

Article

Estimation of C-Reactive Protein, Calcium, and Glucose Levels in the Blood of Patients with Rheumatoid Arthritis in Salah Al-Din Governorate

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Abstract: This study aimed to evaluate serum C-reactive protein (CRP), calcium, and glucose concentrations in patients with rheumatoid arthritis (RA) in Salah al-Din Governorate and to compare these concentrations with those of a healthy control group. The study included 90 samples: 60 persons afflicted with rheumatoid arthritis (RA) and 30 healthy individuals as the control group. We used immunohistochemical, colorimetric, and enzymatic methods to measure the parameters. Then we used statistical analysis to look at the differences and relationships between the variables. The findings indicated a notable increase in CRP levels among patients, particularly in males and the elderly. This proved that CRP is a protein that is present in the acute phase and is a sign of inflammation. As people got older, their calcium levels dropped a lot, but there wasn't much difference between men and women. This is because more calcium is lost and its connection.

Keywords: C-Reactive Protein, Calcium, Blood Glucose, Rheumatoid Arthritis.

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1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that primarily presents with joint inflammation, and can have extra-articular manifestations. This is a long-term inflammatory disease that, in many cases, develops due to an interaction between genetic and environmental risk factors (e.g. smoking) and primarily affects the synovial joints [1]. It typically starts in the small peripheral joints, is frequently symmetrical and can progress to involve adjacent joints over time without treatment [2], [3], [4]. With time, the arthritis causes destruction of joints – cartilage loss and bone erosion. Early-onset RA is defined as having symptoms of rheumatoid arthritis for less than six months, while stable RA is when the condition persists for more than six months. RA is progressive, and untreated RA carries increasing morbidity and mortality risk [5]. RA is thought to be a multifactorial disease and the etiology appears to be mainly determined by genetic factors, with an interaction between the patient's genotype and environmental factors. In a national study involving 91 pairs of identical (monozygotic, MZ) twins and 112 pairs of fraternal (dizygotic, DZ) twins in the UK, the overall concordance rate was 15% among MZ twins and only 5% among non-MZ twins. [6] The heritability of rheumatoid arthritis is estimated between 40% and 65% in seropositive cases, and approximately 20% in seronegative. Various HLA-DRB1 alleles, including: Plasmoidal HLA-DRB1045482; HLA-DRB1015585; and, HLA-DRB1*10 have also been associated with the risk of developing the disease. These alleles have a common five-amino

acid segment, termed with the shared epitope (SE), within the third hypervariable region of the DRB1 sequence that has been associated with an increased risk for rheumatoid arthritis [7], [8], [9], [10].

C-reactive protein (CRP), as its name implies, is an acute-state protein that was first identified as a serum substance reacting with the somatic carbohydrate antigen C of the pneumococcal capsule in patients with acute inflammation. CRP was first isolated in 1930 by Francis Telet [10], and it was assumed to be a disease-associated secreted protein since its levels are elevated in many diseases including cancer [11]. Later discovery of its hepatic synthesis identified it as an indigenous protein [12]. CRP is a circular five-mer protein in blood plasma whose level rises when inflammation occurs. It is the hepatic acute-phase protein whose concentration increases after secretion of interleukin-6 by macrophages and T cells. Its main function as stated above is to bind to lysophosphatidylcholine present on the surface of apoptotic or necrotic cells (and some bacteria) and activate complement system [13].

Calcium is very important in many biological process. Calcium in the blood consists of three components: 50% calcium ions in its active form, 40% bound to serum proteins (mainly albumin), and other anions such as bicarbonate and citrate which account for 10% [14]. Among other things, calcium: regulates ion transport across cell membranes; acts as an intracellular coagulation factor; is a coupling messenger; activates blood clotting processes and neurological and muscular excitability [1]; provides strength and rigidity to bones and teeth [15]. Calcium is critical for bleeding termination as it serves as a cofactor in the coagulation cascade [16]. Over 99% of the total calcium in the body is stored in bones and teeth, its purpose being to help form structures permeated with it. The rest, 1 percent, is found in the blood throughout the body. There are three forms of calcium in blood plasma, which balance with each other. Monosaccharides obtained through the digestion of carbohydrates include glucose, and others as well; namely, fructose and galactose (which are converted to glucose in the liver before accessing the bloodstream). The second source of glucose is from the breakdown of glycogen in the liver [17] and muscles [18]. Glucose is a fuel for tissues and directly associated with sever body metabolic process, thus any perturbation of glucose homeostasis leads to injury and alteration in numerous other metabolic pathways [19]. Glucose is also derived from noncarbohydrate sources including cholesterol, lactic acid, and amino acids. Once glucose enters the blood circulation, it serves as a fuel used by the body to propel the body's diverse tissue metabolic processes.

