Evaluation of Some Cytokines in Rheumatoid Arthritis

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Abstract
Rheumatoid arthritis (RA) a class of systemic auto immune disturbances and manifest its Self with progression inflammation of several Joint in the body. The present results was co conducted to evaluate some hematological parameters and some biomarkers in Patients affected with RA of both Sexes (men and women) .The individuals who had involved in the present Study were 80 subject of Patients and healthy individual and they classified according to gender into two Subclasses, the first one involved 40 males that divided into 20 Males Patients with RA and 20 Males healthy control. The second class had also 40 females of them 20 females affected with RA and 20 healthy females as a control group .All ages of Subject were ranged between 40-79 years old and then according to their age's war classified into four age groups (40-49, 50-59 60-69, 70-79 Years old .) The demographical data that obtained from the Present study indicated that the Percentage ratio of women with RA was 60% higher than Men with RA who had recorded 40% The distribution of Patients according to their age groups the Percentage ratios were the following to 49 first group 30%, Second group 30% Third group 25% fourth group 15% respectively). In addition the Percentage ratios of RA Patients according to body Mass index (BMI) it was found Patients with that had BMI equal to 25 -33 kg/m² comprised 70% higher than those patients who had BMI ranged between 19-23 kg/m² (30%). The changes of hematological Parameters had been exhibited a significant increase (P<0.001) in the Levels of blood platelets and Erythrocyte sedimentation rate (ESR) in all RA patients groups when compared with those healthy one. The correlation Coefficients occurring among Studied hematological Parameters recorded the following data, it was found that then is a significant positive correlation (r=0.52, Sig=0.001) between ESR and Platelets in RA patients. Also, a significant positive correlation (r=0.43, Sig=0.005) occurring between ESR and WBCs. At the same results were found between ESR and granulocytes recorded a significant Positive Correlation (r=0.42, Sig=0.007) in RA Patient. Inversely, a significant negative correlation (r = -0.43, Sig=0.006) between ESR and lymphocytes in RA patients. The observations recorded around studied biomarkers including rheumatoid factor (RF), C-reactive Protein (CRP) and fibrinogen, were showed a significant elevation (P<0.05) in all levels, of these biomarker of all patients groups compared to healthy subjects .These studied biomarkers appear closely associated with BMI therefore their levels were significantly high P<0.05 in RA Patients with BMI 25-33kg/m² Comparing to those patient had BMI ranged between 19-23.
Keywords: Rheumatoid arthritis, erythrocyte sedimentation rate (ESR), Platelets, rheumatoid factor (RF), C - reactive protein (CRP) and fibrinogen

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that preferably affects small joints. RA can also cause many extra-articular manifestations such as pericarditis, pulmonary fibrosis, and peripheral neuropathy, etc. Most often, the diagnosis is made when patients with pain and swelling in the peripheral joints, as well as joint stiffness in the morning, seek medical help. Pain due to RA is typically worse in the morning and the evening and improves during the day. Awakening in the night because of pain in the joints has also been described as a common symptom of RA, as in most inflammatory rheumatic diseases [1].

In parallel to the gut microbiota, several viral and bacterial infections seem to be responsible for the development of autoimmune diseases, including RA [2]. Characterized the infection events in a longitudinal cohort of first-degree relatives of patients with RA, evaluating their associations with the development of the disease [2, 3].

The correlation between bone loss and clinical disease activity, as well as the protection from bone erosion progression by a tight therapeutic control of synovitis, support the concept that chronic inflammation is the major mechanism involved in bone loss in RA [4].

Fibrinogen synthesis is regulated by acute-phase proteins, mainly by IL-6 (derived by monocytes, macrophages, and vascular endothelial cells), which induces its synthesis in the liver, while IL-1β and tumor necrosis factor-alpha (TNF-α) suppress its synthesis [5, 6].

2. Methods

Collection of blood samples

The present work was performed in many locations including hospitals (Marjan teaching hospital and Imam Al–sadiq hospital) and location including Babylon university/college of science for women and private laboratories. The present study was initiated at a beginning of November 2021 to April 2022.

