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Study the Effect of Zinc in Patient with Cardiovascular Disease

Hassan Ahmed Hassan, Muhammad Abboud Sattam, Mustafa Mohi Arnous, Tabark Abdulkareem Sabaa

Abstract: In this research the effect of zinc in Patient with cardiovascular disease where studied . The levels of TC, TG, , LDL-C, and VLDL-C ,respectively compared with control. The mean levels of TC, TG, LDL-C, and VLDL-C were increased in CVD cases as compared to controls and were statistically highly significant ($p<0.01$). the levels of HDL and Zinc compared with control, where as mean level of HDL-C and zinc highly significantly decreased in CVD cases as compared to controls ($p<0.01$). the correlation between zinc and TC , the correlation between zinc and TG, the correlation between zinc and LDL and correlation between zinc and VLDL-C are studied .

Introduction

Zinc (Zn) is one of the most essential micronutrients involved in numerous crucial biological functions, i.e., cell differentiation and proliferation, cellular transport, DNA synthesis, endocrine, immune, and central nervous system functioning, reproduction, gene expression, and homeostasis [(1)]. With the capacity to bind more than 300 enzymes and over 2,000 transcriptional factors, it is often regarded as a multipurpose trace element [(2)]. Zn is a major antioxidant mineral responsible for inhibiting expansion and negative effects of free radicals and regulating the oxidant-antioxidant balance of cells [(3)]. Zn deficiency significantly affects the functioning of biological systems, creates dysfunctions in humoral and cell-mediated immunity, consequently, increases the vulnerability to infections—predisposing people to disturbances in gut microbiota activity, increases the incidence of bacterial, viral, and fungal infections, and leads to the progression of chronic and degenerative diseases, i.e., type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVDs), and cancers ([3]).

Zinc regulates various physiological responses, including growth, differentiation, maturation, and immune responses. Approximately 60% of the zinc in the body is stored in skeletal muscles, 30% in bones, and 5% in the skin and liver

CVDs are the leading cause of morbidity and mortality worldwide, and 17.9 million people died from CVDs in 2016, representing 31% of global deaths [(4)]. CVD-related deaths are projected to reach 23.6 million annually by 2030 [(1)]. Three-quarters of these deaths occur in low-income and middle-income countries [(4)]. The deficiency of Zn affects 17% of the global population, up to 35% in low-income populations, i.e., South Asia and Africa [(1)]. An association between Zn intake and Zn status with the pathogenesis of CVDs is demonstrated by several experimental and clinical studies [(5, 6)]. Imbalances in Zn homeostasis contribute significantly to the development of CVDs, such as coronary heart disease (CHD), congestive heart failure (HF), ischemic cardiomyopathy (CM), myocardial infarction (MI), sudden cardiac death (SCD), and CVD mortality, in general [(5)].Antioxidant and prooxidant functions of Zn may have various positive effects on CV health and could prevent the development of CVDs [(6)].

Cardiovascular disease (CVD) is a general term for conditions affecting the heart or blood vessels.It's usually associated with a build-up of fatty deposits inside the arteries (atherosclerosis)

and an increased risk of clots. It can also be associated with damage to arteries in organs such as the brain, heart, kidneys and eyes.

CVD is one of the main causes of death and disability in the UK, but it can often largely be prevented by leading a healthy lifestyle.

Materials and Methods

Chemicals

The specific chemicals used in this work are listed in table 2-1 with their suppliers.

Table 1. Chemicals and reagents with their suppliers

Chemicals	Suppliers
Cholesterol kit	Human, Germany
Triglyceride kit	Human, Germany
HDL-Cholesterol kit	Human, Germany
Zinc kit	Spinreact,spain

Instruments

The instruments used in this work are listed in table 2-2.

Table 2. Instrument with their suppliers.

Instrument	Suppliers
Water bath	Gemmy, YCW-01 Taiwan
Centrifuge	Hettich GmbH & Co. RG, Germany
Spectrophotometer	APEl Co. LTD, PD-303, Japan

Patients selection and blood sampling

The study included 25 subjects (both sex) with MI of age group 35-60 years. Out of them 25 were normal healthy individuals and they formed the normal control group. 5ml of fasting venous blood sample was taken from CVD patients and controls . Blood sample was collected in plain vial and incubated at 37˚C for 30 minutes. After incubation, clot was removed and remaining sample was taken in centrifuge test tube. Samples were centrifuged at 3000rpm for 10 to 20 minutes. Supernatant was collected in clean and dry test tube for analysis of lipid profile and zinc. Lipid parameters and zinc were estimated .

