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RESEARCH ARTICLE

DETERMINATION OF COPPER, IRON, AND NICKEL IN SERUM OF MYOCARDIAL INFARCTION PATIENTS BY FLAME ATOMIC ABSORPTION SPECTROMETRY.

Alaa Abbas Ibadi¹, Dawood S. Ali² and Hassan Thamir Abdulsahib².

1. Department of chemistry, college of science, Missan University, Missan, Iraq.
2. Department of chemistry, college of science, Basrah University, Basrah, Iraq.

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Abstract

Trace elements may play a vital role in the development of MI. The differences between the concentration of Copper, Iron, and Nickel that has occurred during the Myocardial Infarction by the determination of the concentration of these elements in both patients and healthy control subjects. The study included 60 number of cases of MI and equal number of matched healthy control. The results shows an increasing in the levels of all tested elements Cu, Fe, & Ni with ($P < 0.05$) in MI patients which are found to be statistically significant. In addition, we found slight differences in the elements concentration when compared according to the gender.

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Introduction:-

Myocardial infarction (MI) defined as death or necrosis of myocardial cells. It is a diagnosis at the end of the spectrum of myocardial ischemia or acute coronary syndromes (1). Over the past 50 years, studies on the effect of trace elements in health and disease have led to a good interpretation of their mechanism and essentiality to the life (2).

Trace elements are those found in such small quantities in the living tissues, they are plays a vital role in the structure of proteins, enzymes, and complex carbohydrates to contribute in the biological reactions. Copper and Iron considered a part of the trace elements that appear in the body as essential trace elements, these elements are involved in a number of metabolic activities, including neuroconduction, transport, excretory processes and serving as cofactors for enzymes. (3)(4)

Serum levels of trace elements maybe altered in patients with CAD. The higher or lower levels may be both a cause and effect of atherosclerosis or the result of another unknown parameter (5).

Copper is an important trace element and is associated with a number of metalloproteins (6). Because metals can cause oxidative modification of low density lipoprotein cholesterol and the formation of free radicals, the effect of several prooxidant metals, including copper, on cardiovascular disease has come under investigation, studies support the possibility that increased copper concentration may increase the risk for cardiovascular disease (7). Also Iron is a metal that may be important role in the catalytic reaction and reduction reactions, Epidemiological studies have shown that there are relationship between serum iron levels and Ischemic Heart Disease (IHD) (8). In addition to acceleration of the lipid peroxidation, iron through increase of hemoglobin and hematocrit levels, by increasing the viscosity of blood and thus increasing the probability of thrombogenic effects may increase the risk of MI (9).

Corresponding Author:- Alaa Abbas Ibadi.

Address:- Department of chemistry, college of science, Missan University, Missan, Iraq.

Nickel, in addition to Copper and Iron, also can act an important role in human health, it is an essential nutrient for human. The toxic effects of metals, such as Nickel, and its relation to cardiac damage should be establish urgently, the free radical-mediated cellular damage and reactive oxygen species (ROS) contribute to the Nickel mechanism of toxicity, recent investigation that the free radicals may be important contributors to cardiac dysfunction (10).

Nickel is known to bind to specific proteins and/or amino acids in the blood serum and the placenta. These ligands are instrumental in the transport and distribution of nickel in the body. Nickel is not destroyed in the body, but its chemical form may be altered. The metabolism of nickel is most appropriately viewed in light of its binding to form ligands and its transport throughout the body. Various disease states (myocardial infarction and acute stroke) and injuries (burn injury) are associated with altered transport and serum concentrations of Nickel (11).

Determination of the Trace elements in blood serum becomes very important to investigate their vital role in human metabolism, as well as to achieve information concerning with the health status of individuals (12).

This study included the investigation the differences between the concentration of Copper, Iron, and Nickel that has occurred during the Myocardial Infarction by the determination of the concentration of these elements in both patients and healthy control subjects.

Material and Methods:-

All chemicals used in this study with highly purified material and no farther purification done.

Sample collection and analysis:-

Venous blood samples of (5 ml) drawn from patients of MI, The blood was allowed to clot at 37 °C for 10-15 minutes, and then centrifuged in (402 X g) for approximately 10-15 minutes then the sera were obtained and stored at 0 °C until analysis.

