F	40
26	DM 26	31	50	F	42
27	DM 27	34	48	F	40
28	DM 28	32	31	M	43
29	DM 29	29	68	M	41
30	DM 30	30	44	M	46
31	DM 31	27	52	M	50
32	DM 32	27	38	M	45
33	DM 33	29	27	F	46
34	DM 34	33	39	F	45
35	DM 35	30	30	M	35

SUMMARY

The objectives of the present study were to assess serum zinc and HDL cholesterol concentrations in type 2 diabetes mellitus patients and to compare the values of the above parameters with that of controls.

The present study was conducted in Department of Biochemistry, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum between January 2012 to December 2012 with 35 cases of type 2 diabetes mellitus patients on oral hypoglycemic drugs and 30 healthy controls with age and sex match. 10 ml of blood was collected from the patients and controls under aseptic precautionary measures using disposable syringe plain tubes, centrifuged, serum separated and kept at 4^0C which was analyzed for HDL immediately and for serum zinc within 30 days of collection of the sample using atomic absorption spectrophotometer.

Our study revealed that there was positive co-relation between the levels serum zinc and serum HDL-cholesterol concentration. Further on statistical analysis it revealed that it was not statistically significant and thus findings of this study could be used to use zinc as supplementation in the type 2 diabetic patients to keep the blood glucose levels under good control.