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The total number of individuals was eighty (80) of men and women of these twenty (20) men were complained from Rheumatoid arthritis and twenty (20) men were apparently healthy were selected as a control group.

The remaining individuals (40) women, of them, twenty (20) women were affected with Rheumatoid arthritis and twenty (20) women also they were selected as a control group.

All persons of study, had ages ranged between 40-79 years old. The subjects (patients and healthy control) of the present study were classified according to their ages in to four categories (40-49, 50-59, 60-69, 70, 79 years old). Excluded criteria (diabetes melitus,osteoporosis ,thyrotoxicosis ,malignant diseases and pregnancy).

All patients were admitted to hospital and health care centers to check up their own healthy and received therapeutic options. Concerning control subjects, they were selected from public health centers, workers in hospitals, and person who have normal medical history of both sexes.

Determination of rheumatoid arthritis (RF), CRP and fibrinogen:

Measurement of rheumatoid factor (RF) and CRP was carried out according to instruction applied by Biotech Company whereas determination of fibrinogen was performed according to instructions of Hemostat Company.

Statistical Analysis

Results of the present study were illustrated as means ± standard

Deviation (SD). The values were statistically analyzed by using SPSS 23

Program and analysis of variance were explained. The lowest significant

Differences (LSD) among studied groups was p< 0.05.

3. Results

1. Distribution of patients according to body mass index (kg/m2)

From this study it had been that people with high body mass are affected more than people who have normal body mass, with an average of 70% for people who have excessive body mass and 30 % for people with normal body mass. This was showed in Figure (1)

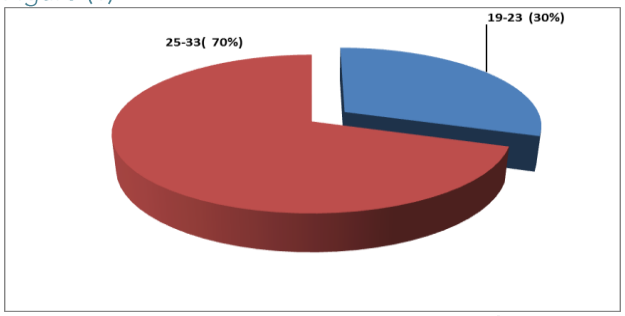

Figure (1): Shows the percentage ratio (%) of body mass index for patients affected with rheumatic arthritis.

2. Blood parameters in rheumatoid arthritis patients and healthy people.

This results that were shown in table (1) explained a significant heightening P<0.01 in the levels of ESR (29.60±2.85 mm/hr) in matching with those healthy control groups 12.59±2.73 mm/hr), platelets indicated a significant different p<0.05 (275.25±17.40 cell/mm3) in matching with those healthy control groups (239.48±15.60 cell/mm3). Table (1): Shows the results of hematological parameters (platelets and ESR) of patients affected with rheumatic arthritis and healthy subjects.

Groups Parameters	Patient (n=40)	Control (n=40)	Pvalue≤0.05
	Mean ±S.E		
PLT (cell/mm3)	275.25±17.40	239.48±15.60	0.034*
ESR (mm/hr)	29.60±2.85	12.59±2.73	0.0001**
-All values are mean ±SE - Results with two a strikes are significantly different at P≤0.01 -Results with one a strike are significantly different at P≤0.05			

3. Illustrate the biochemical markers in Rheumatoid Arthritis Patients and Healthy People.

The results that were shown in table (2) explained a significant heightening p<0.01 in the levels of tumor necrosis factor (, fibrinogen, rheumatic factor (RF) and C-reactive protein (CRP) (, 393.25±13.29 mg/dl, 25.11±2.47 IU/l and 30.26±2.79 IU/l, respectively) in matching with those healthy control groups (, 212.65±14.01 mg/dl, 11.23±1.42 IU/l and 8.59±2.68 IU/l, respectively).

Table (2): Illustrate the biochemical markers (TNF, fibrinogen, Rf, CRP and Gm-CSF) of patients affected with rheumatic arthritis and healthy control.