Analytical methods and procedures

Determination of total serum cholesterol (TC)

Total serum cholesterol was determined utilizing a ready made laboratory kit for this purpose; the principle of determination was based on the enzymatic hydrolysis according to the following reaction ;

$$\text{Cholesterol ester} \xrightarrow{chelesterol\ esterase} \text{Cholesterol} + fatty\ acids$$

$$\text{Cholesterol} \xrightarrow{chelesterol\ oxidase} \text{Cholest} - 4 - en - 3 - one + H_2O_2$$

$$2H_2O_2 + phenol + 4 - aminoantipyrine \xrightarrow{peroxidase} quinoneimine + 4H_2O_2$$

Quinoneimine, a red complex absorbing light at 500nm.

Reagent

Reagent type	Material	Concentration
Reagent (1) Buffer	Phosphte buffer Phenol Sodium cholate surfactant	100 mmol / L 5 mmol/L 23 mmol/L 1.5 mmol / L
Reagent (2) Enzymes	4-aminoantipyrine Peroxidase Cholesterol oxidase Cholesterol esterase	0.25 mmol/L ≥ 1200 Iu/L ≥ 100 I u/L ≥170 Iu/L
Standard	Cholesterol	200 mg/dL (5.17 mmol / L)

Procedure

Working solution: The contents of one vial of reagent 2 was reconstituted with the contents of one vial of reagent 1, mixed well by inventing and store in the reagent 1 bottle.

	Blank	Standard	Test
Standard	---	10 µl	--
Serum	---	---	10 µI
D.W	10 µl	--	--
Working solution	1 ml	1 ml	1 ml

Mix and measure after incubation at 37 ^{0}C for 5 min, adjust to zero by blank then read at 500 nm.

Concentration of cholesterol (mg/dl) = $\frac{Atest}{Astandard} x\ 200$

N.V = 150-250 mg /dl

Determination of serum triglycerides (TG)

Some triglycerides were determined utilizing a ready mode laboratory kit for this purpose. Principle determined based on the enzymatic hydrolysis according to the following reaction

$$Triglycerides \xrightarrow{lipase} Glycerol + fatty\ acids$$

$$Glycerol{+}ATP \xrightarrow{Glycerokinase} Glycerol - 3 - phosphate + ADP$$

$$Glycerol\text{-}3\text{–}phosphate \xrightarrow[oxiase]{Glycerol-3-phosphate} dihydroxy\ acetone\ phosphate + H_2O_2$$

$$2H_2O_2 + parachlorophenol + 4 - amino\ antipyrine \xrightarrow{peroxidose} quinoneimine + 42H_2O_2$$

The intensity of the color formed is proportional to the triglycerides concentration in the simple

Reagents

Reagent type	Material	Concentration
Reagent (1) Standard	Glycerol equivalent to 200(mg/dL) of TG (µw=875)	2.29 mmol/L
Reagent (2) Buffer	Tris buffer Parachlorophenol	50 mmol, pH 7.5 2 mmol/L
Reagent (2) Enzymes	4-amino antipyrine Lipase Glycerokinase Glycerol-3-phosphate oxidase Peroxidase	0.1 mmol/L 150 000 u/L 500 u/L 2500 u/L

	ATP	440 u/L 0.1 mmol/L

Procedure

Working solution: One bottle of reagent (3) was reconstituted with one bottle of reagent (2). Mixed well by inventing and store in the reagent 3 bottle.

	Blank	Standard	Test
Standard	---	10 µl	--
Serum	---	---	10 µI
D.W	10 µl	--	--
Working solution	1 ml	1 ml	1 ml

Mix and incubate for 5 min at t 37 ^{0}C; adjust to zero by blank and measure light absorbance at 500 nm.

Calculation

Concentration of cholesterol (mg/dl) = $\frac{Atest}{Astandard} x\ 200$

N.V = 65-180 mg /dl

Determination of serum HDLc

The chylomicrons and lipoproteins of VLDL, and LDL contained in the serum sample were precipitates by the addition of 4% phosphotungstic acid solution, which contain 10% magnesium chloride pH 6.2. The supernatant obtained after centrifugation contains the HDL, from which the cholesterol can be determined by complementary kit used in determination of total serum cholesterol as described in .

