A Study of Some Biochemical Tests in Blood Choosing the Most Useful Diagnostic for Rheumatic Disease Patients in Ninevah Governorate

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Abstract

The research was concerned with haematological/biochemical studies in plasma and serum of rheumatic disease investigation for patients in Al-Salam General Hospital over the period starting from January 2007 till August 2007.

The present study included (45) patients, their ages ranged between (30 to more than 50) years, also its included (20) blood samples from healthy persons with similar age groups as a control group. The results showed that higher incident of the disease was in age group (30-40) and more than (50) years. The ration of the male to female was (1:3). Higher incident were found in low social classes. The study also showed that (57.78%) of patients were suffering from increasing weight (obesity). The diagnostic investigations revealed that (60%) of patients exhibited positive result for rheumatoid factor (RF) in comparison to control group. It also showed that (62.2%) of patients had positive result of C-reactive protein (CRP).

The results predicted that erythrocyte sedimentation rate (ESR), Haemoglobin (Hb), Haematocrit (PCV) and some trace elements (Calcium, Iron, Zinc, Manganese, Copper and Sodium) statistically showed significant differences with rheumatic disease patients, while potassium showed no significant difference.

2007   -2007

.   (50   – 30)   (45)

.   (20)

(50   ) (40-30)

.(%3:1)

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.(   )   (%52.78)

(%60)

(%62.2)   (   )

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Introduction

Rheumatism can be explained as a condition in which a person experiences severe body pain in bone, stiff muscles, joints pain, swollen and stiff shoulder. Patient can also go to severe stages like coma, other symptoms include inflammation at the time of urination and improper urination[1, 2].

Rheumatoid arthritis (RA) is a chronic and progressive inflammatory disorder resulting in destructive polyarthiritis[3].

Diagnosing rheumatic disease can be difficult because some symptoms and signs are common to many different disease. Many of the clinical feature are used in the clinical diagnosis of RA. Several tests can be used to determine what type of arthritis you have. They include blood urine tests. X–rays and magnetic resonance imaging (MRI) and joint aspiration (removing fluid from an affected joint)[4, 5, 6, 7]. After a medical history and physical examination have been completed, your doctor will likely need more information.

Blood tests can provide more specific information and often serve to confirm what doctor suspects is the diagnosis.

Blood tests are also used to monitor disease activity and treatment effectiveness after a diagnosis has been established. Serum rheumatologic tests are generally most useful for conforming a clinically suspected diagnosis. Testing for rheumatoid factor (RF) is appropriate when (RA). An elevated erythrocyte sedimentation rate (ESR) is a diagnostic criterion for polymyalgia rheumatica and temporal arthertitis; however specificity is quite low. C – reactive protein (CRP) making this test a better indicator of disease activity than the sedrate which changes more gradually. The complete blood count (CBC) determines the WBC (White blood cell count), RBC (red blood cell count), haemoglobin, haematocrit (PCV) suggested the possibility of an active infection[2 ,6 ,8]. Urine tests may also be recommended. Testing of the fluid inside a joint, called the synovial fluid, is often helpful in determining the cause of a person's arthritis[7].

A single symptom or a single test result is not enough to diagnosis arthritic[2].

Blanced diet is very important for good health. Patient suffering from rheumatism should take proper care of his health. One should stick to green leafy vegetables, fresh, fruits, whole wheat grains, salads, cereals, etc. Also junk foods oily and spicy food should be strictly avoided. Red meat, mutton is not good for patient suffering from rheumatism[2 ,9]. Several metal ions such as sodium, potassium, magnesium and calcium are essential to sustain

biological life, at least six additional metals, chiefly transition metals are also essential for optimal growth, developments and reproduction, i.e. manganese, iron, cobalt, copper, zinc and molybdenum. An elements, which is required in amounts smaller than 0.01% of the mass of the organism, is called a trace element. Trace metals function mostly as catalysts for enzymatic activity in human bodies. However, all essential trace metals become toxic when their concentration becomes excessive[10]. The role of trance metallic elements (copper, selenium, zinc, gold) in chronic inflammatory states is of great intrest because many of them are co-factors in metabolic processes involving articular tissue and immune system function. Deficiencies of several of these have documented in patients with Rheumatoid arthritis (RA)[11].

The role of trace elements in human health has become an important area of scientific research.

