Determination Of Copper Trace Element In Rheumatoid Arthritis Patients In Some Areas Of Saudi Arabia Kingdom

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Abstract: Due to the importance of trace elements, different analytical methods using many reagents, have been used on this study. Copper trace element (Cu) have been determined in blood, serum, plasma, plasma clot and serum clot samples. The determination was done by using Inductively coupled plasma – Atomic Emission Spectrometer (ICP – AES) and voltammetry techniques. Effect of age, work, monthly income, period of disease, degree of disease, The Social State and Residential Area on concentration of Cu trace element in both,healthy people and patients with rheumatoid arthritis in Jizan and Jeddah regions, were studied. Statistical analysis was made to analyze the results which were gotten for comparisons between the samples concentrations and correlation coefficient in different studied samples.

1.Introduction

The importance of trace elements in chronic inflammatory diseases is related to their cofactor role in immune system functions and in different metabolic processes in articular tissues. Serum levels of Cu, Zn and Zn/Cu ratio in Rheumatoid Arthritis (RA) patients was compare with healthy volunteers in Sari Rheumatology clinic, 2007. Zn and Cu plasma concentrations were assayed using atomic absorption spectrophotometery in 40 selected RA patients sera based on sex and age compared with healthy volunteers. Statistical analysis was performed by SPSS 10 software using independent sample t-test. That study showed that spreading of RA in Iranian Society is related to age, sex, career and nutrition of the patients (Edmonds and Hughes, 1985). One hundred and five specimens of blood serum from patients with RA, and an equal number of specimens from normal volunteers were analyzed by emission spectrometry for 14 different trace metals. In blood serum from patients with RA, concentrations of copper were high (Al-Dalaan *et al.,* 1998). An *in vitro* and *in vivo* study of some copper chelating anti-inflammatory agents for alleviation of inflammation associated with (RA) has been conducted (Xuqi, *et al.,* 2002). The study synovial fluid and plasma concentrations of copper (Cu) in patients with RA and compared them with sex- and age-matched healthy subjects. Plasma Cu concentrations were determined by atomic absorption spectrophotometry. Cu concentrations were significantly higher in patients with RA than those of healthy subjects ($p <$

0.05). That results showed that synovial fluid and plasma trace element concentrations, change in inflammatory RA. These alterations in trace element concentrations in inflammatory RA might be a result of the changes of the immunoregulatory cytokines (Edmonds and Hughes,1985). Copper and zinc were studied in (RA). Metal levels were measured by atomic absorption spectroscopy in the plasma, whole blood cells and 24 hrs urine of 120 RA patients. In the plasma of RA patients copper and ceruloplasmin levels were found to be significantly increased whereas zinc levels were significantly decreased. No major variations were in the blood cell and 24 hrs urine copper and zinc levels (Kruusma *et al.,* 2004). The food diaries were analyzed together with clinical and laboratory data by means of stepwise multiple linear regression to clarify the effects of both diet and the inflammatory disorder on the plasma concentrations of zinc and copper. There was a strong correlation between plasma copper-copper intake ratio and zinc intake both in univariate (r = -0.638, *P* less than 0.001) and multivariate analysis. This suggests that zinc depresses copper absorption with intakes in normal, physiological ranges (Zaksas *et al*., 2010). (RA) is characterized by low serum Zn and high serum Cu **(**Qing-hua *et al.,* 2012**).**

2.Material and Method:

Samples Collection: 47 blood samples were collected (7 samples from healthy, 20 samples from R.A. patients in Jeddah area and 20 samples from R.A. patients in Gizan region.

Preparation of the samples: 1 ml sample is treated with 5 ml concentrated HNO3 acid in the digestion vessel. Steaming the solution is made on hotplate until the volume of solution is reduced to 1 ml. Then, 5ml of double distilled water is added. This addition of water is repeat many times to get rid of the extra HNO$_3$ acid.

The devices used in the study:

Trace elements were measured by inductively coupled plasma – atomic Emission spectrometer (ICP – AES), the storage of information is done by a computer of Dell company joined with the device. While Polarograph instrumental 746 VA trace analyzer with 747 VA stand from Metrohm company. The information storage is done by a computer, from Toshiba company 757 VA joined with the device.

3.Results and discussion:

This research describes combined effects of inflammation and copper intake on Cu-Zn and extracellular (EC) superoxide dismutase (SOD) activities. Studies in humans found a group of RA patients to possess relatively low erythrocyte SOD and relatively high ceruloplasmin activities (Roitt, 1980).

