Determination of Some Trace Elements in Blood of Rheumatoid Arthritis Patients in Some Areas of Saudi Arabia Kingdom

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Abstract: For importance of the trace elements they have been studied with different analytical methods by using many regents. In this research the trace elements (Cu, Zn, Se, Fe, Cd, Pb) have been determined in blood samples of the healthy and Rheumatoid Arthritis patients in Jeddah and Gizan. This determination was made by using Inductively coupled plasma – Atomic Emission Spectrometer (ICP – AES) and voltammetry techniques, This method is characterized by accuracy, simplicity, speed and high sensitivity. As well, a lower detection limitations have been obtained. Statistical analysis was made to analyze the results which were gotten for comparisons between the elements concentrations and correlation coefficient in different studied samples. Also some study was made to show the accuracy and precision of the used ways.

1.Introduction

Rheumatoid Arthritis (R.A.) is considered one of the diseases that have been associated with autoimmune diseases which attack men, women and children. This disease is the most painful and dangerous because it destroys and deforms joints. This pain can make some people unable to do their daily works(Roitt,1980 ; Edmonds, and Hughes, 1985). There is no statistical evidence of the magnitude of the problem in Saudi nationals. Conducted study to determine the prevalence of RA in the Al Qassim Region of Saudi Arabia. Five thousand eight hundred and ninety-one Saudi adults, aged 16 years and over, were studied in a house-to-house survey in the Al Qassim Region. Of the 5891 adults studied, 13 cases of RA were identified, using the criteria set for the diagnosis of RA by the American College of Rheumatology. Thirty-five percent of our patients showed positive rheumatoid factor. The prevalence of RA in Al Qassim was estimated at 2.2 per thousand people. It was also noted that the prevalence of the disease increased with age, and that it was more common in females. (Al-Dalaan *et al.,* 1998). Most the drugs concentrate on the medical and physical role. The need to find new drugs used in the treatment of chronic inflammatory (R.A.) which affect the population, Metals play important roles in many normal physiological and pathologic processes in all biological systems. They participate in nutrient and gas transport, thermoregulation, pH maintenance (Zaksas *et al.,* 2010). This need made many researchers studied trace elements (Cu, Zn, Fe, Se, Pb, Cd) in different environmental samples (blood, serum, plasma, urine, synovial fluid, tissue etc.). This done by using spectrometric methods, chromatography methods, Radiochemical Analysis etc.. Stripping voltammetry analysis is a powerful and simple tool with which to determine trace target metal species. Because of its inherent sensitivity the technique has been widely used in environmental or clinical samples (Wang *et al.,* 2001); electrochemical methodologies have potential for 'in the field' applications as a result of the portable, inexpensive and easy to operate equipment(Kruusma *et al.,* 2004). The methods of determining major elements and trace elements are inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Xuqi *et al.,*2002), (Rong-fei *et al.,* 2006) and (Zhang and Rui, 2010). ICP-AES method with the advantages of low cost, rapid analysis and wide linear range, low detection limit and simultaneous determination of multi-element has been widely used. (Qing-hua *et al.,* 2012). The aims of this research is to show the main role of trace elements and its relation with the disease or its development. Knowing these element in natural concentrations help to understand the role of these elements and help treat the disease overcome it with its side effects on the human being. Relationship between the change in trace elements and correlation clinical activity disease have been studied. Also the effect of supplementation of trace elements on patients was studied with its relation of the danger of

R.A. The abnormality of autoimmune response was studied.

2.Material and Method:
Collection of the samples:
47 blood samples were collected (7 sampler form healthy, 20 sample 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 HNO3 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. and Polarograph instrumental 746 VA trace analyzer with 747 VA stand or from Metrohm company. The information storage is done by a computer, from Toshiba company 757 VA computrace joined with the device.

3. Results and discussion:
By used ICP method:
First: The Healthy Samples:
Table 1. and Figure 1. Shows the elements concentrations studied in the blood samples of the healthy. And that is by Friedman Test, Significantly ($P < 0.01$). The highest concentration of Fe is (11.1714 ppm) then, Zn (0.4042 ppm) then, Cd. (0.0052ppm) next, Pb. (0.0038 ppm) then, Cu (0.0026 ppm) respectively.

During studying the correlation between the different elements in the healthy samples, the study approves that there is a strong positive correlation between Cu and Fe ($r = 0.849$) and between Cu and Se ($r = 0.949$). Where as the correlation is weak positive between Cu and Pb. ($r = 0.054$).

Second: Samples of R.A. patients in Jeddah region:
Table 1. and Figure 2. Show: that elements concentration differ in the sample of R.A. patients in Jeddah region. That the concentration of Cu is (0.0432 ppm) but it is less concentrated comparing with the other elements. Where as the Fe concentration is (9.7270 ppm) and it is more concentrated comparing with the other elements.

