Evaluation of Trace Elements in Iraqi Patients with Rheumatoid Arthritis by using Atomic Absorption Spectrophotometer (AAS)[#]

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Abstract

Zinc, Copper, Selenium, Magnesium, Manganese, Chromium, Iron, Nickel, Cobalt, Vanadium and Germanium were determined by atomic absorption spectrophotometer (AAS) in blood serum of patients with rheumatoid arthritis, (30) patients (14male and 16female) with age range (37-60) years compared with normal tensive control. The analysis of results showed that the mean value of concentration (Magnesium, Manganese and Nickel) were significantly higher in patients with rheumatoid arthritis compared to that of healthy, while the mean levels of serum (Zinc, Copper, Selenium, Chromium, Iron, Cobalt and Germanium) were significantly lower than controls. There were no significant changes in overall mean concentration of serum Vanadium in patients with rheumatoid arthritis and control group. There were no significant variations in trace elements levels in relation to sex.Our results suggest that low level of trace elements of Zinc, Copper, Selenium, Chromium, Iron, Cobalt and Germanium , and high levels of Magnesium, Manganese and Nickel may provide good clues to the physician about the high probability of the individual for developing of rheumatoid arthritis disease unless the imbalance of these elements in blood serum to be corrected.

Key words: Trace elements, Rheumatoid arthritis,Atomic absorption spectrophotometer (AAS).

تقدير العناصر النادرة في المرضى العراقيين المصابين بالتهاب المفاصل الرثوي باستخدام مطياف الامتصاص الذري

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الخلاصة

تم قياس العناصر النادرة ذات الاهمية البالغة في وقاية وعلاج أمراض المفاصل والتي تم استخدامها عالميا على شكل عقاقير مع الفيتامينات وهي Zinc, Copper, Selenium, Magnesium, Manganese, Chromium, Iron, Nickel, Cobalt, Vanadium and Germanium بواسطة جهاز الامتصاص الذري اللهبي وغير اللهبي, تم قياس هذه العناصر في مصل دم المرضى المصابين بالتهاب المفاصل الرثوي, (٣٠ مريض) (١٤ ذكر و ١٦انثى) وفي اعمار تتراوح بين (٣٧-٦٠) سنة, وتم مقارنة نتائج العناصر النادرة مع المستوى الطبيعي.أوضحت نتائج التحليل أن مستوى العناصر Magnesium, Manganese and Nickel ذات معدل مرتفع بشكل معنوي لدى المرضى مقارنة بالأصحاء بينما كان معدل Zinc, Copper, Selenium, Chromium, Iron, Cobalt and Germanium منخفض بشكل معنوي عند مقارنتها بالأصحاء. ولم يتم التوصل الى فرق ظاهر بين المرضى والأصحاء في معدل Vanadium. لايوجد هناك اختلاف في تركيز العناصر بين الذكر والانثى. قد تعطي هذه العناصر دليلا واضحا للطبيب عن وجود احتمالية كبيرة لإصابة الأفراد بمرض التهاب المفاصل الرثوي ما لم تعدل هذه النسبة إلى الوضع الطبيعي وذلك بواسطة اخذ هذه العناصر حسب الحاجة.

الكلمات المفتاحية: العناصر النادرة ، التهاب المفاصل الرثوي ،جهاز الامتصاص الذري.

Introduction

Rheumatoid Arthritis had a worldwide distribution and affects all ethnic groups. Rheumatoid arthritis is a chronic inflammatory, systemic disease that produces its most prominent manifestations in the diarthrodial [(1,2)] . Typical form of the disease is symmetrical, destructive and deforming polyarthritis affecting small and large synovial joints with associated systemic disturbances, a variety of extra-particular features and the presence of circulating anti globulins antibodies (rheumatoid factor) [(3)].Meanwhile, trace elements are widely distributed in a variable proportion in human body and they play a vital role in growth. Zinc is a part of every cell in the body and forms a part of over 300 enzymes that have functions ranging from proper action of the body hormones to cell growth. Zinc deficiency can cause growth retardation [(4,5)].