The advent flame photometry and the development of the graphite tube in atomizer of atomic absorption techniques has provided more accurate determination of low levels of metals in human body fluid (serum)[12, 13].

The purpose of this study was to determine the usefulness of blood biochemical markers for the screening and diagnosis of (RA).

Materials and Methods
Patients

Patients were enrolled in the present study to the Rheumatoid arthritis (RA) to the consultant Rheumatic disease in department Al-Salam General Hospital in Ninevah Governorate, over the period starting from January 2007-till August 2007. The study sample included (45) blood samples from patients aging between (30 to more than 50) years of both sexes also its included (20) blood samples from healthy persons with

similar age group as a control group. Several studies have suggested that overuse of common plasma and serum rheumatologic tests, (described below) including [rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), C–reactive protein (CRP), Complete blood count such as Haemoglobin (Hb) and Haematocrit (Hct or PCV)] have been assesed as indicated in form (1). Also all these patients were indicated that are not elevated of urine acid in blood.

Collection of Blood Samples

Venous blood samples (5)ml were drawn from each patients after end confirmation of Rheumatic disease, then transferred immediately to a clean (heparinized and non heparinized) plain tube after removing the needle, the blood was divided into two portions one for heparinized tube to give plasma second non heparinized and allowed to clot for at least (10-15)min at room temperature and then centrifuged for (10)min at (4000Xg). Serum was separated using micropipete[14], and was used for determination of some Haemotological and biochemical measurements.
Rheumatoid factor was assayed by manufactured kit by (Linear) (Spin).[15, 16].
Erythrocyte sedimentation rate (ESR) was assayed using Westegren method by. [14, 17].

C–reactive protein (CRP) was assayed by manufactured kit by (Linear) (Spin).[18]. Haemoglobin (Hb) was determined by Drabkin's cyanmethaemoglobin method.[19, 20]. Haematocrit (Hct, PCV) measure the percentage by volume of packed red blood cells (RBCs) in whole blood sample[21].

Blood calcium was determined by using kit manufactured biolabo (France)[22].

Trace Elements Measurement

Several analytical methods are normally needed for the determination of Copper (Cu), Zinc (Zn), Manganese (Mn), Iron (Fe)[23], Sodium (Na) and Potassium (K) in serum[24, 25].

Even with determination of these elements by flame photometry (Sherwood 410) and flame atomic absorption spectroscopy by manufactured in (Pye Unicom, Philips). Varian hollow cathode lamps were used for each determination. Each determination was carried out by the standard addition method of calibration using peak height absorbance measurements [12, 26].

Form 1

University of Mosul
With assistance Al-Salam General Hospital
- Case No.:
- Age:
- Body size (obesity):
- Living state:　　　　rich ☐　　　　　　moderate ☐　　　　　　poor ☐
- Symptoms:
- Name of primary doctor:
- Medical history:

Treatment (Current Medication)

Post medical condition	Present medical condition	Medication allergies

Physical Examination:

[Inflammation☐, Tenderness☐, Swelling☐, Nodules☐, Fever☐, Fatigue☐, … etc.].

Medical Imaging: X-Ray or MRI
Laboratory Test:

Blood test/Haematological/Biochemical parameters	N.R	results
1- Rheumatoid factor (RF)		
2- Erythrocyte sedimentation rate (ESR)		
3- C – reactive protein (CRP)		
4- Complete blood count (CBC)		
* Haemoglobin (Hb)		
* Haematocrit (PCV)		
5- Trace elements (Calcium , Iron, Zinc, Manganese, Copper, Sodium, Potassium)		

Results and Discussion

In this study descriptive and statistical analysis, paired t – test was used to measured blood parameter. The difference is considered significant at p≤0.05[27] of data described below.

In figure (1) showed the percentage differences in Rheumatic disease patients according the age. Showed the (42.23%) in age between (30-40) years, (33.33) more than (50) years and (24.44) between (40-50) years. This confirmed by researchers[28, 29].

In figure (2) the percentage difference in Rheumatic disease patients according the sex, (75.56%) in female patient while lowest percent in male (24.44%) [29,30]. Also the study evidence the higher incidence were found in low social classes[30,31].

In figure (3) showed the percentage of the relationship between the body size (obesity) and rheumatic patients, showed the (57.78%) of patients with weight increased[8].