Then Pb concentration (0.165 ppm) and Zn (0.1610 ppm) next, Se (0.1168 ppm) then, Cd (0.0437 ppm). The study of correlation has been done between the different elements in samples of R.A. patient's blood in Jeddah region. The study approves the strong positive correlation between Cu and Se ($r = 0.846$) and between Cd and Fe ($r = 0.897$). while the correlation in the case of Se and Zn is medium positive ($r = 0.1$).
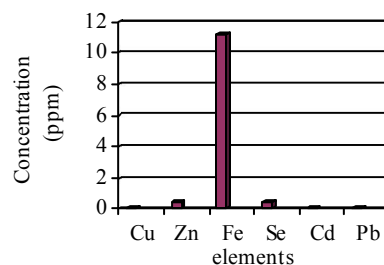

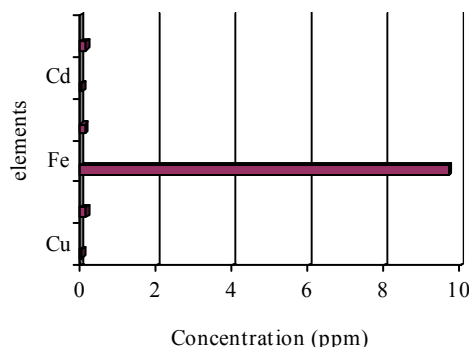Figure 1.Mean concentration of elements in blood samples of the healthy.

Figure 2.Mean concentration of elements in blood samples of R.A. patients in Jeddah region.

Third: Samples of R.A. patients in Gizan region:
Table 1. and Figure 3. Shows that the elements concentrations differ in the blood samples of R.A. patients of Gizan region. The concentration of Cd (0.0447 ppm) but it is less concentrated comparing with the other elements. Whereas Fe concentration is (8.4645 ppm) and it is more concentrated comparing with the other elements. Then, Cu concentration which is (0.0472 ppm) next, Se (0.0849 ppm) then Pb (0.1643 ppm) and Zn (0.1895 ppm).

The study is done about the correlation of the different elements with each other in R.A. blood samples in Jeddah region. This study approves the strong positive correlation between Cu and Se ($r = 0.826$) and between Cd and Fe ($r = 0.867$) while in

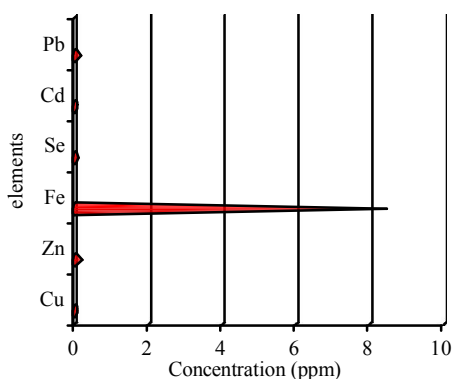the case of Se and Zn, the correlation is medium positive (r=0.454).

Figure 3.Mean concentration of elements in blood samples of R.A. patients in Gizan region.

T able 1. Concentrations of Trace Elements:

Element	Mean ± std. deviation		
	Healthy	R.A.Jeddah patients	R.A.Jizan patients
Cu	0.0026± 0.0023	0.0432± 0.006	0.0472± 0.0139
Zn	0.4042± 0.0179	0.1610± 0.0172	0.1895± 0.0632
Fe	11.1714± 0.3039	9.727± 0.1932	8.4645± 0.8366
Se	0.3971± 0.1150	0.1168± 0.0931	0.0849± 0.0127
Cd	0.0052 ±0.0039	0.0437± 0.0931	0.0447± 0.0253
Pb	0.0038± 0.0013	0.1650± 0.0135	0.1643± 0.0168

Table 1. Shows the difference in the elements concentration between the healthy and patients of both regions: It is as follows:

Cu: The concentration of Cu in the patients of Gizan region is higher than that of Jeddah region patients. The concentration of Cu in the patients is higher than that in the healthy this in blood samples.

Zn: They study shows that there is difference in Zn concentration between patients and healthy. And that there is difference in concentration between patients themselves in both areas. We find Zn concentration in patients is low comparing with the healthy and we find Zn concentration declines more in Jeddah area patients comparing with Gizan area and that is in blood samples.

Fe: the concentration of Fe in the patients less than that in the healthy. Also, Fe concentration in Gizan region patients is less than that in Jeddah region patients.

Se: There is difference in Se concentration in the healthy comparing with the patients in both areas. The concentration of Se is low in the patients comparing with the healthy. Also Se is much lower in Gizan region patients than in Jeddah region patients.

Cd: This research approves that the Cd concentration is higher in the patients, than in the healthy, and the concentration of this elements is clearly higher in Gizan region patients comparing with the patients of Jeddah region. This is in blood samples.

Pb: Concentration differs in the healthy and patients that means that Pb concentration is high in the patients comparing with the healthy. Also it is high in Jeddah region patients comparing with Gizan region patients.