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Zinc is important in the maintenance of proper immune response [5]. Copper is an essential part of key metalloenzymes as ceruluplasmine, cytochrome, oxidase, tyrosinase and monamine oxidase[6]. Copper enters in a large number of enzymes in addition ceruloplasmin, as it will be necessary for the work of an enzyme super oxide dismutase (SOD) as well as an oxidation enzyme Lysyl oxidase, which is one of the necessary enzymes in the synthesis of connective tissue, it is believe that lack of this enzyme leads to decrease of Copper, which leads to adverse effects in bone and connective tissue [7]. Excess Copper as with excess Iron can cause free radical production and damage, also deficiency of Copper results in poor collagen integrity with resultant blood vessel rupture [7,8].Selenium is an essential element of one of the main anti-oxidant enzymes in the human body, glutathione peroxidase (GSH-PX). This explains its benefit in a number of "oxidative" conditions including cancer, cardiovascular disease and rheumatoid arthritis [1], Selenium is incorporated at four active site in the antioxidant enzyme glutathione peroxidase (GSH-PX). This enzyme protects against free radical and oxidative damage by catalyzing the destruction of hydrogen peroxides (H_2O_2) and lipid peroxides, this in turn protects membrane lipids and hemoglobin against oxidation by peroxides[9,10]. Magnesium is involved in at least 300 enzymatic processes; Magnesium participates in a number of biochemical reactions that take place in bone. Alkaline phosphatase, enzyme involved in forming a new calcium crystals, is activated by Magnesium. The conversion of vitamin D to its biologically active form 1,25 dihydroxy vitamin D3, also appears to require Magnesium. Deficiency of Magnesium can produce a syndrome of vitamin D resistance. The concentration of intracellular Magnesium is very high as compared with concentration of extracellular Magnesium for that reason can be attributed the increase in the output cell concentration to the damage done to the cells and then increase its concentration in the blood serum[1,11]. Magnesium an important in bone structure. Deficiency results in tetany and can lead to calcium deficiency. Magnesium is essential to maintain both acid- base balance in the body, and healthy functioning of nerves and muscles [12]. Diets that provide recommended levels of Magnesium are beneficial for bone health, but further investigation on the role of Magnesium in bone metabolism and osteoporosis is needed[8,13]. Manganese is biochemically essential as a constituent of metalloenzymes and as an enzyme activator[14]. A deficiency of Manganese concentration may affect brain health and skeletal and cartilage formation, antioxidant enzyme superoxide dismutase (SOD) which prevents damage by superoxide free radicals [15,16].Chromium is one of the newer essential trace elements. Have a great role in maintaining good health; Chromium may have a function in the control of glucose and lipid metabolism [17].Iron carries oxygen to the cells and is necessary for the production of energy [18-19]. Iron is available in both a ferrous and ferric. Iron in the ferrous form is better absorbed than ferric Iron. Many people with Iron deficiency anemia die from infection because of weak end immune systems. Iron's role in maintaining immunity covers every aspect of how the systems work[20]. Iron is also needed to help produce antibodies and to maintain your white blood cell count[18].A significant amount of Iron is stored as ferritine and hemosiderin. Iron played a potential role in oxidative stress mediated injuries and pathologies e.g. rheumatoid arthritis [21,22]. Nickel has been shown to be essential to man, is present in all tissues of the body. It is firmly attached to DNA and a protein that binds to it in the blood [23] . Nickel is used for increasing Iron absorption, preventing Iron-poor blood (anemia) and treating weak bone and bone structure, it may also be involved in Iron metabolism, as it influences Iron absorption from foods and may also play a role in production of red blood cell. Nickel may then bind with one of the body neutral proteins, this Nickel-protein complex may not be recognized by the immune system, and this may tiger signals to the body's defense mechanism to respond to the complex as if were an intruding antigen. Nickel may deplete glutathione and protein-bound sulf hydryl group, resulting in the production of reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl radical. Other changer observed in a Nickel deficient state include change in skin color, hormone imbalance and abnormal bone growth. liver function is impaired and Iron metabolism is affected, resulting in poor absorption of Iron. Several publication review the evidence for the essentiality and proposed functions of this elements[24,25].Cobalt is essential for humans only as an integral part of vitamin B12 (cobalamin), no other function for Cobalt in the body is known. Deficiency of the vitamin B12 causes a megaloblastic anemia and in several cases, sub acute combined degeneration of the spinal cord [18,12]. Cobalt essential for the growth and development of