When diagnosing the (RA), your doctor will look for very specific signs, symptoms, and disease characteristics, your doctor may order some laboratory tests to help confirm a diagnosis. Samples of blood may be needed for the test. Patients with arthritis will probably have blood tests as a part of the initial evaluation and follow-up care. This is because blood is the most easily and safety sampled body tissue, and it contains traces of material from every other part of the body.
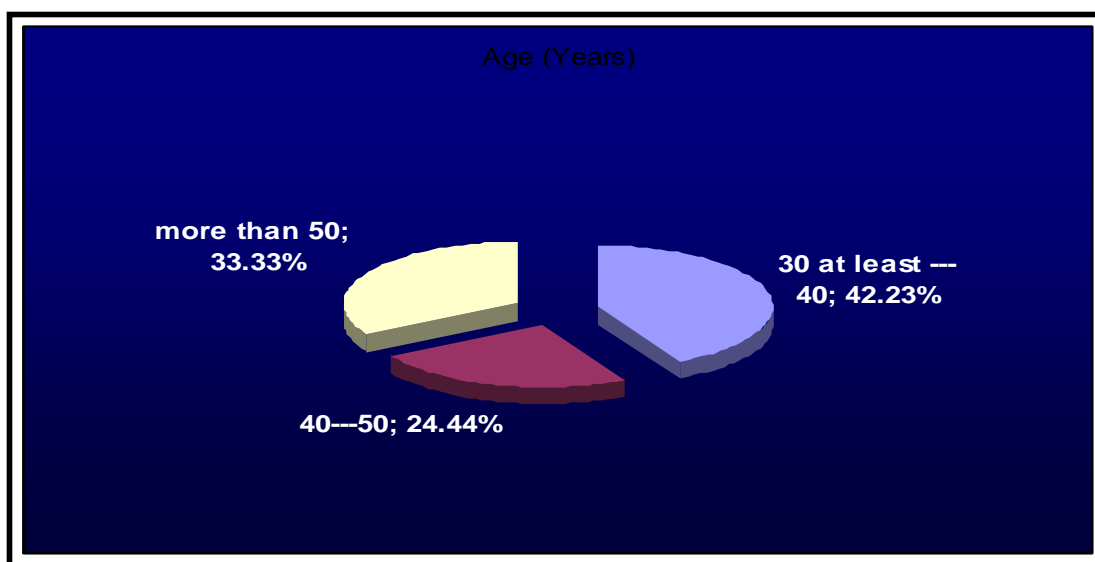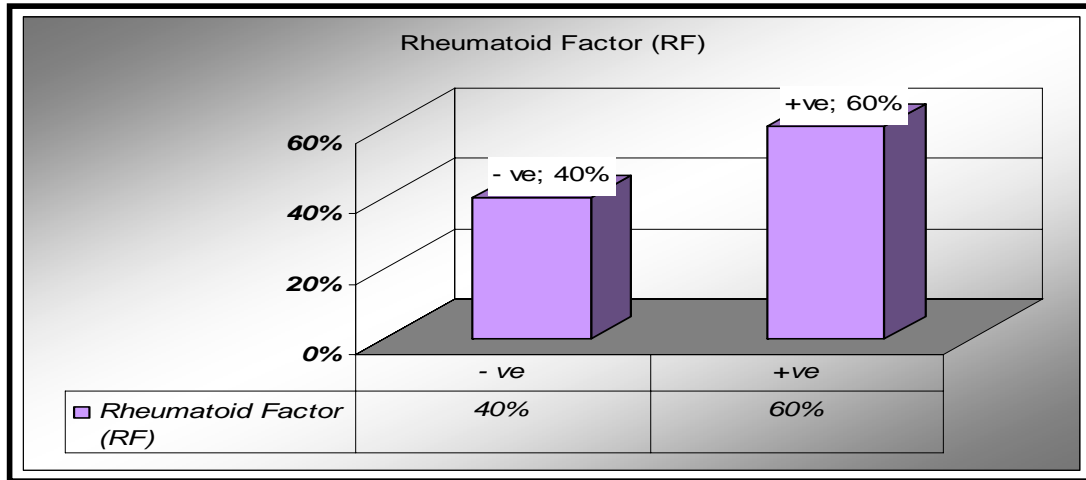