Determination of serum levels of trace elements:-

Serum concentrations of Cu, Fe, and Ni were determined by using Flame Atomic Absorption Spectrometry (AAS), determination method is based on the principle that atoms of the element when aspirated into AAS vaporized and absorbed light of the same wavelength as the emitted by the element when in the excited state.

Statistical Analysis:-

Data were analysed by using SPSS software (Version 19) and the values were expressed as mean and standard deviation. Pearson's correlation analysis was carried out. All comparison were 2-tailed, and p value of <0.05 or <0.01 were considered significant

Results:-

From 60 patients with Myocardial Infarction, 35 (58.33%) subject were males and 25 (41.66%) were females. The average age of patient's males and females were 54.75±14.9 and 62.08±12.0 years, respectively. From 60 healthy group, 37 (61.66%) subject were males and 23 (38.33%) were females. The average age of healthy males and females were 40.59 ± 14.3 and 40.59 ± 13.3 years, respectively.

A comparison of levels of Copper, Iron, and Nickel in study cases shown below in the Table 1, also the Table 2 shows a comparison of levels of Copper, Iron, and Nickel in study cases according to the gender. Also Figure 1 shows the comparison of levels of Copper, Iron, and Nickel in study cases, Figures 2,3, and 4 shows the Concentration of Copper, Iron, and Nickel related to life spans, finally, Figure 5 shows Copper, Iron and Nickel concentration related to the gender.

Table 1:- comparison of levels of Copper, Iron, and Nickel in study cases

Parameter (µg/ml)	Controls	Patients	P-Value
Copper	0.929±0.544	1.375±0.604	P<0.05
Iron	2.862±1.38	4.297±0.202	P<0.05
Nickel	2.539±1.616	6.381±1.44	P<0.05

Values reported as mean± Standard deviation (SD); n= no. of cases P<0.05 highly significant