healthy nervous system. Deficiency of vitamin B12 and hyperplesia of the bone narrow. Cobalt is one of the essential trace elements, human body need essential elements to grow. It is also necessary co-factor for making the thyroid hormone and deficiency lead to slow growth and development conditions[26]. Cobalt as a co-factor in antioxidant therapy for the enzyme catalase. Deficiencies are reflected in abnormal bone development[26].Vanadium biochemical function as peroxide of vanadate activated insulin receptor in animal studies [27]. Vanadium is another trace mineral that we do not understand the role of very well yet. It clearly has an effect on the health of our bones. In research with goats scientists have noted skeletal deformations in the legs of Vanadium a compound containing Vanadium has been shown to stimulate bone cell proliferation. It was also shown to stimulate collagen (the organic part of bone that provides strength and flexibility) synthesis. Vanadium compounds were shown to increase bone formation without any adverse health effect [27,28]. Vanadium is necessary for bone and tooth development. High doses of Vanadium improve the strength of bone and teeth in experimental [27]. It is needed for cellular metabolism and for the formation of bones. Vanadium May be required for glucose, cholesterol and bone metabolism[28]. High doses of Vanadium may deplete vitamin C [29]. The chemical properties of Germanium are similar to those of silicon [30]. According to Kidd[31], Germanium normalizes many physiological functions such as lowering high blood pressure in human. Seaborn and Nielsen[30] investigated whether Germanium could substitute for silicon in bone formation. A most interesting characteristic of Germanium is it ability to relieve a great deal of pain it does this by inhibiting the natural body enzyme that in turn inhibits production of the body endorphins[28]. Germanium has the ability of stimulate immune function, supplement tissue oxygen and help inhibit rheumatoid arthritis. It has been suggested that Germanium deficiency could be a contributory factor in Kashin-Beck disease (arthritis disease)[31]. Arthritis is a disease of the immune system, commonly referred to as an (autoimmune) disorder, the membrane surrounding a joint becomes inflamed, resulting in a build up of lymphoid cells, resulting in the degeneration of bone, cartilage, ligaments and tendons. Some agent, perhaps the Epstein-Barr virus, may tigger the initial joint inflammation, resulting in the production away results in the painful swelling and of antibodies. This immune response gone inflammation characterized by arthritis.

Materials and Methods

Patients and Method

A shimadzu model AA- 6200 Flame Atomic Absorption Spectrophotometer FAAS and Graphic Furnace Shimadzu flameless Atomic Absorption GFA- 6200 were used for analysis of blood serum samples, which were centrifuged through the preparation of samples then dilution the serum with deionized water for analysis. Samples were taken from patients who had been admitted to Baghdad Teaching Hospital with positive diagnosis of rheumatoid arthritis disease from (February - December) 2010, the analysis of trace elements was done in Ministry of Science and Technology labs.Zinc, Copper, Magnesium and Iron were analyzed by flame atomic absorption spectrophotometer, but Selenium, Manganese, Chromium, Nickel, Cobalt, Vanadium and Germanium were analyzed by flameless atomic absorption spectrophotometer at their specific wavelength.Standard of sample Zn, Cu, Se Mg, Mn, Cr, Fe, Ni, Co, V and Ge are obtained from analytical reagent using solution (Aldrich, 1000µg / L) for each, and subsequent dilution is then carried out to obtain a calibration curve. All other reagent used was of analytical grade, distilled deionized water was used to ensure no leaching of any trace elements to the measured standard and samples polyethylene containers are used to maintain clean samples.Serum samples were obtained from 30 patients (14 males and 16 females) their ages ranged between (37-60) years, with means ± SD (45.667±6.875). The mean duration of the disease was one year.The control group consisted of 30 healthy (14 male and 16 female) individuals with no general complications and who were receiving no medication, their ages ranged between (35-55) years, with means ± SD, (44.667 ± 66.874) . All statistical work and reporting of obtained data were carried out by using SPSS program version 10.0. Difference of the means considered of significance according to the t-test at level of $p<0.05$ and $p<0.01$.