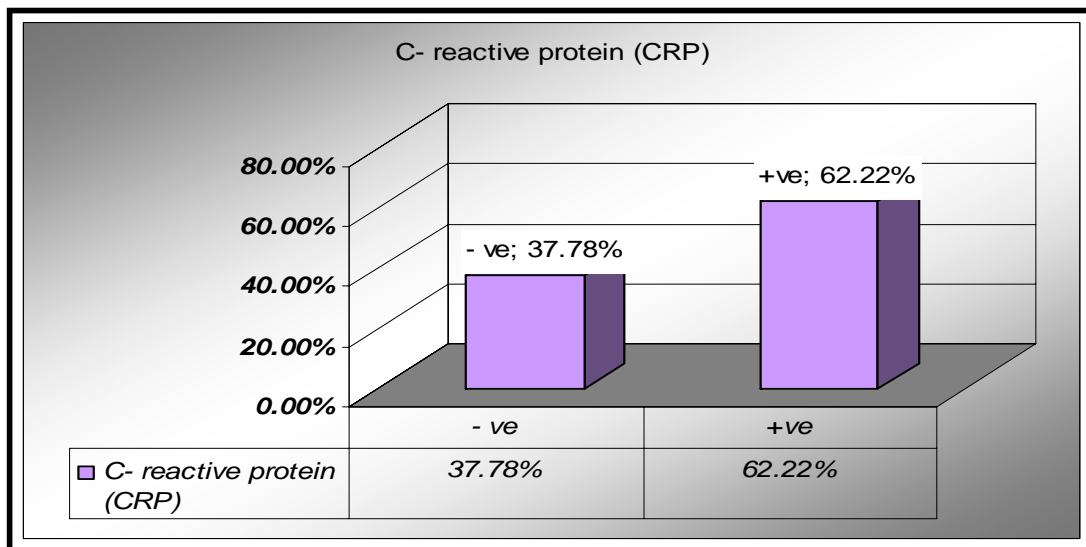**Fig. (1): Percentage in Rheumatic Disease patients according to Age.**
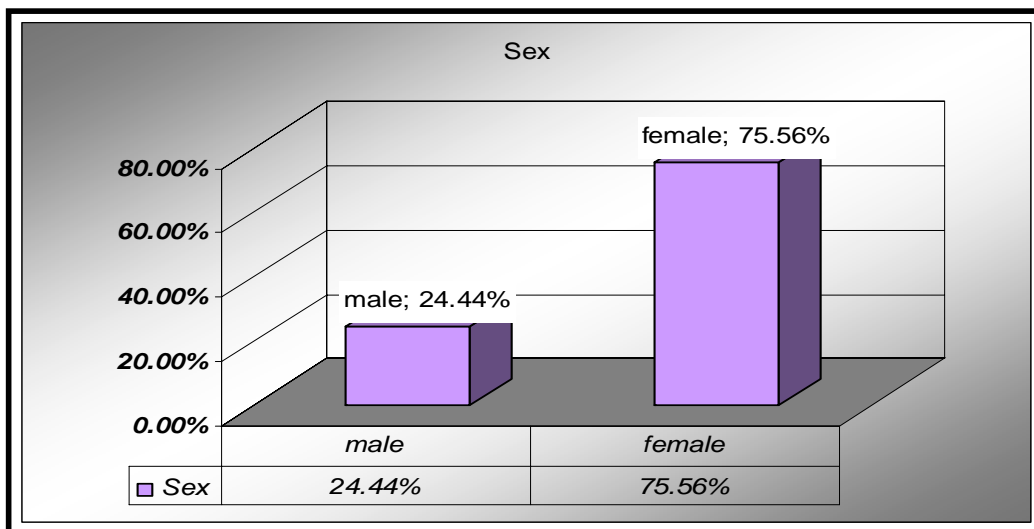
Fig. (2): Percentage the Rheumatic Disease patients according to Sex.
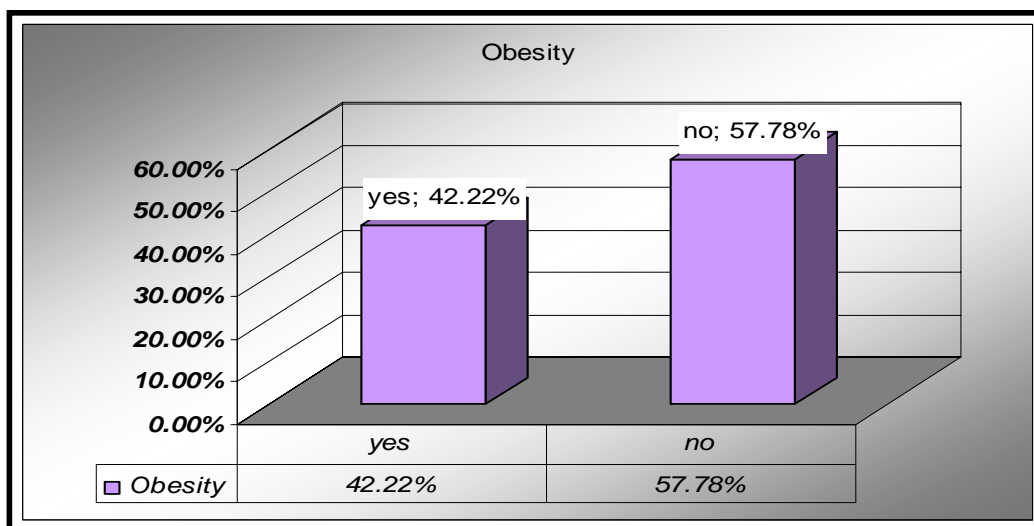
Fig. (3): Percentage the Rheumatic Disease patients according to Body size. (Obesity).