Reagents:

Reagent type	Material	Concentration
Reagent (1) Precipitating agent	Phosphotungstic acid $MgCl_2$	0.55 mmol/L 25 mmol/L
Reagent (2) Standard	Cholesterol	50 mg/dl or 1.29 mmol/L
Reagent (2) Cholesterol enzymatic working solution		

Procedure

Serum 200 µl

Reagent (precipitant) 500 µl

	Blank	Standard	Test
D.W	0.05 ml	---	---
Reagent(2)	---	0.05 ml	---
Supernatant			0.05 ml
Cholesterol enzymatic working solution	1 ml	1 ml	1 ml

Mix and incubate for 5 min in 37 ^{0}C; adjust to zero by blank, and then measure light absorbance at 500 nm.

Calculation

Concentration of HDLc (mg/dl) = $\frac{A\ test}{A\ standard} x conc\ of\ st.$

N.V = 35-65 mg/dl

Determination of serum LDL-cholesterol

LDL-cholesterol was estimated in directly by the use of friedewald formula [29]:

LDLc = TC - HDLC-VLDLc

= TC - (HDLc+VLDLc)

and VLDLc was calculated as:

Concenteration of VLDLc (mg/dl) = $\frac{TG}{5}$

N.V of LDL = 65-160 mg /dl

N.V of VLDL= 25-50 mg /dl

Determination of serum Zinc

Zinc reacts with 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino) -phenol (5-Br-PAPS) to form a red chelate complex in pH = 9.8 . The increase of absorbance measure at 560 nm is proportional to the concentration of total zinc in the sample.

Reagent R	Concetration
Bicarbonate buffer, pH = 9.8	500 mmol/L
5-Br-PAPS	0.03 mmol/L
Sodium citrate	68 mmol/L
Dimethyl glyoxime	4 mmol/L
Detergent	
Standard	200 mmol/L

Procedure

Wavelength Spectrophotometer Hg (560 nm)

	Blank	standard	sample
Distill water	50 µl		
standard		50 µl	
sample			50 µl
Reagent	1000 µl	1000 µl	1000 µl

Mix, incubate for 8 min. at 20 – 25 °C. Read the absorbance (A). The final colour is stable for at least 30 min.

Calculation

Concentration of zinc (µg/dl) = $\frac{Atest}{Astandard} x200$

N.V; 46- 150 µg/dl

Results

Table 1 and figures (1),(2),(3), and (4) shows the levels of TC, TG, , LDL-C, and VLDL-C ,respectively compared with control. The mean levels of TC, TG, LDL-C, and VLDL-C were increased in CVD cases as compared to controls and were statistically highly significant ($p<0.01$).

Table 1 and figures (5),(6) shows the levels of HDL and Zinc compared with control, where as mean level of HDL-C and zinc highly significantly decreased in CVD cases as compared to controls ($p<0.01$).

Table 1. Mean levels of lipid profile, and Zinc in healthy subjects and CVD patients

groups	cholesterol	Triglyceride	HDL	VLDL	LDL	zinc
Healthy subjects	171.50±7.25[a]	104.80±15.3[a]	53.0±4.98[a]	20.3±2.49[a]	97.60±10.78[a]	96.91±19.28[a]
patients	306±28.51[b]	353±40.49[b]	27.3±3.23[b]	70.60±8.09[b]	208.9±27.26[b]	35.4±7.45[b]

Values represent mean ±SD; values with non-identical superscripts (a,b) indicated significant differences between groups ($P<0.01$)