Result and Discussion

This study was performed for to compare between in apparently healthy subject with patients with rheumatoid arthritis. The role of trace metallic elements in chronic inflammatory states is of great interest because many of them are co-factors in metabolic processes involving articular tissues and immune system function [18]. However, many studies showed that there was a relationship between trace elements levels and period of

rheumatoid arthritis diseases.The role of Zinc and Copper in chronic inflammatory disease is of interest because they are co-factor of important enzymes involved in collagen and bone metabolism, immune functions and antioxidant protection. An excess of Zinc may cause anemia or reduce bone formation. Zinc also plays an important role in the catabolism of RNA by regulation RNAase activity. The Zinc and Copper metals prevent the formation of free radicals capable of inducing mutation and have antioxidant effects [7,8,20].Results of these studies have demonstrated that both Copper and Zinc alteration can be explained by the active inflammatory process and that serum trace elements are measures of disease activity, and immune system function [7].One hypothesis mentioned that a decreased Zinc and increase of Copper in sera of acute or chronic inflammatory processes cause an accumulation of Copper and Zinc in many body compartments and in the inflamed areas [7]. Supporting the hypothesis that the development of inflammation induces an increase body requirement of Copper and Zinc. The results obtained for Zinc concentrations in healthy and patients group which are shown in table (1), show high significant difference in Zinc levels decreased ($p<0.001$). Concentration of Zinc is so low in patients group as compared with those in control group. The result obtained in this study are similar to those published in the literature [7,8,26]. Zinc deficiency is common that effect obviously apparent in figure (2) which represented a histogram for data of table (2) means of Zinc Colak et al. [7] reported two effect of Copper deficiency on Iron metabolism, the first occurring early was an adverse effect of Copper deficiency on Iron absorption (or mobilization), the second was inadequate erythropoiesis, even in the presence of a abundant Iron stores. Serum Copper level for patients and controls are shown in table (1), and figure (2) a histogram indicates decrease serum Copper levels in the patients groups in comparison with controls groups in both sex.The decrease of Copper level was statistically significant ($p<0.001$). The results of this study indicate that Copper serum level decrease significantly during rheumatoid arthritis these results are in agreement with the reported [6,8].protects cells from oxidative damage [7]. Selenium has been shown to have anti (proliferative, inflammatory, viral) and immune altering effects. Dietary Selenium is essential for an optimum immune response, although the mechanisms of this requirement are not always fully understood [18]. Figure (1) represents a histogram for data of Table (1) shows the result of Selenium determination in human sera. The mean±SD for control group is (102±15) serum Selenium, high significant differences were found ($p<0.001$), decreased in all patients group compared with control group, these results proved the possible relationship between low levels of serum Selenium and rheumatoid arthritis [7] .Table (1) comprise the results obtained for the Magnesium content in the sera of all groups involved in the present study. Therefore, the difference in the mean serum Magnesium concentration between the two groups is highly significant increased ($p<0.001$). These results are in agreement with the previous studied [8]. Figure (3) shows a histogram which contains the mean values of serum Magnesium concentration in control and patients groups.Manganese is one of several trace elements that are necessary for bone health. One study [15] found that taking a combination of Calcium, Zinc, Copper and Manganese helped lessen spinal bone loss in a group of post menopausal women. People with arthritis tend to have low level super oxide dismutase (SOD) (an oxidant that helps protect the joints from damage during inflammation). Manganese is a required mineral in the metabolism of protein, fat, healthy immune also required in normal bone growth, energy production. The estimated results of serum Manganese are listed in table (1), these results clearly show that high significant differences, elevated ($p<0.001$) in patients group with rheumatoid arthritis as compared with control group, Very little information was found in the literature about this point [8] . Manganese is required for the utilization of vitamin B1 and vitamin E, it is used in the formation of cartilage and synovial fluid of the joints. Manganese deficiency can lead to improper bone formation and production disorder. An Excess of Manganese can lead to poor Iron metabolism [14].Chromium is widely distributed throughout the body, infants have a higher Chromium concentration than adults [1]. The basis for the suggestion that Chromium may effective in preventing rheumatoid arthritis is that post menopausal women taking a Chromium supplement exhibited increased plasma dehydro epiandrosterone, a precursor of estrogen which inhibits bone loss, and decreased urinary calcium and hydroxy proline excretion, which are indirect rather variable indicators of bone loss [26]. These provocative findings need to be confirmed, and the prevention of bone loss needs to be validated by the use of methods that can directly detect