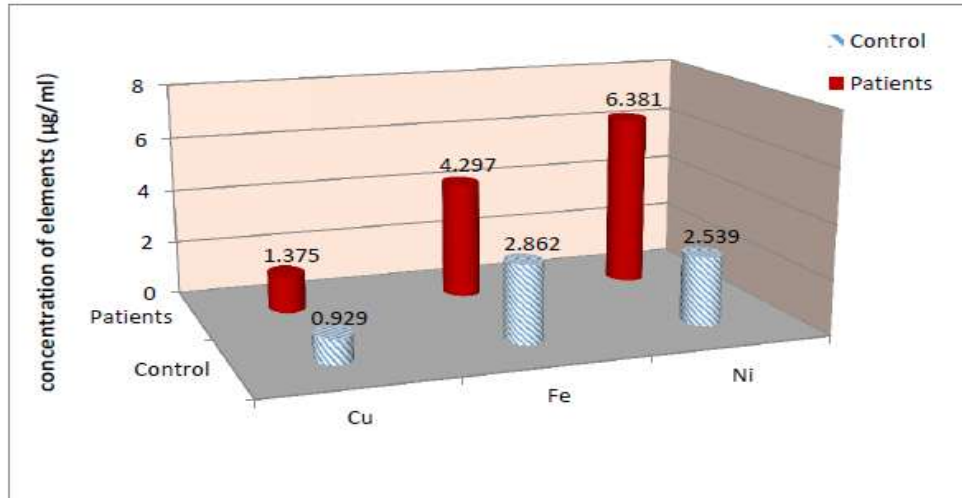


Figure 1:- comparison of levels of Copper, Iron, and Nickel in study cases

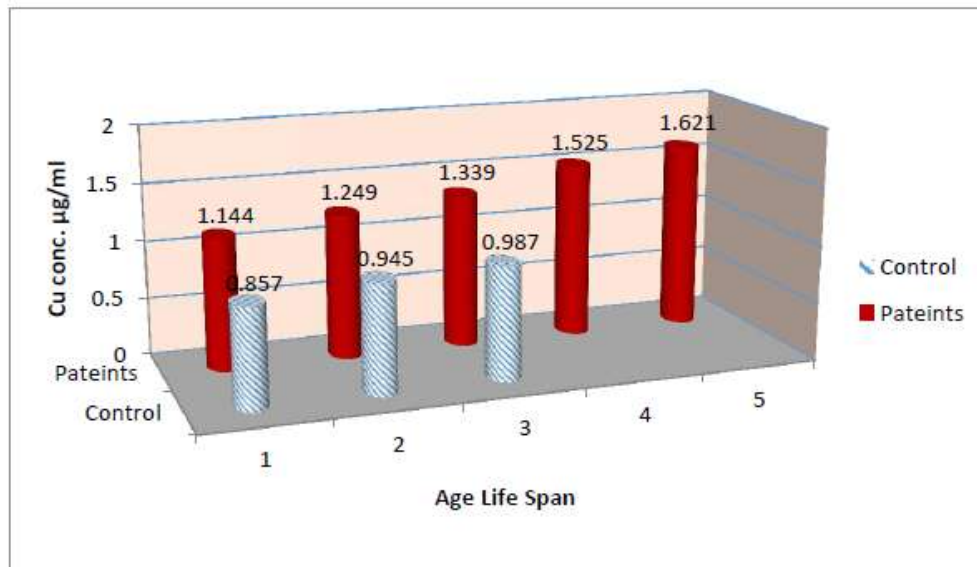


Figure 2:- Concentration of Copper related to life spans

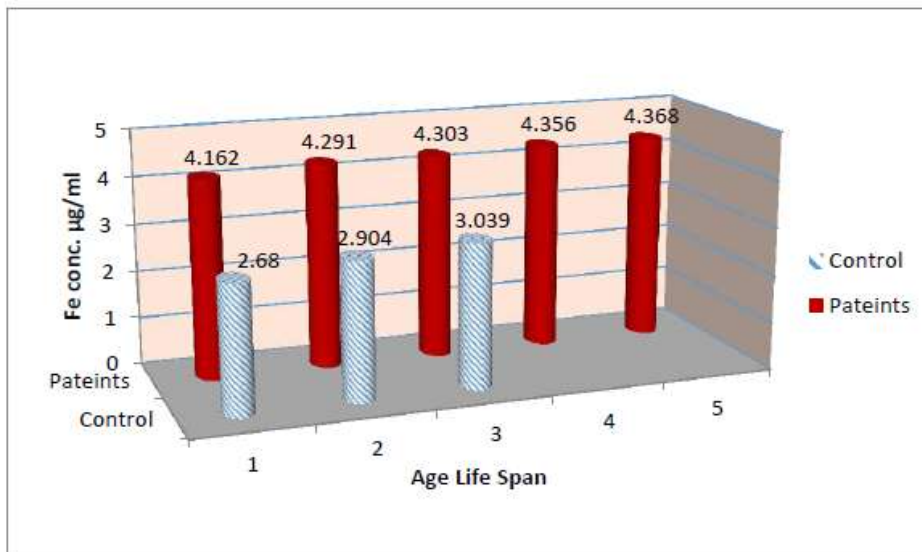


Figure 3:- Concentration of Iron related to life spans

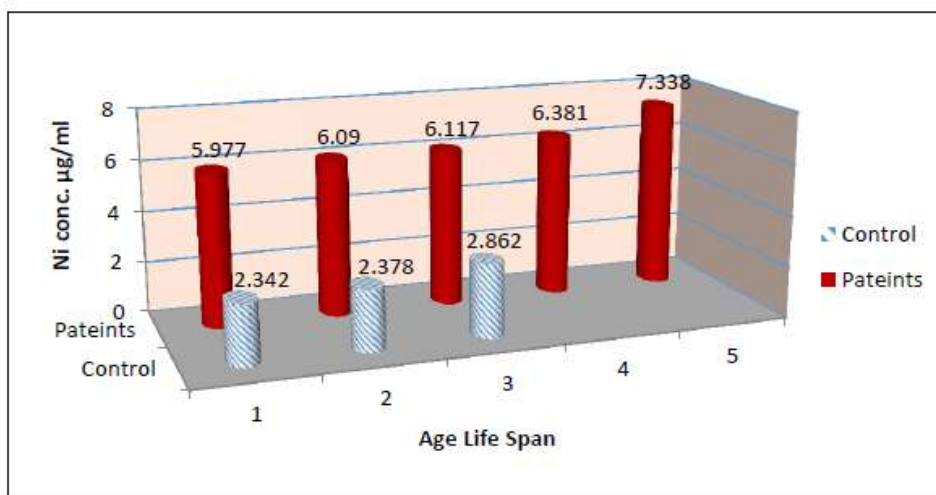


Figure 4:- Concentration of Nickel related to life spans

Table 2:- comparison of levels of Copper, Iron, and Nickel in study cases according to the gender