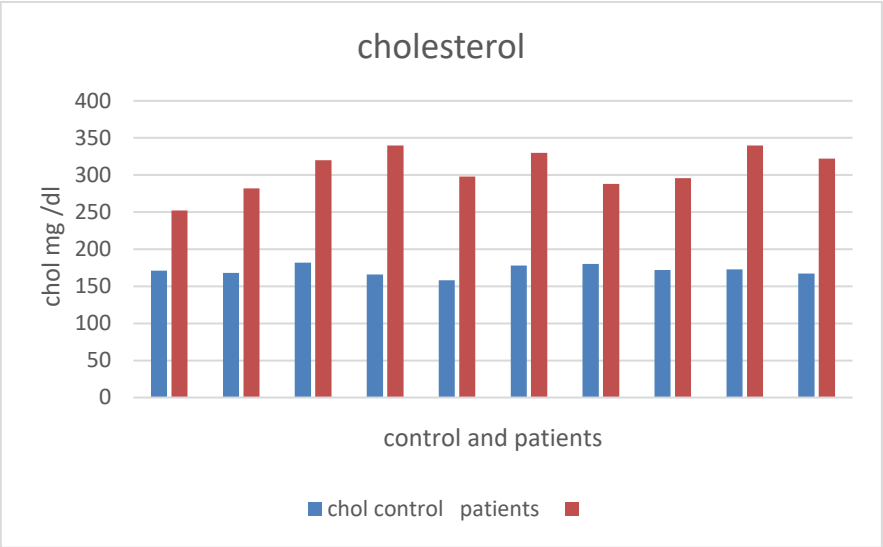


Figure 1. differences in serum cholestero levels of CVD patients and controls

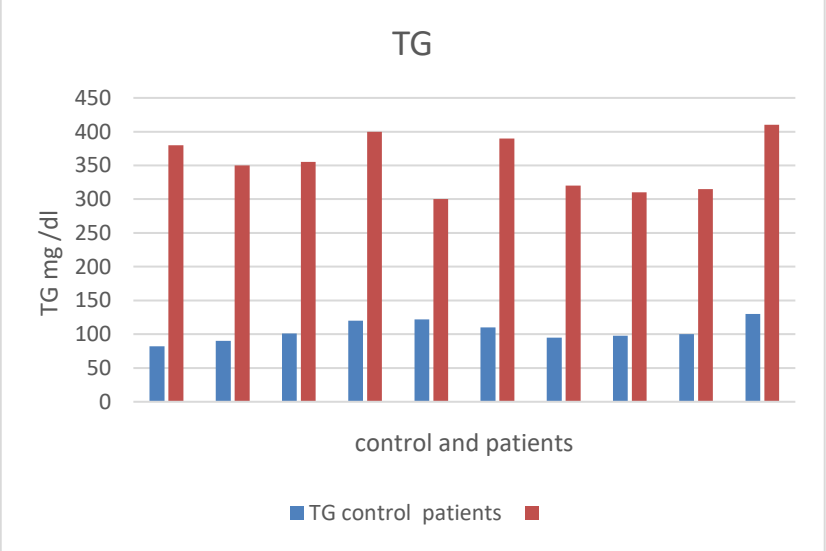


Figure 2. differences in serum TG levels of CVD patients and controls

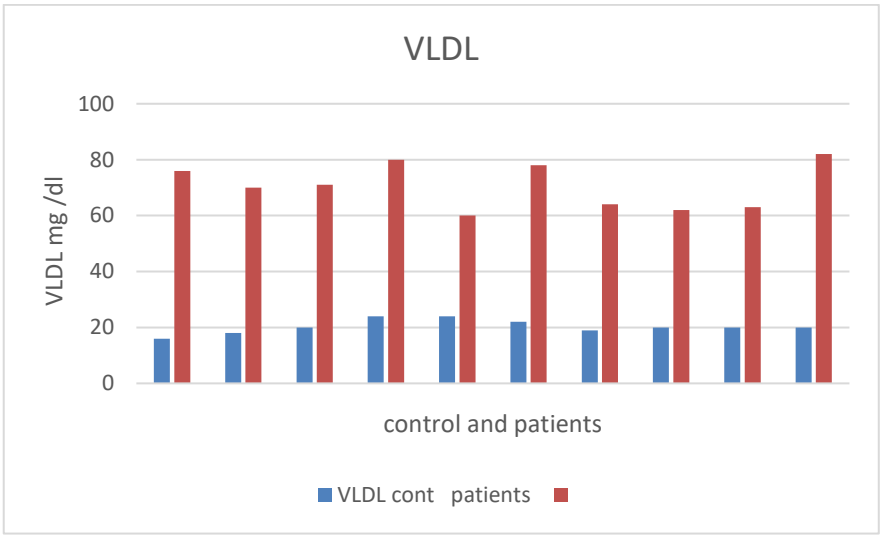


Figure 3. differences in serum VLDL levels of CVD patients and controls

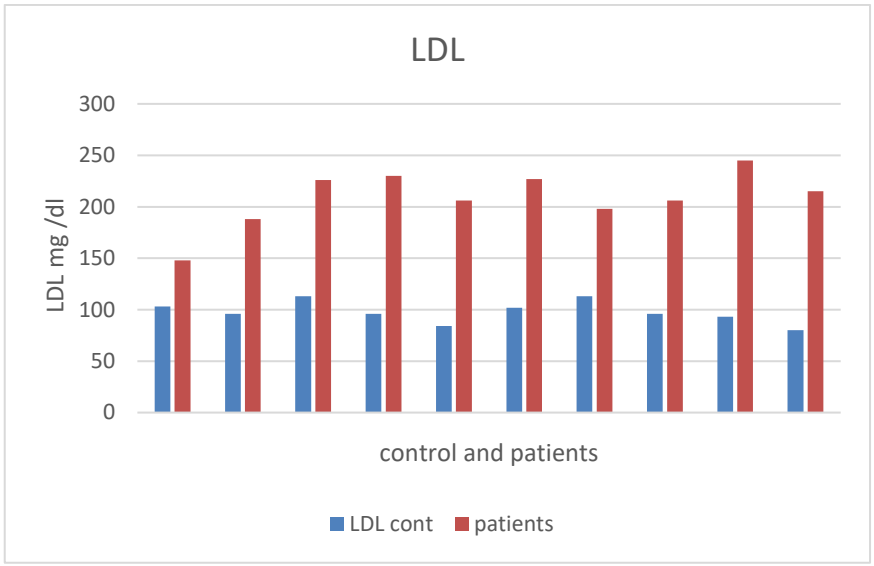