Groups Parameters	Patient (n=40)	Control (n=40)	Pvalue≤0.05
	Mean ±S.E		
Fibrinogen (mg/dl)	393.25±13.29	212.65±14.01	0.0004**
Rf (IU/l)	25.11±2.47	11.23±1.42	0.0001**
CRP (IU/l)	30.26±2.79	8.59±2.68	0.0001**
All values are mean ±SE -Results with two a strikes are significantly different at P≤0.01. -Results with one a strike are significantly different at P≤0.05.			

4. Blood parameters in rheumatoid arthritis patients and healthy people according to gender.

Data that were illustrated in table (3), showed a significant increase P<0.05 in levels, of platelets , and ESR (273.80±33.2 cell/mm3 and 28.91±4.7 mm/hr respectively) in male Patient , while indicated a significant increase P<0.05 in levels of platelets and ESR(276.70±12.6 cell/mm3 , and 12.08±9.6 mm/hr respectively) in female Patient , when compared with healthy control group in levels of platelets , and ESR(237.05±16.4 cell/mm3 , 12.08±9.6 mm/hr respectively) in males healthy control group , and the levels of platelets , and ESR for females healthy

control group(, 241.90±18.8 cell/mm3 , and 13.09±1.3 mm/hr respectively).

Table (3): Shows the results of hematological parameters (PLT and ESR) of patients and control affected with rheumatic arthritis according to gender.

Groups Parameters	Male		Female		LSD(0.05) (gender*group)
	Patient	Control	Patient	Control	
	Mean ±S.E				
PLT (cell/mm3)	273.80±33.2	237.05±16.4	276.70±12.6	241.90±18.8	22.197*
ESR (mm/hr)	28.91±4.7	12.08±9.6	30.30±2.9	13.09±1.3	2.666*

-All values are mean ±SE
-Results assigned with different letters significantly different at p<0.05

5. The results of biochemical markers (fibrinogen, rheumatoid factor and C-reactive protein) of patients affected with rheumatic arthritis and control group according to gender.

Table (4) illustrated the results recorded a significant increase p<0.05 in the levels of, fibrinogen, rheumatoid factor and C-reactive protein (, 395.25±35.4 mg/dl , 24.30±8.2 IU/l and 29.38±5.3 IU/l , respectively) in males patients when compared with control group in the levels of , fibrinogen, rheumatoid factor and C-reactive protein (, 211.95±14.2 mg/dl , 11.07±0.3 IU/l and 8.64±1.1 IU/l , respectively) in males , while in females patient the values pointed out a significant elevation P<0.05 in the levels of, fibrinogen, rheumatoid factor and C-reactive protein (391.25±12.5 mg/dl , 25.93±3.4 IU/l and 31.15±4.1 IU/l respectively) when compared with control group in the levels of fibrinogen, rheumatoid factor and C-reactive protein (, 213.35±12.7 mg/dl , 11.38±1.3 IU/l and 8.55±0.7 IU/l, respectively) in females.

Table (4): Illustrate the biochemical markers (fibrinogen, rheumatoid factor and C-reactive protein) of patients affected with rheumatic arthritis and control groups according to gender.

Groups Parameters	Male	Female			LSD (0.05) (gender*group)	
	Patient	Control	Patient	Control		
	Mean ±S.E					
Fibrinogen (mg/dl)	395.25±35.4	211.95±14.2		391.25±12.5	213.35±12.7	12.397*
Rf (IU/l)	24.30±8.2	11.07±0.3		25.93±3.4	11.38±1.3	1.495*
CRP (IU/l)	29.38±5.3	8.64±1.1		31.15±4.1	8.55±0.7	2.489*

-All values are mean ±SE
-Results assigned with different letters significantly different at p<0.05

6. Hematological parameters according to gender and age groups of patients affected with rheumatic arthritis and healthy control of both sexes.

Results which were illustrated in the following table (5) they explained the effects of age periods on some hematological parameters (platelets, and erythrocyte sedimentation rate (ESR mm/hr) of both patients and control, some of the results significantly different at p<0.05 when compared among different age groups according to LSD values. Table (5): Shows the results of hematological parameters (PLT and ESR) of patients affected with rheumatic arthritis and healthy groups according to gender and age.