Table(1) shows that there is a difference in the concentration of copper element of healthy samples and,both patient samples in Jeddah and Jizan regions.

Table: (1): The Concentration of Cu Trace Element in Healthy, R.A. Jizan and R.A. Jeddah.

Elements	Conc. (Mean ± S.D)/ (ppm)		
	Healthy	**R.A. Jizan**	**R.A. Jeddah**
Blood	0.0028 ± 0.0032	0.0116 ± 0.0060	0.026 ± 0.0013
Plasma	0.0078 ± 0.0055	0.0159 ± 0.0067	0.0024 ± 0.0022
Serum	0.0065 ± 0.0025	0.0192 ± 0.0095	0.0026 ± 0.0021
Clot of plasma	0.0116 ± 0.0013	0.0125 ± 0.0171	0.0087 ± 0.0147
Clot of Serum	0.0047 ± 0.0029	0.0133 ± 0.0097	0.0022± 0.0017

It has been found that the concentration of copper element in blood samples from patients with the Jeddah (0.0260 ppm) was higher than the patients' blood samples of Jizan (ppm 0.0116). Also, in the case of patients and healthy the focus of a copper was higher in patients than in healthy (0.0028 ppm) as well as in serum and plasma samples and plasma clot and serum clot.

Effect of age on concentration of Cu trace element in rheumatoid patients:

In Table (2) the effect of age on the concentration of copper **in Jizan** demonstrated an increase on the concentration of copper element with the increasing age of patients, the lowest concentration of copper element (0.0072 ppm) was for patients age ranges between (20-40/year), while the highest concentration of copper was (0.0216ppm) in patients age ranges between (61-70/years).This study shows the strong positive correlation between increased concentration of copper with increasing age of patients (r = 0.918).

The same results were found for **Jeddah**. Table (2) shows that concentration of copper element **in Jeddah** increases with age of patients where the concentration of copper (0.0027ppm) in patients age between (20-40 years) while high concentration of Cu was (0.0251 ppm) in patients age between (61-70 years). This study approves the strong positive correlation between that increases concentration of copper element with increases age of patients (r = 0.403).

Effect of age on concentration of Cu trace element in healthy people and patients with rheumatoid arthritis in Jizan and Jeddah regions:

-for age from (20-40/years):

Table (2): shows that the concentration of copper element increases in patients (0.0024ppm) compartment of healthy. It is high in Jizan patients (0.0072ppm) than in Jeddah patients (0.0027ppm).

-for age from (41-50/years):

That concentration of copper element is high in Jizan patients (0.015 ppm) than of Jeddah patients (0.0029 ppm).

- for age from (51-60/years):

The concentration of copper element is high in Jizan patients (0.0178 ppm) than that in Jeddah patients (0.011 ppm).

- for age from (61-70/years):

That concentration of copper element is high in Jeddah patients (0.0251 ppm).

Table (2): Effect of Patients Age on the Concentration of Cu Trace Element in Healthy and R.A. Patients.

Patients Age Group (Years)	Conc. (Mean ± S.D)/ (ppm)		
	Healthy	**Jizan**	**Jeddah**
(20 -40/year)		0.0072 ± 0.0016	0.0027 ± 0.0065
(41-50/year)	0.0024 ± 0	0.015 ± 0.008	0.0029 ± 0
(51- 60/year)		0.0178 ± 0	0.0110 ± 0.0032
(61-70/year)		0.0216 ± 0.0041	0.0251 ± 0.0005

Effect of work on the concentration of Cu trace element in patients with rheumatoid arthritis in Jizan and Jeddah regions:

The study of the effect of work on the concentration of Cu trace element for rheumatoid arthritis patients in the two regions, table (3) shows that the concentration of copper element is high in not working patients (0.0184ppm) than that of working patients (0.0140ppm).

Table (3): Effect of Working Patients on the Concentrations of Cu Trace Element in R.A Patients.

Element	Conc. (Mean ± S.D)/ (ppm)	
	Work	Not Working
Cu	0.0140 ± 0.0063	0.0184 ± 0.0059

Table (4) shows effect of work on the concentration of trace element in not working patients with rheumatoid arthritis is the concentration of copper element is high in work lees Jizan patients (0.0140 ppm) than work patients (0.0037ppm) in Jeddah region.

Table (4): Effect of NonWork for Patients on the Concentrations of Trace Element in R.A. Patients.