Figure-4. differences in serum LDL levels of CVD patients and controls

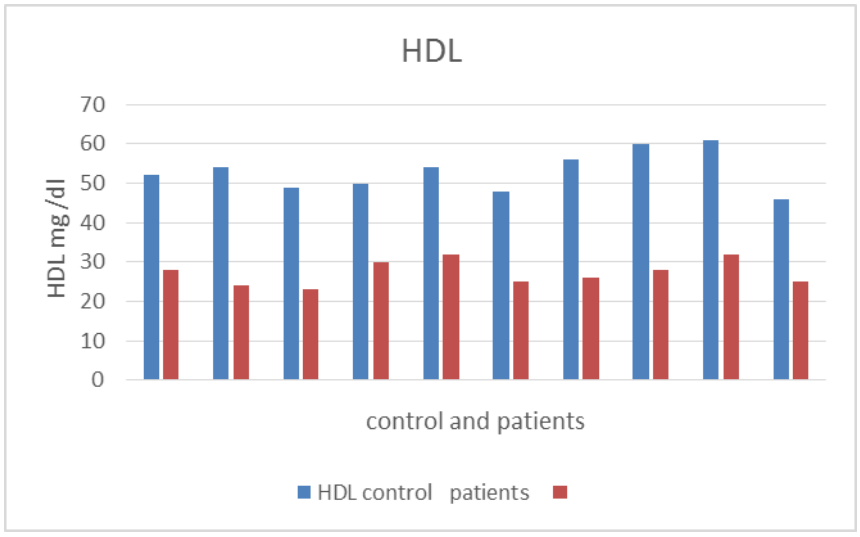


Figure 5. differences in serum HDL levels of CVD patients and controls

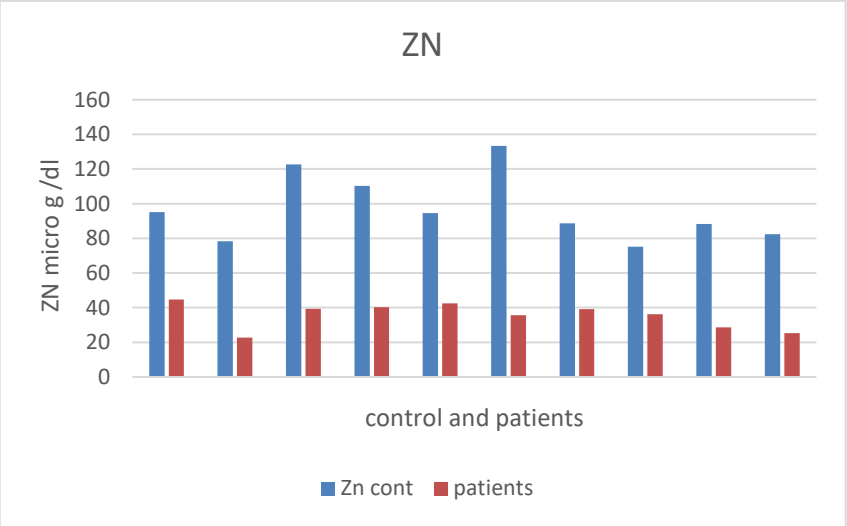


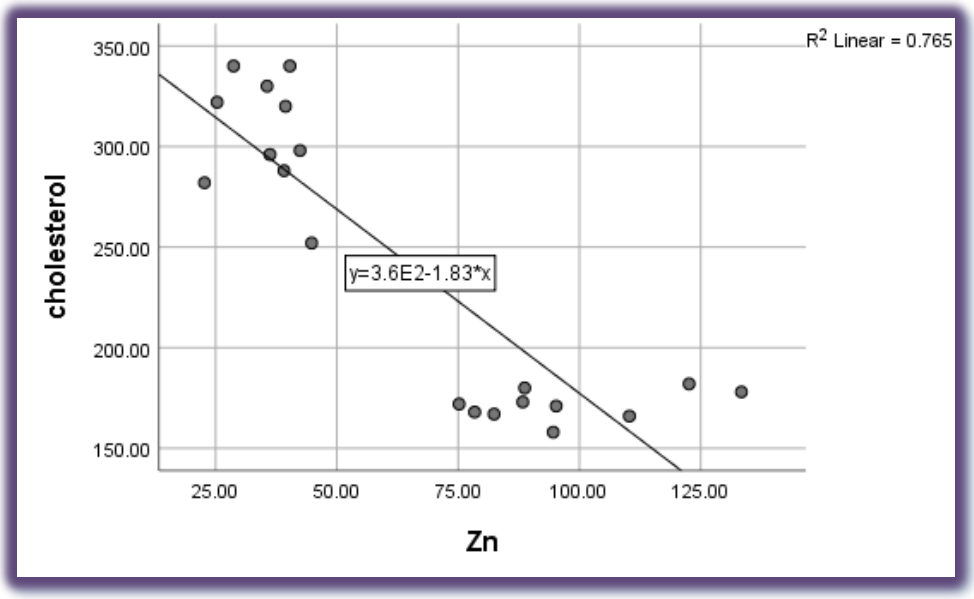
Figure 6. differences in serum Zinc levels of CVD patients and controls