Groups Parameters	Age (year)	Male		Female	LSD(0.05)	
		Patient	Control	Patient	Control	
		Mean ±S.E				
PLT (m/mm3)	40-49	226.00±22.6	259.40±22.1	229.20±12.5	264.60±22.3	35.223
	50-59	256.40±14.6	250.00±14.2	258.20±22.3	257.80±35.1	
	60-69	301.40±17.6	225.80±25.2	303.60±33.1	229.80±17.9	
	70-79	311.40±33.1	213.00±11.7	315.80±31.1	215.40±14.6	
ESR (mm/hr)	40-49	24.82±11.4	9.00±2.1	26.50±2.6	8.86±1.2	4.049
	50-59	25.80±3.3	9.66±1.3	26.18±2.2	11.92±0.9	
	60-69	30.26±2.6	14.42±1.1	32.16±1.9	15.30±1.1	
	70-79	34.76±2.9	15.24±0.8	36.34±1.7	16.28±1.0	

-All values are mean ±SD
-Results assigned with different letters significantly different at p<0.05

7. The results of biochemical markers according to gender and age groups of patients affected with rheumatic arthritis and healthy control of both sexes.

All the results had been recorded in the table(6) were a significantly increase (P<0.05) in levels of (fibrinogen, rheumatoid factor and C-reactive protein) as the values are shown in the table, in all age groups (40-49,60-69,70-79) in male and female with rheumatic arthritis when compared with healthy groups .

Table (6): Illustrate the biochemical markers (fibrinogen, rheumatoid factor and C-reactive protein) of patients affected with rheumatic arthritis and healthy groups according to gender and age.						
Fibrinogen(mg/dl)	40-49	384.80±22.5	193.20±17.8	385.00±35.2	194.20±15.6	19.738*
	50-59	394.20±17.5	201.40±20.1	395.40±17.8	202.80±20.5	
	60-69	397.60±20.1	205.80±15.5	398.80±16.9	207.20±12.6	
	70-79	404.40±33.2	247.40±9.6	385.80±23.1	249.20±22.7	
Rf (IU/l)	40-49	20.84±1.9	7.84±1.1	23.92±2.6	6.94±0.9	1.855*
	50-59	22.26±2.1	11.12±0.7	25.78±1.3	12.26±1.2	
	60-69	26.80±3.3	12.12±1.1	26.14±2.4	12.96±1.4	
	70-79	27.30±4.1	13.20±1.6	27.86±3.3	13.36±1.1	
CRP (IU/l)	40-49	25.92±2.3	6.12±1.2	26.36±2.3	5.14±0.3	4.037*
	50-59	26.44±3.3	7.26±0.8	29.22±1.7	7.36±0.6	
	60-69	30.40±1.9	10.14±0.5	32.22±3.6	10.36±0.1	
	70-79	34.74±4.4	11.04±1.3	36.80±2.7	11.32±2.1	
All values are mean ±SD -Results assigned with different letters significantly different at p<0.05						

7. The results of hematological parameter erythrocyte sedimentation rate (ESR) of patients affected with rheumatic arthritis according to BMI.

The results that were shown in the table(7) indicated a significant evaluation p<0.01 in the level of erythrocyte sedimentation rate (ESR) 34.06±3.1mm/hr in patients when compared with control groups in the level of erythrocyte sedimentation rate (ESR) 20.08±1.6 mm/hr.

Table (7): of hematological parameter according to BMI (kg/m2) of patients with rheumatic arthritis and control groups.			
BMI groups Parameter	BMI (Kg/m2)		P≤0.05
	Patient (25-33)	Control (21+2)	
	Mean±S.E		
ESR(mm/hr)	34.06±3.1	20.08±1.6	0.004**
All values are mean ±SE -Results with two strikes are significantly different at P≤0.01.			

8. The results of biochemical markers (rheumatoid factor, granulocyte monocyte-colony Stimulating factor, tumor necrosis factor, fibrinogen, and C-reactive protein) of patients affected with rheumatic arthritis according to BMI.