changes or no changes in bone composition with Chromium supplementation; Chromium supplementation should view as only one of a number of speculative method that may help in maintaining healthy bones [17,28]. Figure (1) shows a histogram which contains the mean values of serum Chromium concentration in control and patients groups, Chromium deficiency in patients with rheumatoid arthritis disease. Table (1) shows the results for mean values of serum Chromium concentrations of these elements, so low in patients group as compared with those in control group. The results are in agreement with the data [20]. There were no significant difference in serum trace elements level between male and female patient group according table (2) and table (3). Studies show people who have both rheumatoid arthritis and anemia tend to have more severe arthritis than people without anemia. They are more likely to have serious joint symptoms; anemia is the most common problem for people with rheumatoid arthritis. Studies show as many as 60% of people with rheumatoid arthritis are anemic.

Table 1: Serum trace elements concentration in patients with rheumatoid arthritis and healthy control group in serum (ng/ml) *.

Trace elements	Studied groups	No.	Mean(ng/ml) ± SD	Comparison of significance	
				t-test	P-value
Zn	*Control*	30	975.3 ± 170.1	30.45	0.001
	patients	30	710.2 ± 200.76		
Cu	*Control*	30	1110 ± 250.3	20.17	0.001
	patients	30	872.13 ± 146.72		
Se	*Control*	30	102 ± 15	6.82	0.01
	patients	30	92.8 ± 12.13		
Mg	*Control*	30	16526.67 ± 1056	20.72	0.001
	patients	30	20984.92 ± 5260		
Mn	*Control*	30	26.5 ± 6.25	6.45	0.01
	patients	30	31.26 ± 8.14		
Cr	*Control*	30	46.5 ± 3	85.79	0.001
	patients	30	40.2 ± 2.21		
Fe	*Control*	30	1150 ± 300.4	50.69	0.001
	patients	30	712.33 ± 152.06		
Ni	*Control*	30	24.5 ± 4.7	20.12	0.001
	patients	30	33.46 ± 9.88		
Co	*Control*	30	38.5 ± 4.3	7.87	0.001
	patients	30	35.73 ± 3.28		
V	*Control*	30	33 ± 1.2	0.42	0.51
	patients	30	32.66 ± 2.61		
Ge	*Control*	30	42.1 ± 1.6	159.71	0.001
	patients	30	31.73 ± 4.2		

*Concentration are expressed as mean± SD

Table 2 : Mean serum levels (ng/ml) between different genders of patients *

	Zn	Cu	Se	Mg	Mn	Cr
Male	712±220.56	878.15±151.62	93.65±12.5	22113.7±5773	32.4±8.4	40.6±2.92
Female	708±180.83	866.11±143.21	91.95±10.2	19856.14±4939	30..12±7.8	39.8±3.05

*Concentration are expressed as mean±SD

p value >5% for all elements between male and female

Table 3: Mean serum levels (ng/ml) between different genders of patients*

	Fe	Ni	Co	V	Ge
Male	725.82±158.4	34.77±9.23	36.65±3.57	33.48±3.13	32.81±4.5
Female	698.84±146.3	32.15±8.71	34.81±2.98	31.84±2.32	30.65±4.1