Table 2 and figures (7),(8),(9),and (10) shows correlations of zinc with cholesterol,TG,VLDL and LDL, respectively. Zinc was negatively correlated with total cholesterol, triglyceride, LDL and VLDL in CVD patients and was statistically highly significant while,Figure (11) show highly significant positive correlation between zinc and HDL cholesterol.

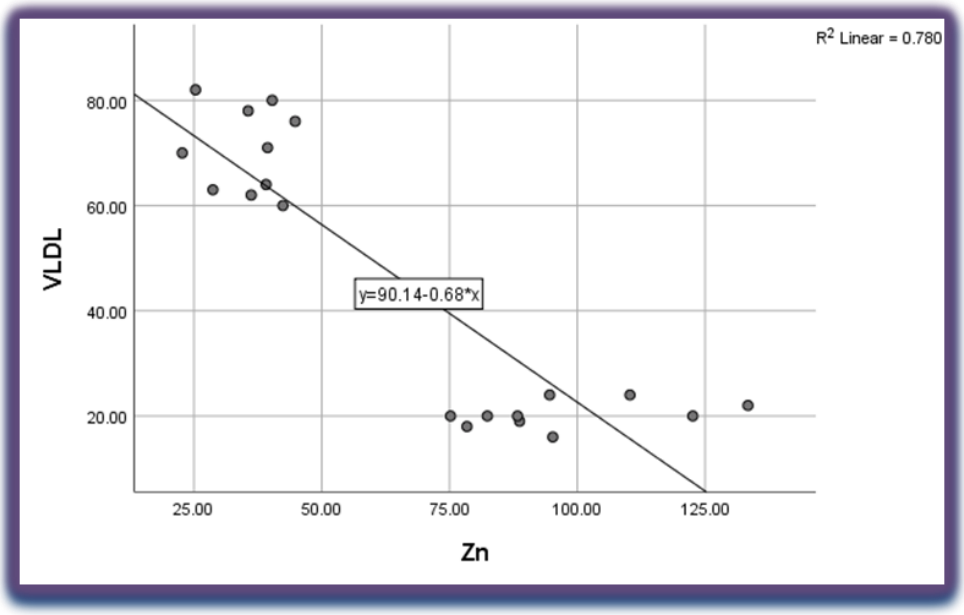
Table 2 ;shows correlations of ZN with lipid profile