The results that were shown in the table (8) indicated a significant evaluation p<0.01 in the level of fibrinogen, rheumatoid factor and C-reactive protein (401.80±10.8 mg/ml, 25.84±1.1 IU/l and 32.94±3.1 IU/l respectively) in patients when compared with control groups in the level of fibrinogen rheumatoid factor and C-reactive protein (121.80±1.9 215.00±12.8 mg/ml,11.44±1.2 and 10.42±2.3 IU/l respectively)

Table (8): Biochemical marker according to BMI (kg/m2) of patients with rheumatic arthritis and control groups			
BMI groups Parameters	BMI (Kg/m2)	P≤0.05	
	Patient (25-33)	Control (19-23)	
	Mean±S.E		
Fibrinogen(mg/ml)	401.80±10.8	215.00±12.8	≤0.0001**
Rf(IU/l)	25.84±1.1	11.44±1.2	≤0.0001**
CRP(IU/l)	32.94±3.1	10.42±2.3	≤0.0001**
-All values are mean ±SE -Results with two strikes are significantly different at P≤0.01 -Results with one a strike are significantly different at P≤0.05			

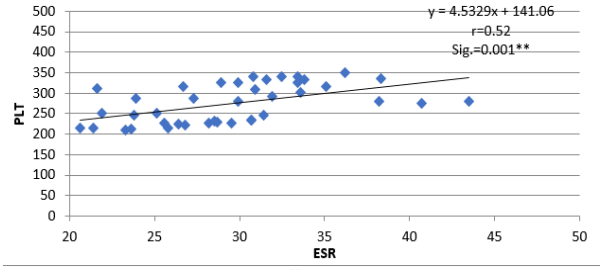

Figure (2): Correlation coefficient between ESR and PLT.

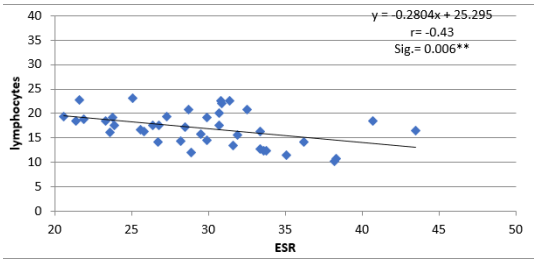

Figure (3): Correlation coefficient between ESR and lymphocytes.

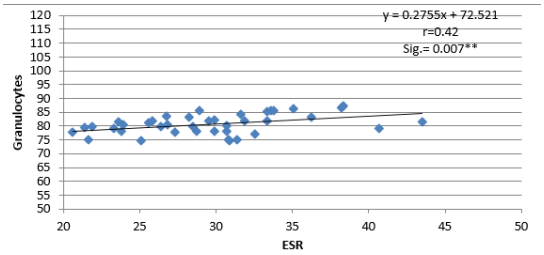

Figure (4): Correlation coefficient between ESR and granulocytes.

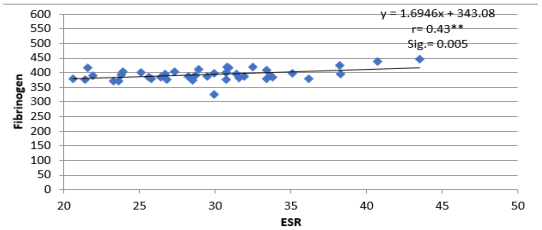

Figure (5): Correlation coefficient between ESR and fibrinogen.

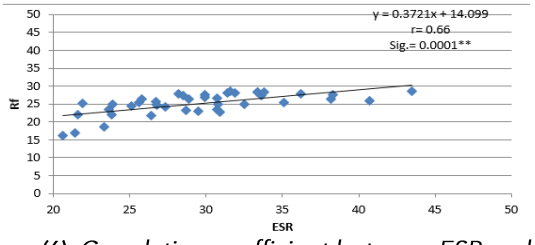

Figure (6): Correlation coefficient between ESR and RF.

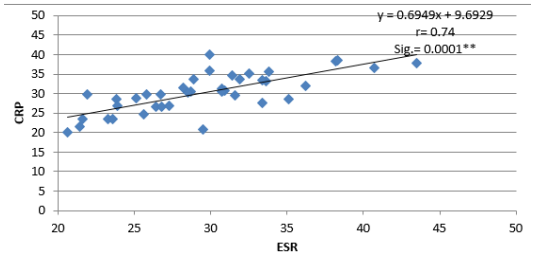

Figure (7): Correlation coefficient between ESR and CRP.