Case / Gender	Control			Patients		
	Cu	Fe	Ni	Cu	Fe	Ni
Male	0.865±0.539	3.161±1.40	2.452±1.67	1.360±0.65	4.333±0.19	6.261±1.48
Female	1.043±0.550	2.695±1.36	2.695±1.53	1.394±0.53	4.270±0.20	6.540±1.39
P-value	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Values reported as mean± Standard deviation (SD); n= no. of cases. P<0.05 highly significant

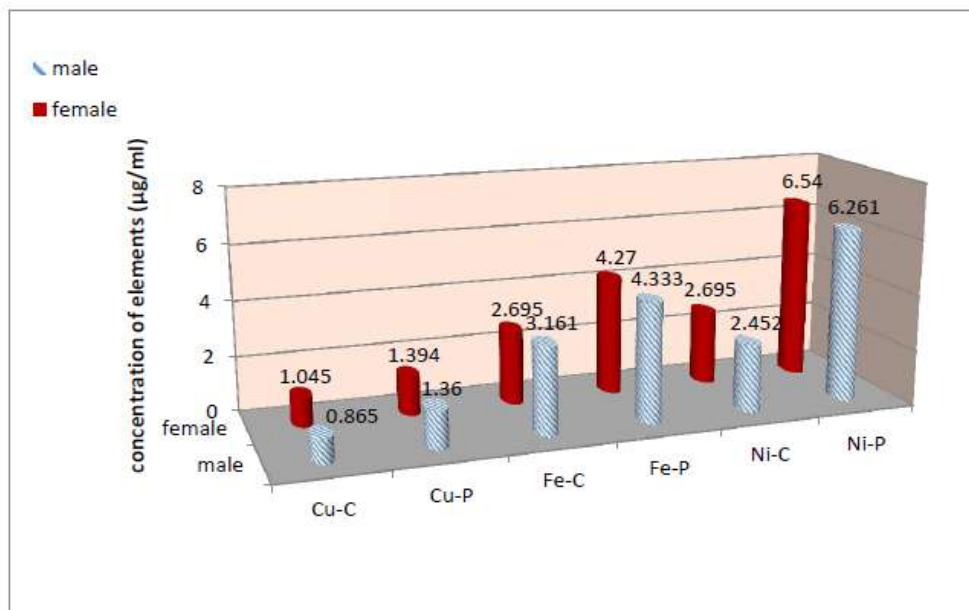


Figure 5:- Copper, Iron and Nickel concentration related to the gender

Discussion:-

Our results shows a highly significant increase in the concentration of Copper, Nickel and Iron in myocardial infarction patients' compared with healthy controls.

A highly significant increase observed in the levels of Copper concentrations in the sera of patients compared with healthy control subjects. The increasing in the Copper levels may be due to rise in the Copper binding capacity of ceruloplasmin which is a Copper containing enzyme and acute phase reactant, also may be due to the injury and subsequent necrosis of myocardial cells. Increased serum Copper levels are a part of a specific defense mechanism to provide more Copper at the site of infarction to reduce its size and the extent damage (13) (14).

It is well known that copper plays a vital role in oxidative stress in which copper in its free form is a potent cytotoxic element because of its redox chemistry. It readily participates in Fenton and Heiber Weiss reactions to generate reactive oxygen species, a high level of copper enhances the toxic effect of metal dependent free radicals (15).

In addition, it acts as pro-oxidants and may participate in metal catalysed formation of free radical, it is very potent to generate reactive oxygen species (ROS) after a reaction with oxygen. Free Cu(II) ion can interact with hydrogen peroxide (H_2O_2) leading to the formation of the deleterious hydroxyl radical via the Fenton reaction. Bound to proteins, copper is generally less susceptible to participate in the Fenton reaction (16)

The second element in our study is Iron, which plays an important role in heart disease, the relationship of serum Iron status and cardiovascular disease was demonstrated by the epidemiological studies.

Iron concentrations was significantly higher in the patients whom suffering from myocardial infarction compared to healthy control that's agree with the studies of (Rostami M. *et al*; 2013), (Vijaya L. *et al*;2016), and (Pradhan D.P. *et al*;2016).