Element	Conc. (Mean ± S.D)/ (ppm)	
	Not Working	
	Jizan	Jeddah
Cu	0.0140 ± 0.0063	0.0037 ± 0.0012

Effect of monthly income on the concentration of Cu trace element in patients with rheumatoid arthritis in the Jeddah area:

The obtained results from the study in table (5) shows low concentration of copper trace elements (0.0012 ppm) in patients with monthly income (5000 S.R.≤) and (0.0041ppm) with monthly income (2000-5000 S.R.) then concentration of copper trace element increases (0.0045ppm) in patients with monthly income (1000-2000 S.R.) while high concentrations of copper trace element was (0.005ppm) in patients with monthly income (1000 S.R. ≥). The studied approved negative medium correlation between increase concentration of copper trace element and low monthly income in patients (r = - 0.564).

Effect of monthly income on the concentration of copper trace element in patients with rheumatoid arthritis in Jizan area:

The study in table (5) shows low concentration of copper trace element (0.0024ppm) in patients with monthly income (5000 S.R.≤) and (0.0029ppm) with monthly income (2000-5000 S.R.) then concentration of copper trace element was increased (0.0167ppm) in patients with monthly income (1000-2000 S.R.) while high concentration of copper trace element was (0.018ppm) in patients with monthly income (1000 S.R. ≥). The studied approved negative correlation

medium between increase concentration of copper trace element and low monthly income in patients (r = - 0.757).

Effect of monthly income on the concentration of copper trace element in patients with rheumatoid arthritis in Jizan and Jeddah area:
-for monthly income (1000 S.R. ≥):

In table (5) the study proved increase concentration of copper in patients Jizan area (0.0180ppm) compared with patients Jeddah area (0.0050ppm) and who have the same patients standard of living and monthly income.

-for monthly income from (1000-2000 S.R.):

Increase concentration of copper in patients Jizan area (0.0014ppm) compared with patients Jeddah area (0.001ppm).

-for monthly income from (2000-5000 S.R.):

Increase concentration of copper in patients Jeddah area (0.0014ppm) compared with patients Jizan area (0.001ppm).

-for monthly income (5000 S.R. ≤):

Increase concentration of copper in rheumatoid patients Jeddah area (0.0002ppm) compared with healthy.

Table(5): Effect of Family Income on the Concentrations of Cu Trace Element in R.A. patients.

Family Income (S.R.)	Conc. (Mean ± S.D)/ (ppm)	
	R.A. Jeddah	R.A. Jizan
1000≤	0.0050 ± 0.0087	0.0180 ± 0.0012
1000-2000	0.0045 ± 0.0055	0.0167 ± 0.0011
2000-5000	0.0041 ± 0.0071	0.0029 ± 0.0012
5000 ≥	0.0012± 0.0036	0.0024 ± 0.0015

Effect of period of disease on the concentration of copper trace element under study for patients with rheumatoid arthritis in Jeddah and Jizan regions:

In table (6) the study proves that a copper concentration increases with increasing duration of disease in Rheumatoid patients where positive medium 1 (r = 0.675).

-for period of disease from (1-5/years):

Table (6) shows increasing the concentration of a copper with increase period of disease in Rheumatoid patients which (1-5/year). In region of Jizan study showed greater concentration of copper element in Rheumatoid patients (0.0249 ppm) than Rheumatoid patients of Jeddah (0.0125 ppm).

-for period of disease from (6-9/years):

This study showed high concentration of copper element in Rheumatoid patients in Jeddah (0.0139 ppm) than patients with Jizan (0.0032 ppm).

-for period of disease from (10-25/years):

The high concentration of copper element in Rheumatoid patients in Jeddah (0.0162 ppm) compared to Jizan (0.0043 ppm).

Table (6): Effect of Period of Disease on the Concentration of Cu Trace Element in R.A. of Jizan and Jeddah.

Period of Disease	Conc. (Mean ± S.D)/ (ppm)	
	R.A. of Jizan.	R.A. of Jeddah
1-5	0.0249 ± 0.0121	0.0125 ± 0.0075
6-9	0.0032 ± 0.0111	0.0139 ± 0.0070
10-25	0.0043 ± 0.0075	0.0162 ± 0.0051

Effect Degree of disease on concentration of copper element in Rheumatoid patients in Jeddah:

In table (7) the study shows that the positive medium correlation between increased of concentration of copper element and increased disease degree in rheumatoid patients in Jeddah (r = 0.494). Also the study reveals that concentration of copper element (0.0113 ppm) in patients with weak symptoms while the concentration of copper element in patients with middle symptoms of the disease (0.0124 ppm) and attains highest concentration of copper in the patients with strong symptoms of the disease (0.0190 ppm).