By used Polarography method:
Firstly: Samples of the Healthy:
        Table 2. Shows elements concentrations studied in the healthy blood samples and this is by Friedman Test. Significantly ($p < 0.01$). The highest concentration of Fe is (11.1714 ppm), then Zn (0.4042 ppm) next. Se (0.3971 ppm) then, Cd (0.0052 ppm) then, Pb (0.0038 ppm) and Cu (0.0026 ppm) on sequence.

        The study of correlation between different elements in healthy blood samples, approves that there is strong positive correlation between Cu and Fe (r = 0.849) and between Cu and Se (r = 0.949). The correlation is weak positive between Cu and Pb (r=0.054).

Secondly: Samples of R.A. Patients in Jeddah region :
        The research shows, as in schedule 2, that the concentration of elements differ in the blood samples of R.A. Patients in Jeddah area. Cu concentration is (0.0432ppm) but it is less concentrated comparing with the other elements. While Fe concentration is (9.7270 ppm) and it is more concentrated comparing with other elements. Then, Pb concentration is (0.0165 ppm) next. Zn concentration which is (0.1610 ppm) then Se (1168 ppm) then, Cd (0.0437 ppm).

        A study has been done about correlation between the different elements with each others in blood samples of R.A. patients in Jeddah area. This study shows the strong positive correlation between Cu and Se (r = 0.846), and between Cd and Fe (r = 0.897) while in the case of Se and Zn, the correlation is medium positive (r = 0.474).

Thirdly: Samples of R.A. Patients in Gizan region:
 The research shows that the concentrations of the elements differ in the blood samples of R.A. patients in Gizan region. Cd concentration is (0.0447 ppm) but it is less concentrated comparing with the other elements. Fe concentration is (8.4645ppm) but it is more concentrated comparing with the other

elements. Then, Cu concentration which is (0.0472) next, Se which is (0.0849ppm) then, Pb (0.1643 ppm) and Zn (0.1895 ppm).

A study has been made about correlation between the different elements with each others in the blood samples of R.A. patients in Gizan region. This study approves the strong positive correlation between Cu and Se (r = 0.826) and between Cd and Fe (r=0.867). whereas in case of Se and Zn, the correlation is medium positive (r = 0.454).

T able 2. Concentrations of Trace Elements.

Element	Mean ± std. deviation		
	Healthy	R.A. Jeddah	R.A. Jizan
Cu	0.0026 ± 0.0023	0.0432 ± 0.0060	0.0472 ± 0.0139
Zn	0.4042 ± 0.0179	0.1610 ± 0.0172	0.1895 ± 0.0632
Fe	11.1714± 0.3039	9.7270± 0.1932	8.4645 ± 0.8366
Se	0.3971 ± 0.1150	0.1168 ± 0.0931	0.0849 ± 0.0127
Cd	0.0052 ± 0.0039	0.0437 ± 0.0212	0.0447 ± 0.0253
pb	0.0038 ± 0.0013	0.1650± 0.0135	0.1643 ± 0.0168

Table 2. Shows that there is difference in elements concentration between the patients and between the healthy and patients in both regions, as follows:

Cu: The Concentration of Cu in Gizan region patients is higher than that of Jeddah region patients. Also Cu concentration in patients is higher than that in the healthy. This is in blood samples.

Zn: The Study shows that there is difference in Zn concentration between the patients and the healthy. Also, there is difference in concentration between the patients themselves in both regions. We find that Zn concentration in patients is low comparing with the healthy. The concentration of Zn gets much lower in the patients of Jeddah region comparing with Gizan region and that is in blood samples.

Fe: The concentration of Fe in the patients is less than that in the healthy. The concentration of Fe in Gizan region patients is much lower than that in Jeddah region patients.

Se: There is difference in Se concentration in the healthy if we compare them with the patients, in both regions, that Se concentration is low in the patients comparing with the healthy. Also, Se element is much lower in Gizan region patients than in Jeddah region patients.

Cd: The study approves that Cd concentration is higher in the patients than in the healthy. Also, the concentration of this element is clearly much higher in the patients of Gizan region comparing with that in the patients of Jeddah region, and that is in blood samples.

Pb: There is difference between the patients and the healthy in Pb concentration, That the concentration of Pb element is high in the patients comparing with the healthy. Also, it gets higher in the patients of Jeddah region comparing with Gizan region patients**.**

Summary

In this study, some trace elements (Cu, Zn, Se, Fe, Cd, Pb) have been determined in blood samples of Rheumatoid Arthritis patients in both Jeddah and Gizan regions comparing with the healthy. This is done by using Inductively coupled plasma – Atomic Emission Spectrometer (ICP – AES) and voltammetry techniques, The study showed that the concentrations of Cu, Cd and Pb are significantly higher (p<0.001) in the Rheumatoid Arthritis patients comparing with the healthy. The raise of the concentration in Gizan region is more than that of Jeddah region. The study showed also that the concentrations of Zn, Se and Fe is lower in patients than that in the healthy. This low is higher in Gizan region comparing with Jeddah area.

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9/1/2013