parameters	r value	P value
TC	-0.875**	<0. 01
TG	-0.886**	<0.01
HDL	0.824**	<0.01
VLDL	- 0.883**	<0.01
LDL	- 0.852**	<0. 01

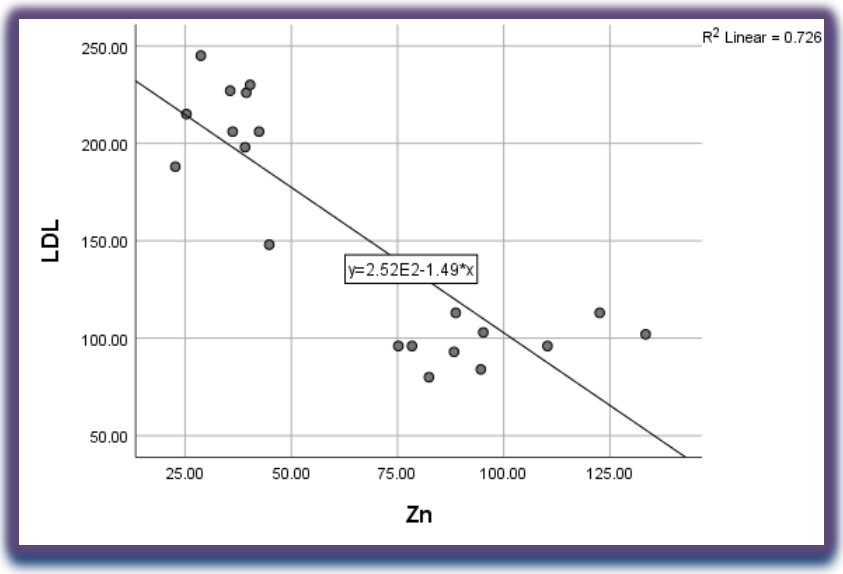
Correlation** is significantat at the <0.01 level, Correlation* is significantat at the 0.05 level, TC=Total Cholesterol, TG=Triglyceride, HDL-C=High density lipoprotein cholesterol, LDL-C=Low density lipoprotein cholesterol, VLDL-C=very low density lipoprotein cholesterol, and Zn,zinc