2. Materials and Methods

Practical part

Study Samples

The study was conducted on (90) samples: (60) from patients with rheumatoid arthritis (RA), and the other 30 were healthy individuals as a control group whose samples have been taken from a variety of public laboratories in Salah al-Din Governorate during the period from November 2024 to March 2025. The patients selected had a mean age of 35-60 years with RA, while healthy individuals have been recruited, and their Mean age is in the range of ~35–60 years. Diagnosis in the patients was performed by specialists at private laboratories.

C-RP Test in the Acute Phase

Serum is prepared based on the diagnostic kit supplied by Biorex Francis and is used for measurement. This test is based on the principle of immune response to C-RP, as an antigen and its specific antibody, immobilized on the surface of biologically inert latex particle [20].

- 1- Fill a 50L sample and load one drop of both the positive and negative control on the slide, each in its own cycle. 2. In your sample to be examined, supplement one drop (50 μ L) of latex reagent.
- 2- Stir the drops with a wooden stick and cover up the entire area of your circle.
- 3- The liquid itself will show granulation when the result is positive (+) or no (granulation -).
- 4- This test is capable of detecting inflammation as a result of protein in affected patients.

Determination of serum calcium

A- Method principle:

In a normal acidic medium, calcium forms a stable blue complex with arsenazo III. The intensity of the color is directly proportional to the amount of calcium in the sample [21].

B- Solutions used

Table 1. Solutions and concentrations of materials used.

Reagents	Concentration
buffer (pH:7.5)	100 mmol/L
Arsenazo III	0.13 mmol/L
Calcium	2.5 mmol/L
Sodium azide	14 mmol/L

C- Method of work

Three test tubes were used, and these tubes are (the model, the standard solution, and the efficient solution (Blank)) as shown below.

Solution	Blank	Sample	ard	Standard
Reagent(A)	1000 μ l	1000 μ l		1000 μ l
Water	10 μ l	-		-
Sample	-	10 μ l		-
Standard	-	-		10 μ l

After mixing and shaking the contents of each tube well, they are put in an incubator at 37°C for five minutes. Then, at a wavelength of 600 nm, the samples' absorbance is read against a blank solution. The hue stays the same at room temperature for around 15 minutes.

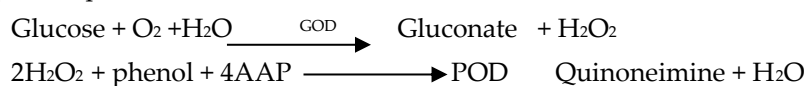
D -Calculation

$$\text{Calcium } \left(\frac{\text{mg}}{\text{dl}} \right) = \frac{\text{Abs Sample}}{\text{Abs Standard}} \times \text{Standard Value } \left(10 \frac{\text{mg}}{\text{dl}} \right)$$

Determination of Glucose Concentration:

Basic Principle

Blood serum glucose concentration was measured with a ready-made kit-based enzymatic calorimetric method (GIESSE, Italy). This method is based on the enzymatic oxidation of glucose (Glucose Oxidase) to gluconate and hydrogen peroxide (Trinder reaction), then in the presence of the enzyme Peroxidase (POD) to form the colored compound quinonimin [22].



Reagents

	Buffer	100 mmol/l
Reagent (A) GLU Volume =100/250/1000ml	Glucose oxidase	10000 U/l
	POD	2000U/l
	4-AAP	1 mmol/l
	Phenol	10 mmol/l
Standard GLU Volume=10ml	Glucose	100 mg/dl 5.56 mmol/l

Procedure

Three test tubes were used (sample, standard solution, efficient blank), and each contained the following solutions:

Pipette	Blank	Sample	Standard
Reagent	1000 ul	1000 ul	1000 ul
Water	10 ul		
Sample		10 ul	
Standard			10 ul

The solutions are thoroughly mixed and then incubated for 10 minutes at 37°C. The absorbance of the standard solution and the sample is then measured against the blank solution at a wavelength of 510 nm using spectroscopy.