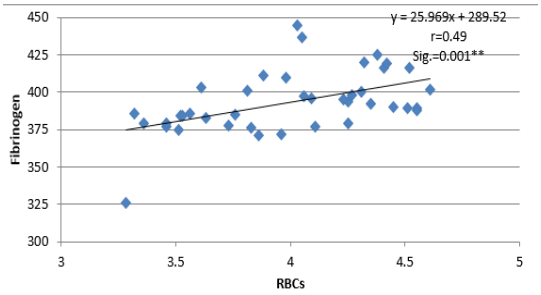

Figure (8): Correlation coefficient between RBCs and fibrinogen.

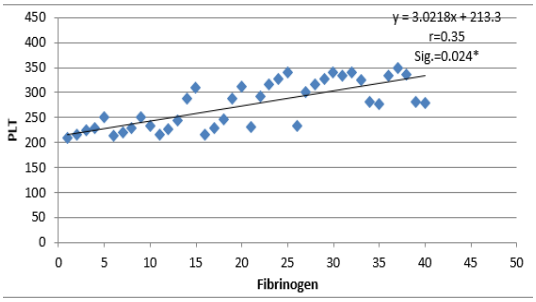

Figure (9): Correlation coefficient between Fibrinogen and PLT.

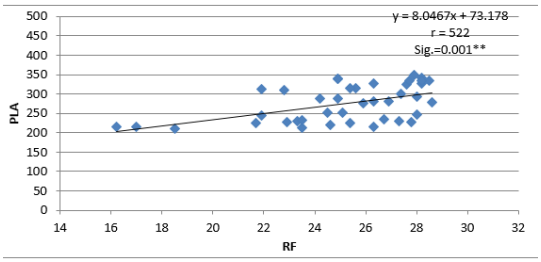

Figure (10): Correlation coefficient between RF and PLA.

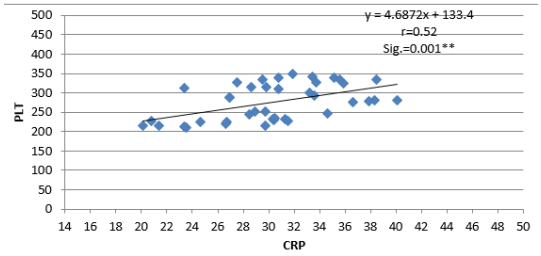

Figure (11): Correlation coefficient between CRP and PLT.

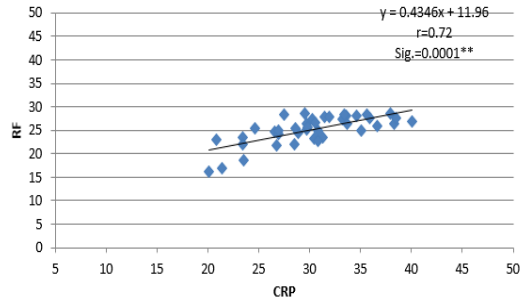

Figure (12): Correlation coefficient between CRP and Rf.

Table (9): the best cut off, sensitivity and specificity for prediction of the disease activity by parameters.						
Parameter	Sensitivity	Specificity	AUC	Cut off	95% confidence	p-value
Fibrinogen	0.800	0.450	0.775	205.00	0.666-0.883	≤0.0001
RF	0.825	0.675	0.864	12.700	0.779-0.950	≤0.0001
CRP	0.800	0.475	0.728	7.850	0.612-0.844	≤0.0001