Figure(7) Correlation between zinc levels and cholesterol levels in CVD patients



Figure(8) Correlation between zinc levels and TG levels in CVD patients



Figure(9) Correlation between zinc levels and LDL levels in CVD patients

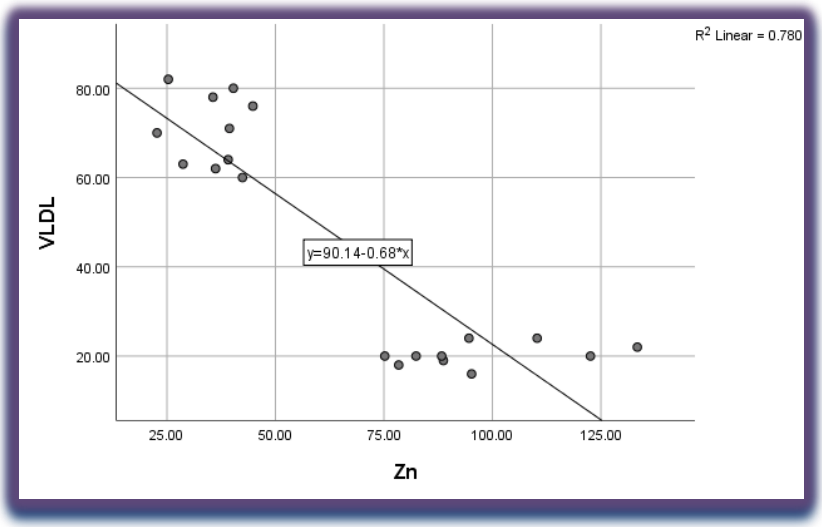
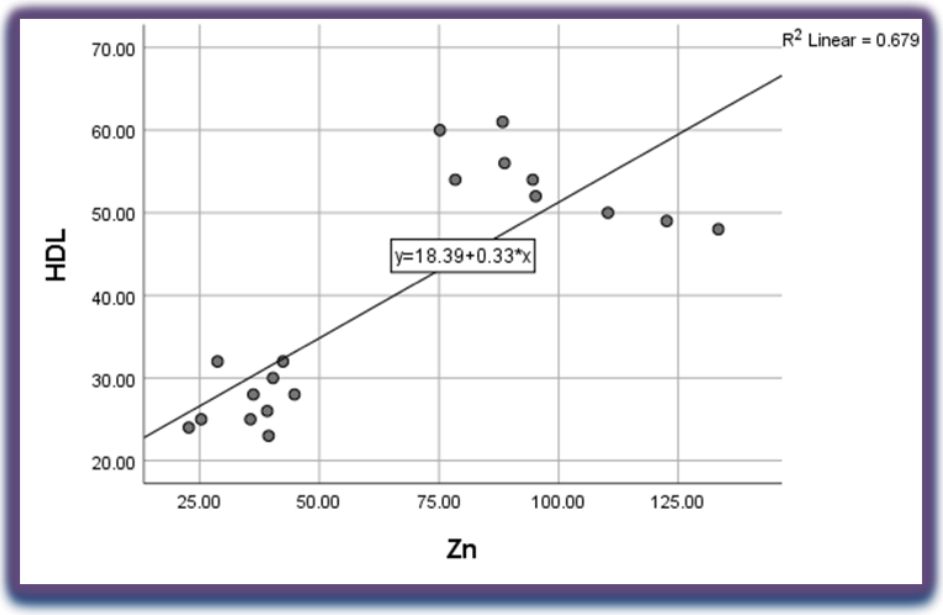


Figure (8) Correlation between zinc levels and TG levels in CVD patients



Figure(8) Correlation between zinc levels and HDL levels in CVD patients

Disscusion

Zinc is an essential trace element that plays an important physiological role in numerous cellular processes. Zinc deficiency can result in diverse symptoms, such as impairment of the immune response, skin disorders, and impairments in cardiovascular functions. Recent reports have demonstrated that zinc acts as a signaling molecule, and its signaling pathways, referred to as zinc signals, are related to the molecular mechanisms of cardiovascular functions. Therefore, comprehensive understanding of the significance of zinc-mediated signaling pathways is vital as a function of zinc as a nutritional component and of its molecular mechanisms and targets. Several basic and clinical studies have reported the relationship between zinc level and the onset and pathology of cardiovascular diseases, which has attracted much attention in recent years.

In the present study, we found strong significant negative correlation of serum zn levels with total cholesterol, triglyceride, LDL and VLDL in AMI patients. In the other hand, we found significant positive correlation of serum zn levels with HDL-cholesterol. The findings of our study support the fact that ,Zinc is a vital element in maintaining the normal structure and physiology of cells. The fact that it has an important role in states of cardiovascular diseases has been studied and described by several research groups. It appears to have protective effects in coronary artery disease and cardiomyopathy. Intracellular zinc plays a critical role in the redox signaling pathway, whereby certain triggers such as ischemia and infarction lead to release of zinc from proteins and cause myocardial damage. In such states, replenishing with zinc has been shown to improve cardiac function and prevent further damage. [(5)]

Myocardial tissue needs zinc in small quantities to maintain the extracellular structure formed by matrix metalloproteinasesand to serve as a cofactor in free radical oxidation reactions catalyzed by superoxide dismutase. [(6)]

Conclusions

In our study, we found alterations in the lipid profile and zinc in CVD cases, which play significant role in incidence of CVD. Hence, all the people over 40 years should undergo regular checkup including lipid profile evaluation and zinc to decrease the incidence, morbidity and mortality from the disease, because low plasma(serum) zinc levels increase the risk of developing cardiovascular diseases, so supplementation of zinc may decrease this risk, which indicate the importance of appropriate zinc management in disease prevention.

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