Calculations

Glucose concentration is calculated using the following mathematical relationship:

$$\text{Conc. of Glucose (mg/dl)} = (\text{Abs (sample) / Abs (standard)}) \times \text{Standard conc.}$$

Statistical Analysis

XL-SPSS statistical software was used to perform a statistical analysis of the study results. A t-test was applied to test the difference between both groups. Table values are meant to be mean standard deviation, and probability was taken in account. P 0.05 represent the non-significant significance Statistical analysis was conducted to find the correlation coefficient and relationship between C-reactive protein (CRP) and other variables.

3. Results and Discussion

C-Reactive Protein (CRP) Levels

Results: The RA patient group had a significantly increased serum CRP concentration. Table: Distribution of CRP among Patients (Elevated vs. Non-elevated) Though elevated CRP levels were present in 65% and absent in 21% patients.

Table 2. Standard Deviation of CRP.

%	NO.	CRP
65	39	patients Ve+
35	21	patients Ve-

This finding is in line with Sura Zahim et al., the significance content of acute-phase protein playing a role in rheumatoid arthritis where increased active levels of C-protein (CFP) was found together with disease. CFP is an acute-phase protein that plays a

significant role in the disease process [23] and thus it was shown to be a rheumatoid arthritis marker in this study. Elevated CFP levels were found in 65% of patients while 35% did not have elevated levels but no elevated levels found in healthy individuals which is different from the current study. This correlates with a study by Alhial et al. that examined associations between the levels of acute-phase proteins and bone biochemical variables of subjects with type 2 diabetes [24]. In addition, significant gender differences were observed between male and female subjects in RA patients, with males showing higher values of CFP than females (Table 3).

Table 3. Levels of active protein (C) in the blood serum of male and female patients.

Total		Females		Males		CRP
%	NO.	%	NO.	%	NO.	
%65	39	%30	18	% 35	21	patients +Ve
%35	21	%15	12	%20	9	patients -Ve

For example, increased C-reactive protein levels (CRP) in rheumatoid arthritis results in higher coronary heart disease risk with aging. It was found to be higher with the increasing age, where it appeared in the second group in (26) of (38) with a percentage of (64.3%) and shown in first group with (13) of (19), then the percentage was 46.6%, presented as table [4].

Table 4. represents the level of active protein (C) in the blood serum of patients with age.

Total		Group 2 (65-46)		Group 1 (45-25)		CRP
%	NO.	%	NO.	%	NO.	
65	39	%43	26	%21	13	patients Ve+
%28	29	%20	12	%13	6	patients Ve-
		63.3%		42.6%		Visibility

Blood calcium levels in patients vs healthy controls

Control group calcium mean \pm standard deviation: 9.0931 ± 0.17511 ; Rheumatoid arthritis mean \pm standard deviations: 8.9233 ± 0.19430

Table 5. Mean \pm Standard Deviation Calcium in Sera of the Studyed Samples.

Group parameter	Control	Patient	P \leq
	Mean \pm S.D		
NO.	30	60	0.05
S.ca (mg/ml)	9.0931 \pm 0.17511	8.9233 \pm 0.19430	

N.O = Number of samples

The findings also show a marked decrease in blood calcium levels, since patients with rheumatoid arthritis undergo physiological changes that include an increased

metabolism of calcium as well as swelling of extracellular fluid and improvement in glomerular filtration rate (GFR), which leads to loss of calcium from the body through urine and correlated by an increase phosphate levels ((storing compound) in the blood due to reduced excretion in the kidney. Vitamin D and parathyroid hormone regulate calcium metabolism, which is tightly linked to phosphorus metabolism. Intestinal absorption of calcium and phosphorus [25], [26]

Table 6. Calcium levels in the blood serum of healthy male and female patients.

P	(Mean \pm SD) Mm/1hr	NO.	Groups	
P \leq 0.05	8.9233 \pm 0.19430	06	patients	Total
	9.0931 \pm 0.17511	30	Control	
P \leq 0.846	8.9444 \pm .18160	30	females	patients
	9.0136 \pm .22056	30	males	

No notable difference was also observed with regard to sexes at (p \leq 0.846): for male, (9.0136 \pm 0.22056) and female (8.9444 \pm 0.18160).