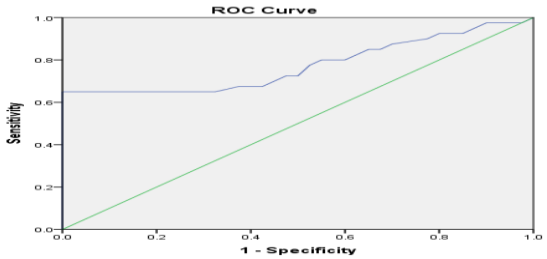

Figure (13): ROC curve for prediction of the disease activity by Fibro

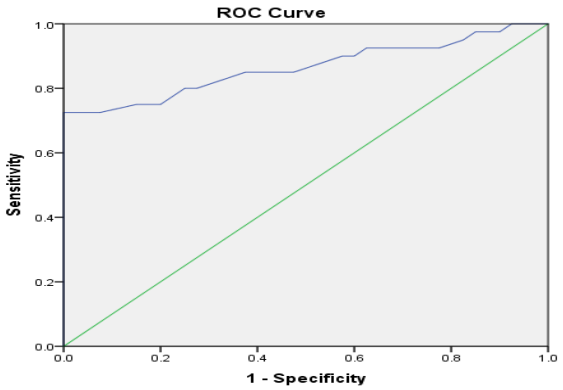

Figure (14): ROC curve for prediction of the disease activity by RF

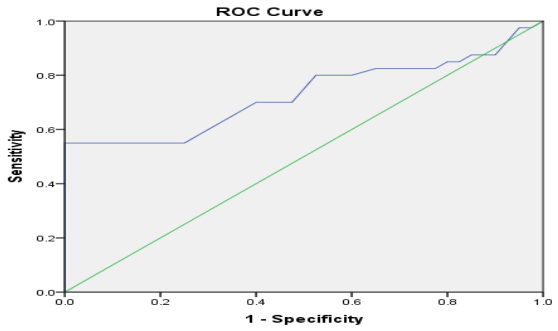

Figure (15): ROC curve for prediction of the disease activity by CRP.

4. Discussion

Without doubt , the old age is associated with different chronic diseases of both men and women, previous figure (1) established that RA affects human over 65 years old, and also the sex plays pivotal role in the incidence of this disease, the yield data confirm that patients men become improved more than women pf all age groups that treated with anti-inflammatory drugs, and there for the incidence and severity of RA is age and sex dependent [7]. It has