Iron is a transition metal, which can important role in the catalytic reaction of poisons, reduction reactions and is involved in many biological processes and harmful diseases to humans. Excess iron of body is a risk factor for incidence of CVD (17)

Also, Iron induced oxidative stress in the form of increased generation of reactive oxygen species (ROS), in a series of Fenton like reactions, it's through increase of lipid peroxidation and increased of haemoglobin and haematocrit levels and therefore increasing the viscosity of blood and increasing the likelihood of thrombogenic effects, can

increase the risk of myocardial infarction. It is the free ionic form of iron that is harmful due to its pro-oxidative properties, which generates reactive free radicals. Superoxide produced during oxidative stress can mobilize free catalytic iron from ferritin and facilitate the formation of Hydroxyl ion. Reactive oxygen species, superoxide cause lipid peroxidation and endothelial dysfunction in vessels (18)(9).

In addition to Copper and Iron effect in myocardial infarction case, also Nickel plays a vital role in this case, the rapid increase in the concentration of nickel in the blood may perhaps be used as a diagnostic indicator of recent myocardial infarction, that's according to study of (Hegde *et al.*, 1961)

Nickel may bind to DNA-repair enzymes and generate oxygen-free radicals to cause protein degradation *in situ*. This irreversible damage to the proteins involved in DNA repair, replication, recombination, and transcription could be important for the toxic effects of nickel (19)

Ni (II) can also generate free radicals from lipid hydroperoxides in the presence of several oligopeptides. Hence, free radical generation from the reaction of Ni (II)-thiol complexes and molecular oxygen, and/or lipid hydroperoxides, could play an important role in the mechanism(s) of Ni (II) toxicity. The results of a series of studies using cultured human peripheral blood lymphocytes also suggest that nickel induces oxidative stress in humans (20-22)

Our results shows a high significant increasing in Nickel concentrations in patients compared with controls, which agree with a study of (Charles N. L. *et al* 1985) which also observed an increase in Nickel concentrations in the sera of patients with myocardial infarction, their study hypothesises three explanations for this increasing:-

First, the myocardium or coronary arteries of patients may contain abnormally increased concentrations of nickel and when stunned by ischemia, may release nickel into the circulation.

Second, hypernickemia may be a secondary phenomenon, mediated by release of nickel from another organ, in response to stress, the lung would be a likely source of nickel, because human lung tissue accumulates nickel with advancing age

Third, nickel may be bound to clotting factors or complement components, and released into serum during the coagulation cascade that occurs during coronary thrombosis or during the activation of the alternative complement pathway that follows myocardial inflammation. (23)

Also we compared our results as a life spans, we found an increase in serum Copper concentration during age that's agree with study of Yunico *et al.*,1974 (24).So we observed an increase in Iron and Nickel concentration during age life spans, that's corresponds to studies shown that age accompanied by increasing Iron levels (25). So Nickel accumulates with advance aging, that's agree with our results.(23)

When we compared our results according the gender, we found the females have a higher Copper and Nickel concentrations compared with males in both cases patients and healthy control, and the opposite will found in Iron concentration, which tend to be higher in males than females in both patients and healthy control subjects.

The increase in Copper concentration in females can be attributed to estrogen, a hormone which alters the distribution of copper in the liver and increases its plasmatic levels (26).

It is generally considered that women, especially before menopause, have lower levels of iron stores than age-matched men. Although periodic iron loss (menstruation) or childbirth has long been, consider the protective mechanism against Iron overload in women (27).

A slight increase in Iron concentrations observed in male patients compared with females, this can attributed to the fact that nearby 80% of the females in our study are over the age 50 years, in menopause, it is expected that there will be convergence of iron concentration in both sexes and therefore Copper as well.

The normal ranges of nickel concentrations in body fluids or tissues (serum, blood, lung, and kidney) are not significantly influenced by age, sex, or pregnancy (28).

In our study, a very slight difference observed in Nickel concentrations in males and females in both cases. Females of all ages, children, youths, and adults are more exposed to Nickel than men are, that maybe due to direct and prolonged skin contact with items that release nickel ions. The causes vary on fashion and other factors influencing exposure. Cheap jewellery, precious-metal jewellery, watches, spectacle frames, buckles (29).

Conclusion:-

It is concluded that the trace elements such Copper, Iron, and Nickel considered being a major risk factor for myocardial infarction. The results of the present study confirm the hypothesis that the increasing in the levels of both Copper and Iron catalyse the Fenton reaction in generation of free radicals, also Nickel have the ability to generate free radicals from lipid hydroperoxides in the presence of several oligopeptides, which in turn increase free radical mediated oxidative stress.

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