Effect Degree of disease on Concentration of copper element in Rheumatoid patients in Jizan:

The study shows higher correlation and positive between increased concentration of copper element and increased disease degree in Rheumatoid patients in Jizan (r = 0.833). The study also reveals that the concentration of copper element (0.0024 ppm) in patients with weak symptoms while the copper concentration in patients with middle symptoms of the disease (0.0032 ppm) and attains the highest concentration of copper element in the patients with strong symptoms of the disease (0.0043 ppm).

Table (7): Effect Degree Of Disease on the Concentration of Cu Element in R.A. Patients of Jeddah and Jizan.

Patients	Conc. (Mean ± S.D)/ (ppm)		
	Degree of Disease		
	Weak	Middle	Strong
R.A. Jeddah	0.0113 ± 0.0023	0.0124 ± 0.0068	0.0190 ± 0.0049
R.A. Jizan	0.0024 ± 0.0002	0.0032 ± 0.00002	0.0043 ± 0.0013

Effect of The Social State on the Concentration of Copper Trace Element in rheumatoid of Jeddah and Jizan.

On comparing patients unmarried with married patients in Jeddah and Jizan regions, table (8) shows that high concentration of copper element in patients unmarried (0.0039 ppm) compared with patients who are married (0.0032 ppm).

Table (8): Effect of The Social State on the Concentration of Cu Trace Element in R.A. of Jeddah and Jizan.

Element	Conc. (Mean ± S.D)/ (ppm)	
	The Social State	
	Married	Unmarried
Cu	0.0032 ± 0.0	0.0039 ± 0.0014

Also, Table (9) shows that the concentration of Cu Trace element was high in patients who are married in Jizan (0.0145ppm) over Jeddah (0.0072ppm) compared to healthy controls (0.0039ppm).

Table (9): Effect of The Social State (Married) on the Concentration of Cu Trace Element in Healthy and R.A. of Jeddah and Jizan.

Element	Conc. (Mean ± S.D)/ (ppm)		
	The Social State(Married)		
	Healthy	Jizan	Jeddah
Cu	0.0039 ± 0.0014	0.0145 ± 0.0063	0.0072 ± 0.001

Effect of Residential Area on the Concentration of Copper Trace Element in R.A. of Jizan:

Table (10) indicates the comparison between concentration of copper trace element of rheumatoid patients living in villages and city in Jizan.
High concentration of copper trace elements of rheumatoid patients in Jizan (0.0045 ppm) who living in villages compared to patients who live in city (0.0027 ppm).

Effect of Residential Area on the Concentration of Copper Trace Element in R.A. of Jeddah:

On Table (10) the comparison between concentration of copper trace element of rheumatoid patients living in the villages and the city of Jeddah.
High concentration of Copper Trace Element of rheumatoid patients in Jeddah (0.0149 ppm) who living in villages compared to patients who live in city (0.0136 ppm).

Table (10): Effect of Residential Area on the Concentration of Copper Trace Element in R.A. of Jeddah and of Jizan:

Cu Element	Conc. (Mean ± S.D)/ (ppm)	
	Residential Area	
	City	Village
R.A. Jeddah	0.0136 ± 0.0061	0.0149 ± 0.0066
R.A. Jizan	0.0027 ± 0.0003	0.0045 ± 0.0011

Effect of Residential Area (City) on the Concentration of Copper Trace Element in

Rheumatoid patients of healthy and patients of Jeddah and Jizan region.

Table (11) shows high concentration of copper trace element in rheumatoid patients of Jeddah area (0.0136 ppm) compared to Jizan area patients (0.0027 ppm) and healthy (0.00045ppm).

Table (11): Effect of Residential Area (City) on the Concentration of Cu Trace Element in Healthy and R.A. of Jeddah and Jizan.

Element	Conc. (Mean ± S.D)/ (ppm)		
	Residential Area (City)		
	Healthy	Jizan	Jeddah
Cu	0.00045 ± 0.0011	0.0027 ± 0.0003	0.0136 ± 0.0061

Effect of Residential Area (Village) on the Concentration of Copper Trace Element in Rheumatoid patients of Jeddah and Jizan who living in village.

The study reveals as in table (12): High concentration of copper trace element in rheumatoid patients of Jeddah villages (0.0149 ppm) compared to Jizan villages patients (0.0045 ppm).

Table (12): Effect of Residential Area (Village) on the Concentration of Cu Trace Element in R.A. of Jeddah and Jizan.

Element	Conc. (Mean ± S.D)/ (ppm)	
	Residential Area (Village)	
	Jizan	Jeddah
Cu	0.0045 ± 0.0011	0.0149 ± 0.0066

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