*Concentration are expressed as mean±SD

P value >5% for all elements between male and female

Table (1) and Figure (2) show a histogram which contained the mean values of serum Iron concentration in control and patients groups, Iron deficiency in patients with rheumatoid arthritis disease [18,28]. Iron plays potential role in oxidative stress, mediated injuries and pathologies e.g. rheumatoid arthritis, decades ago it was suggested that Iron may have a crucial role in progression of inflammation in rheumatoid arthritis. Indeed free radical generated by Iron can cause damage to lipids, proteins, carbohydrates and DNA. It is this destruction process that it believe to occur in rheumatoid joint [1,18].Research showed that Nickel was to be found in blood and tissue at quite consistent physiological significance. Nickel is required for normal growth and reproduction in animals and presumably in human being as well it appears to have role in the modulation of the immune system. Nickel plays some important role in biological system such as in enzyme activity, hormonal control also in structure or function of RNA, DNA and protein [24,25].Metabolism of other nutrients like Calcium and vitamin B12 is also altered due to Nickel deficiency. Bone development, resistance to infection and immune function are some of the problems associated with Nickel. Table (1) Figure (1) represented a histogram for mean values of serum Nickel concentration in blood sera of control and patients groups.Table (1) gives serum Cobalt concentrations in patients with rheumatoid arthritis and control subject. Attempt to measure serum Cobalt levels in patients with rheumatoid arthritis disease were reported only by few investigators [18,11].Table (1) contains the results of serum Vanadium concentrations in controls and patients with rheumatoid arthritis disease. As shown above these results are also included in figure (1) which represented a histogram for serum Vanadium levels of control and patients groups. This study involved measurement of serum Vanadium in patients described above, the results no- significant difference decrease (P>0.05) compared with control.Figure (1) represents a histogram for data of table (1) which contains all results of Germanium determination in blood sera of control and patients.