Fig. (4): Percentage the Rheumatiod Factor (RF) in the Rheumatic Disease patients.

Fig. (5): Percentage showed the relationship between (CRP) and Rheumatic Disease patients.

The most common blood test used to assist in the diagnosis and management of arthritis include: Rheumatoid factor (Rf), Erythrocyte sedimentation rate (ESR), C–reactive protein (CRP), Complete blood count (CBC) such as haemoglobin (Hb) and haematocrit (PCV) and uric acid[3,32].

Rheumatoid factor (Rf). This test determines whether rheumatoid factor is present in the blood. (Rf) is an antibody or immunoglobulin found in the blood most (but not all) people who have (RA)[8].

(Rf) may be found in many other diseases besides (RA), and sometimes in normal, healthy people. (Rf) is present in about 70 to 80 of adults who have (RA). It is one of several criteria used in diagnosing (RA)[2].

Figure (4) showed the percentage of the (Rf), 60 percent of patients with (RA) are positive results, 40 percent of patients with (RA) may be seronegative early in the course of the disease ([33]). The test of (Rf) is most useful when there is a moderate level of suspicious for (RA)[33] as the specificity of (Rf) for (RA) ranges from 80 to 95 percent, depending on the age and health of the population studied[34,35].

(Rf) testing in these circumstances may influence the physician away from the true diagnosis but these test are not widely used clinically. In addition the monitoring blood for (RA) rheumatiologists will also typically order blood tests such as a (ESR) and (CRP) in (RA) patients.

The erythrocyte sedimentation rate (ESR) is a test that involves placing a blood sample in a tube of anticoagulant blood and determining how far the red blood cells settle in one hour[36]. When there is inflammation in the body, it produces proteins in the blood, which make the red cells clump together, causing them to fall faster than healthy blood cells. Since inflammation can be caused by conditions other than arthritis. Higher sed rates indicate the presence of inflammation and are typical of many forms of arthritis, such as (RA), and many of the connective tissue diseases. This tests is an indicator of the presence of nonspecific inflammation[2]. Factor that may increase or decrease (ESR) values are showed in reference[37]. In this study (28) percent of patients an elevated (ESR) value and (ESR) had a significant effect ($p \leq 0.05$) in (RA) patients as shown in table (1). The ESR is a mean for staging (RA) rather than a major diagnostic criterion[8].

C–reactive protein (CRP) is a protein which is produced by the liver following tissue injury. Plasma levels of (CRP) increased quickly following periods of acute inflammation or infection, making this test a better indicator of disease activity than the sed rate which changes more gradually[2]. The result of (CRP) in (RA) patients are shown in figure (5), (62.22) percent positive test in (CRP) than (37.78) percent is a negative test.

Complete blood count (CBC), in addition of white blood cell count (WBC), also measures haemoglobin (Hb), the iron-containing component of red cell that carriers oxygen. The haematocrit (PCV, Hct) is the percent of total blood volume is made up of red cells. A lower (Hct) can be caused by a number of factor or conditions including (RA) or anemia, chronic inflammation can cause a low red blood cell count, low (Hb) and (Hct) may be indicative of anemia associated with chronic disease[2].