been found that women with RA worse step by step compared to men and have poor prognosis but the destructions of joins are similar to that of males [8].
Another recent study is designed to investigate whether there are differences in radiological characteristics of ageing individual with RA and compare with young individual, the study determined inflammatory mediators such as CRP, Ferritin, and Fibrinogen develop systemic inflammatory process than of elderly individual [9].
The previous findings are consistent with the present table, especially, the epidemiological studies explained there is a positive relationship and risk of RA incidence, and this relevant is stronger in men and women who have high body mass index [10].
Abnormal accumulation of adipose tissue in people who have heavy weights is associated with activation of inflammatory and autoimmune processes.
Excess adipose tissue are source in secretion of pro-inflammatory and inflammatory mediators and chemokines [11, 12].
It is not surprising that obesity is physiological problem when become over 30 kg/m3, it is associated with several chronic disease such as autoimmune, diabetes mellitus, and arthritis.
Adipocytes is adipose biomarkers that secreted from adipocytes and act in different part of the body, The levels of these markers is corrected positively with BMI and they are implicated with incidence and development of RA [13, 14].
The results of tables for the gender(3) ,age groups (5), and BMI (7) indicated an increase significant in value of ESR, while the results correlations between ESR and platelets, ESR and lymphocytes , ESR and granulocytes , ESR and fibrinogen , ESR and RF , ESR and CRP (r=0.52 , sig =0.001) , (0.43 , sig =0.006) , (r=0.42 , sig =0.007) , (r=0.43 , sig=0.005) , (r=0.66, sig=0.0001) , (r=0.74 ,sig =0.0001) respectively.
In this study, age and sex were independently associated with the levels of both acute phase reactants in early RA, although the effects appeared to be strongest on the ESR. These results emphasize the need to take these external factors into account when interpreting disease activity in patients with early RA. Because the acute phase reactants tend to increase with age, independent of other core measures of disease activity, the disease activity of older-aged patients might be overestimated. Also, ESR values are more likely to be elevated in women than in men, whereas the opposite appears to be the case for CRP.
Although no significant interactions were found between age and sex, the gradually decreasing differences in ESR and CRP between male and female patients with age are consistent with findings from.
Furthermore, the finding that the ESR tends to be more elevated in women than in men is consistent with the results of previous studies [15].
However, inconclusive results have been reported on sex differences in CRP levels. Where [5] found CRP values to be higher in males than in females, other studies have reported the opposite [16, 17].
The results of tables for the gender (4), age groups (6), and BMI (8) indicated an increase significant in value of RF while the results correlations between RF and platelets, CRP and RF, ESR and RF(r=522, sig=0.001), (r=0.72, sig =0.0001), (r=0.66, sig=0.0001) respectively, and the result of the ROC / AUC =0.675.
It well documented that rheumatoid factor (RF) is not found only in patient complained from RA but it had been diagnosed in wide types of autoimmune diseases, It also found in minimum levels in young and elderly individual [18].
Women appear more susceptible for autoimmune diseases in particular RA, It is well found that incidence of RA is affected by sex differences, that is, women have more affected than men especially women that have high BMI [19].
Recent study indicated the role of sexual hormone in development of autoimmune diseases and pro-inflammatory cytokines, it is well established that estrogen evokes immune system in contrast to that of testosterone [20].
It have been demonstrated that platelets exerts pivotal roles in inflammatory process occurring within the body, since they responsible for regulation and activation of white blood cells by releasing various inflammatory cytokines [21] This appear through p-selection and other adhesion molecules located on platelets, these molecules involved in interactions occurring among platelets and leukocytes including neutrophils, monocytes, and T-cells [22].
Recent evidence indicate that platelets mediate inflammatory processes in different part of the body
It is documented that platelets number tend to be increased in RA patient with high activity of diseases compared to those moderate level of RA [23].
It is suggested that platelets act as a pathway to deliver high amount of inflammatory cytokines, growth factor, and chemokines to maintain and sustain autoimmune inflammatory [24].
The results of tables for the gender (4), age groups (6), and BMI (8) indicated an increase significant in value of CRP and the result of the ROC / AUC =0.675. While the results correlations between CRP and platelets, CRP and RF, ESR and CRP(r=0.52, sig =0.001), (r=522, sig =0.001), (r=0.74, sig =0.0001) respectively.
The data obtained from the present study were consistent with previous study indicated that CRP represent regulator of inflammatory conditions in those patients complained from RA and its levels is accompanied with incidence and comorbidities RA and its levels remain elevated in RA [25] .
In fact, the systemic inflammation of the body is resulted from pro-inflammatory pathway mediator, of these pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 [26].
There several inflammatory cytokines take part in most of joint inflammation, it is well understanding that increased levels of pro-inflammatory cytokines is associated with damage of joint because of

stimulation of proliferation of tissue within joint [27].
The results of tables for the gender(4) ,age groups (6), and BMI (8) indicated an increase significant in value of Fibrinogen and the result of the ROC / AUC =0.775, while the results correlations between ESR and fibrinogen , RBCs and fibrinogen (r=0.43 , sig=0.005) , (r=0.44 , sig=0.001) respectively.
Fibrinogen is clotting factor (FI) of coagulation cascade in addition, it has ability to form link between other mechanism such as thrombosis, proliferation, and inflammatory processes, therefore, it is well documented that levels of plasma fibrinogen are significantly higher in those patients complained from RA compared to those healthy subjects [4].
The present data of study can agree with previous study of [28] , the observations noted in this study involved that RA represents a risk factor in development of cardio vascular diseases because of systemic inflammatory processes, of cardiovascular events, the thrombotic incidence and elevated of fibrinogen levels as a marker of inflammation with other inflammatory markers such as CRP, IL-6, and TNF-α.
In fact and dependent on pathological mechanisms, it obviously demonstrated that coagulation components co-operate with immune system to perform several functions [29].
In returning to obesity, it is well fined that patients with RA and have high BMI they having inflammatory mediators at maximum peaks than those healthy and normal BMI [4].

5. Conclusions

The results obtained, from the parent work indicated increase incidence of arthritis with increase of BMI and the elevation of inflammatory mediators is associated with systemic inflammation because of RA.

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