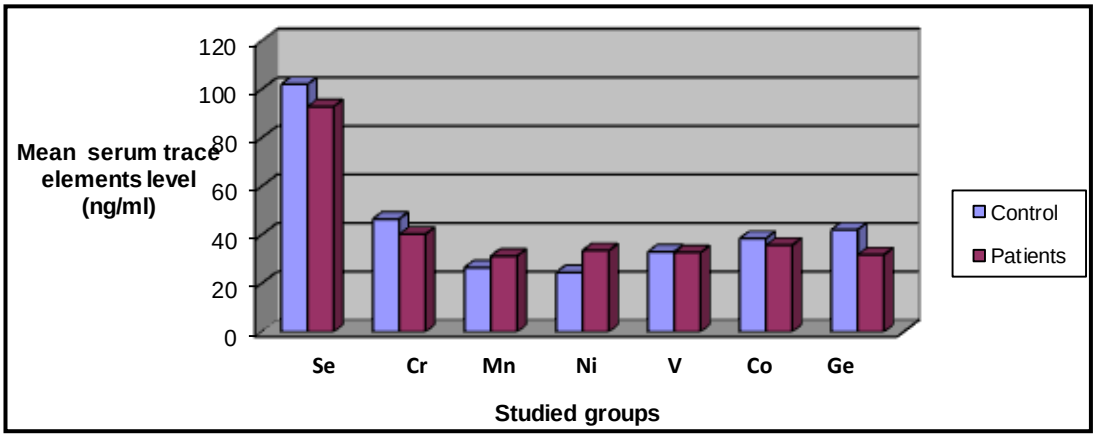

Figure1: Mean serum trace elements level (ng/ml) among the studied groups

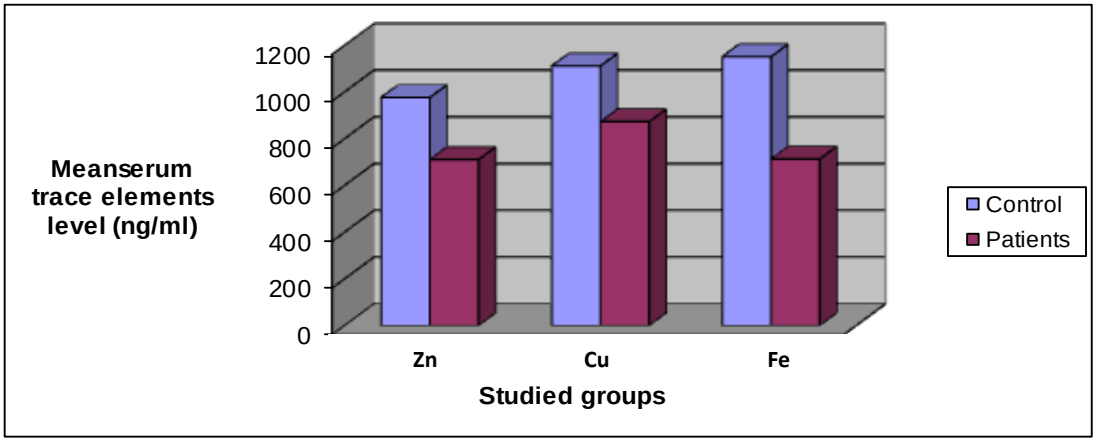

Figure 2: Mean serum trace elements level (ng/ml) among the studied groups

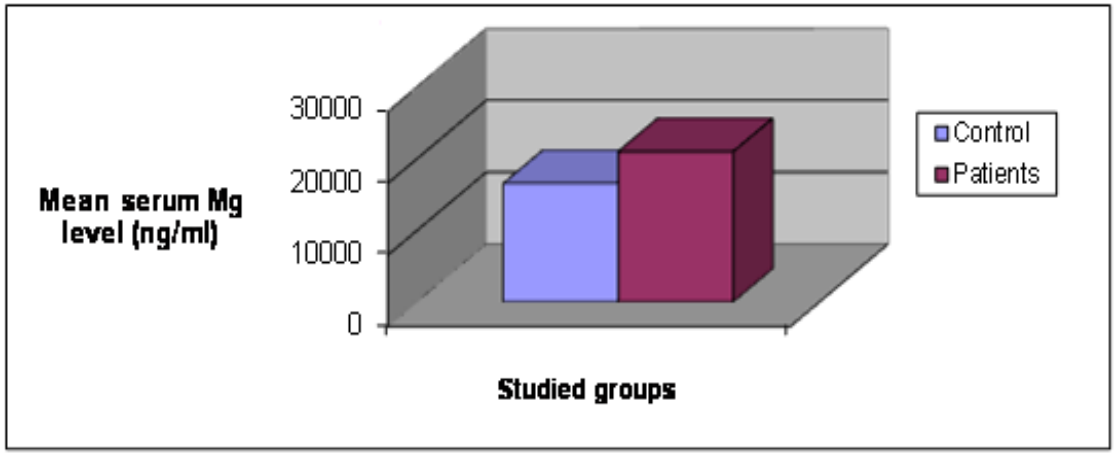

Figure 3: Mean of serum trace elements level (ng/ml) among the studied groups

Conclusion

The conclusions obtained from this study can be summarized:-

Accurate, sensitive and reliable methods had been adopted for measurement of eleven essential and trace elements using both flame and flameless atomic absorption spectrophotometer in blood sera of normal and patients with rheumatoid arthritis.The results observed that the levels of serum Zinc, Copper, Selenium, Chromium, Iron, Cobalt and Germanium were significantly decreased in patients with Rheumatoid arthritis as compared to a control group,The results show that the level of serum Magnesium, Manganese and Nickel were highly increased in the patients. The results of this study show no significant change in the concentrations of serum Vanadium in all patients compared with normal subjects. There were no significant differences in sex related to trace elements. The alteration of trace elements in sera of patients with rheumatoid arthritis can enable us to shed more height on the role of trace elements in both physiological and pathological states. Since there is a significant decrease in Zinc, Copper, Selenium, Chromium, Iron, Cobalt and Germanium, so supplementation of these trace elements could be necessary to get a beneficial from trace elements rebalance in blood serum. We suggest that the deficiency of serum Zinc, Copper, Selenium, Chromium, Iron, Cobalt and Germanium might be used in early diagnosis and treatments of rheumatoid arthritis.

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