In table (1) (Hb) and (Hct, PCV) has a significant effect $p \leq 0.05$ on blood in (RA) patients. A decrease in the number of red blood cell (anemia) is common in people with inflammatory arthritis and rheum atic disease[2].

Table (1): Some Haematological and Biochemical Parameters of Patients with Rheumatic disease using paired t – test

No.	Tests Haemalogical & Biological	Normal value[a]	Mean±SD[b]	t – value	Significance p (-value)[c]	Rationate
1	Rheumatoid factor (Rf)	Negative results	Descriptive statistics			An accurate diagnosis is required for proper of (RA)[2]
2	C – reactive Protien (CRP)	Negative results	Descriptive statistics			
3	Erythrocyte sedimentation (ESR)	0-20mm/hour	29.60±16.36	8.03	0.000	
4	Complete blood count (CBC)					
	* Haemoglobin (Hb)	14-18g/dl for adult males 12-16g/dl for adult females	12.30±1.46 g/dl	-7.95	0.000	
	* Haematocrit (Hct, PCV)	0.4-0.54 for males 0.37-0.47 for females	0.39±0.06	-2.80	0.013	
5	Trace elements					Arthritis may need (Ca, Fe, Co, Mn, Zn) for help person bone, build bone and iron deficiency in rheumatic disease[11 , 42 , 44].
	* Calcium (Ca)	8.9-10.1mg/dl	7.59±2.20 mg/dl	-2.27	0.038	
	* Iron (Fe)	0.65-1.7µg/dl	0.74±0.26µg/dl	-8.97	0.000	
	* Copper (Cu)	0.8-1.5µg/dl	0.71±0.30µg/dl	-2.99	0.008	
	* Manganese (Mn)	0.4-0.85ng/dl	0.55±0.24ng/dl	-9.34	0.000	
	* Zinc (zn)	0.7-1.5µg/dl	0.45±0.14µg/dl	-7.90	0.000	
	* Sodium (Na)	135-145meq/L	122.14±19.31meq/L	-2.27	0.036	is a systemic electrolyte and is essential in corequlating ATP of potassium and sodium[44].
	* Potassium (K)	3.8-5.5meq/L	4.90±0.72meq/L	-1.87	0.087	

a- The detailed information using the reference clinical laboratory tests: value and implication[21], since values may be method dependent.

b- The values are the means of Rheumatic patients from haematological/Biolochemical test

c- p≤0.05 means significant difference, p>0.05 means nonsignificant difference.

Trace Elements Estimations

In recent years, awareness that trace elements play a very important role, either beneficial or harmful, in human health has increased. Many metabolic disorders are accompanied by alterations in the concentration of one or more trace elements in somebody fluid, especially blood serum or plasma[38]. Interest in trace element research in clinical medicine, biology, environmental studies, toxicology, and nutrition has become an exciting frontier, and during the last two decades the number of publications on this subject has progressively increased. Recent developments in instrumentation have lowered the limits for determining many trace elements to low nano gram or even picogram range, thus enabling determination of parts perbillium (ng/g) and, in some cases, even less[39] because more essential trace metals are present in biological specimens in very low concentration, precise and accurate analysis is most essential if meaningful results are to be obtained[10].

In the early to mid-1960s, that the clinical community realized that they had a highly sensitive and diverse trace element technique that could automated. Every time there was a major development in atomic spectroscopic (AS)[40]. The role of trace elements in human health has become an important area of scientific research. The (Pye Unicam, Philips). Spq atomic absorption spectrometer and (Sherwood 410) flame spectrometer with programmable sample dispenser was used in this study. Varian hollow cathode lamps were used for each determination. Each determination was carried out by the standard addition method of calibration using peak height absorbance measurement.

Deficiencies of several trace elements have been documented in patients with (RA)[11, 41].

Today scientists and doctors agree that (RA) a results of the lack of specific nutrients. In this study, observed that diets of patients with (RA) are deficient in zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), Calicum (Ca) and Sodium (Na) the typical Ninevah diet. In table (1) showed significant were absorved (Zn, Mn, Fe, Cu, Ca, Na). no significant change was absorved in potassium in (RA) patients[11 , 42].

Researchers reported that the diets of patients with (RA) are deficient in zinc, manganese, magnesium, copper and folate versus the typical American diet[43, 44].

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