Also, the results showed significant differences with age at a level of (p \leq 0.05), where levels were (9.0304 \pm 0.20707) gm/L in age categories 35-45 years compared with (8.8909 \pm 0.16652) gm/L for patients aged 46-65 living with HCV.

This finding agrees with the study of Boonen [7], which concluded that calcium levels slightly decline with age due to reduced calcium absorption efficiency as shown in Table(7).

Table 7. Calcium levels in the blood serums of healthy and sick individuals with increasing age.

P	(Mean \pm SD) Mm/1hr	NO.	Groups	
P \leq 0.05	8.9233 \pm 0.19430	60	patients	Total
	9.0931 \pm 0.175110	30	Control	
P \leq 0.01	9.0304 \pm .20707	27	Group 1 (45-35)	patients
	8.8909 \pm .16652	33	Group 2 (65-46)	

The table shows the mean \pm standard deviation of random blood glucose in the control group (108.4000 \pm 11.30944) and the mean \pm standard deviation in the rheumatoid arthritis group (150.3167 \pm 45.18643).

Table 8. Mean \pm Standard Deviation of Blood Glucose in the Sera of the Studied Samples.

Group parameter	Control	Patient	P \leq 0.05
	Mean \pm S.D		
NO.	30	60	
RBS(mg/ml)	108.4000 \pm 11.30944	150.3167 \pm 45.1864	

N.O = Number of samples

A higher level of blood glucose was recorded among the group of patients with rheumatoid arthritis. This is similar to the study conducted by Tian, Z. et al (2021) which

proved that risk of diabetes is higher with rheumatoid arthritis. This concept provides support for the role of inflammatory pathways in the pathogenesis of diabetes [28]. David et al. situation with a majority of the patients being screened, in fact, 50% of individuals with rheumatoid arthritis were screened for diabetes risk factors, especially lipid profile, glycosylated hemoglobin (HbA1c), and body mass index (BMI). RA and non-RA groups had similar screening rates. But people with rheumatoid arthritis were more likely to get diabetes. Consequently, it was recommended that patients with rheumatoid arthritis undergo periodic screening to avoid and decrease the possibility of developing diabetes [29].

Table 9. Serum (glucose) levels in healthy males and females.

P	(Mean \pm SD) Mm/1hr	NO.	Patient	
P \leq 0.05	150.3167 \pm 45.1864	60	Patient	Total
	108.4000 \pm 11.30944	30	Control	
P \leq 0.885	136.9783 \pm 43.61014	30	female	Patient
	135.6818 \pm 41.39867	30	male	

There are no differences between sexes our results should be at a probability level (p \leq 0.885) where was for males (135.6818 \pm 41.39867,) and females were (136.9783 \pm 43.61014.) The current study concurs with Telford, M. (30) showing more higher glucometries for females than males in rheumatic diseases as shown on the following figure. Age group differences were also highly significant. At a probability level of P \leq 0.05, patients aged 35-45 years showed decreased glucose levels (117.4737 \pm 29.09903 gm/L) when compared to those between ages 46 and 65 years old (168.9394 \pm 42.03417 gm/L). Table 10 shows that in agreement with the study of Peter (2009) (31), increase in glucose levels were shown to be positively correlated with age among patients diagnosed with rheumatoid arthritis.

Table 10. Serum (glucose) levels in healthy and sick individuals with age groups.

P	(Mean \pm SD) Mm/1hr	NO.	Patient	
P \leq 0.05	150.3167 \pm 45.1864	60	Patient	Total
	108.4000 \pm 11.30944	30	Control	
P \leq 0.05	117.4737 \pm 29.09903	27	Group 1 (45-35)	Patient
	168.9394 \pm 42.03417	33	Group 2 (65-46)	

4. Conclusion

It found that patients with rheumatoid arthritis (RA) experienced a 65% increase in C-reactive protein (CRP), which is a pointer of inflammatory activity, especially in males and older people. The authors also note a dramatic decrease in calcium with age and an increase in random glucose, changes that are consistent with disturbances of mineral and sugar metabolism. Higher levels of CRP, lower calcium and high glucose reflect the chronic inflammation in RA patients.

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