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INVESTIGATION OF HUMORAL IMMUNITY, PHAGOCYTOSIS INDEX & SOME OF HEMATOLOGICAL PARAMETERS IN PATIENTS OF RHEUMATOID ARTHRITIS IN THI-QAR PROVINCE

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ABSTRACT

The current study was conducted at the laboratories of Biology Dept, Collage of education for pure science–Thi-Qar Univ, during the period from October 2014 to May 2015. The study aimed to evaluate immune status of Rheumatoid arthritis (RA) patients by measuring the levels of immunoglobulin's (IgM, IgG) by single redial immune diffusion (SRID) and the study included test phagocytic cells on phagocytosis (coefficient of phagocytosis) and measurement hematological parameters (hemoglobin, packed cell volume and count the total and differential white blood cells). The study included a total of 100 patients with RA (25 males) and (75 females) and there aged between (20-65) years. Levels were measured in serum immunoglobulins for 20 samples of patients with RA, also examined the levels of immunoglobulins in the serum of 10 samples from healthy people in the control group. The results of the statistical analysis showed high significant increase ($P \leq 0.001$) in the immunoglobulins in serum IgM, IgG in patients with RA compared with healthy control group. inecrease coefficient phagocytosis was significantly ($P \leq 0.001$) in all patients with RA compared to the group of control, either with respect to the hematological parameters have explained the results of the current study, the lack of significant differences($P \leq 0.05$) in the rates of the values of Hb and PCV in all patients RA, compared with the control group, also increased the rate of counting the total of WBCs and count differential cells neutrophils, monocyte, lymphocytes and platelets ($P \leq 0.001$) in patients with RA compared with healthy people in the control group.

KEYWORDS: RA, IgG, IgM, Phagocytosis, lymphocytes, WBCs count, PCV.

INTRODUCTION

Rheumatoid Arthritis (RA) RA is the most common systemic autoimmune disease in the world (Haro., et al 2011). It affects approximately 0.5-1% of the world population (Hambright, et al 2011), being more common in women than in men, and in ages between 40 and 60 years (Ebringer., et al 2010). There are skeletal remains from North America showing that the disease occurred at least 3000 years ago, and it was given its name by Alfred Baring Garrod in 1859 (Garrod,1859). Patients developing RA typically present with numerous swollen and painful small joints of hands and feet, fatigue and morning stiffness. If not treated, the disease proceeds to affect more joints, and more constitutional symptoms like weight loss and fever may develop. The joint inflammation often leads to erosions of bone and eventually joint deformities and loss of function. Involvement of other organs such as lungs, kidneys, skin, eyes, heart and blood vessels occurs in some patients. Patients with RA have an increased mortality compared to the general population, especially due to cardiovascular disease (Humphreys., et al 2014). However, rheumatoid arthritis is a heterogeneous disease. Some patients have a mild, self-limiting arthritis and others a rapidly progressing, highly inflammatory disease with extensive joint damage. RA primarily affect joints also affect other organs in 15_25%of individuals. Arthritis of joints involves inflammation of the synovial membrane. joints become swollen, tender and warm, and stiffness limits their movment. Most commonly involvedare the small jointsof the hand, feet and cervical spine. (Davidson, 2010). RA characterized by autoantibody production (rheumatoid factor (RF) and anti–citrullinated protein antibody [ACPA] and systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders (Mcinnes., et al, 2011)

Immunoglobulin's (Ig)

The mature B cells in the immune system produces different types of antibodies molecules (Rolink., et al 1999; Brekke and Sandlie, 2003). Ig antibodies consist of of four polypeptide chains (two identical heavy chains and two identical light chains) joined together by disulphide bonds. Each Ig recognizes a specific antigen unique to its target and is used the immune system to locate and destroy invading microorganisms (Takai, 2002). Serum immunoglobulin levels provide key information on the humoral immune status. (Buckley, 1986; Dispenzieri., et al 2001).

Immunoglobulin M (IgM)
It is a pentameric structure with molecular mass approximately 950 kDa that makes up about 8% of the antibody in the serum (Hayzer and Jaton, 1985; Klaus, **1998)**. It is the first isotype to be generated during a primary immune response and it is predominates in immune response to most antigens, IgM is larger than IgGa.

Immunoglobulin G (IgG)
IgG consider a major effector molecule of the humoral immune response in human, accounts for about 80% of the total immunoglobulins in plasma of healthy persons. The molecular mass of IgG is (150 kD), it is consist of of two light chains and two heavy chains (g). The four polypeptide chains are covalently held together by disulfide bonds. It's the major antibody in the blood. Human IgG consists of four subclasses: (IgG1, IgG2, IgG3, and IgG4). It express predominant activity during a secondary antibody response. IgG antibodies have a relatively high affinity and persist in the circulation for a long time (Abbas., et al 2007).

Phagocytosis
Phagocytosis define as process that involves digestion and deglutition microbes and other foreign molecules, the major phagocytic cells in the body are Neutrophils, Macrophages, Dendritic and Monocytes, cells (Doan., 2008). Phagocytosis is one of the most primordial of all effector mechanisms and probably has its evolutionary roots. (Davidson, 2010; Robbin et al., 2010).

MATERIALS AND METHODS

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This study was performed on (100) Iraqi patients with RA, who attended AL-Husain teaching hospital, AL-Shatra general hospital and AL-Rfai hospital inThi-Qar Province the period from beginning of October 2014 to the end of May 2015. This study included too (50) person apparently healthy individuals as a control group, who have no history or clinical evidence of RA or any other chronic disease, and no obvious abnormalities.

Blood Samples Collection
Blood samples were collected by venipuncture from 100 patients and 50 controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. Were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -10 C°. These sera (20 RA patients and 10 controls) were used for estimating the concentration of Immunoglobulin's (Ig G,Ig M).

METHODS

Determination of serum levels of IgM and IgG, by single radial immune diffusion (SRID) plate.
A-Principle
Kits of (IgG,IgM) provided by Cusabio company. The total serum level of (IgM,IgG) was determined by means of (SRID) assay which was developed by **(**Mancini., et al 1965) for quantitative determination of proteins in the serum. Test sample is added to a well in an agarose gel containing a monospecific antiserum. The sample diffuses radially through the gel and the substance being assayed forms a precipitation ring with the monospecific antiserum. Ring diameter is measured and the concentration is determined from the reference standard curve.

B-Procedure
Before starting the assay , the plates were opened and left for 5 minutes at room temperature (18-25° C), and then 5 ml of serum was dispensed into well in the plate. The plate was incubated in flat position at room temperature to 48 hours for (IgG) and to 72 hours for (IgM). The rnig diameter was measured by an ocular and the concentration was obtained the reference curve.

Hanks Balanced Salt Solution (HBSS)
Prepare this solution according to **(**Gupta, 2009) that contains calcium ions Ca + + and Mg Mg + + dissolving substances listed in the table (1) below in 1000 ml of distilled water and adjust the pH to (PH 7.2) and divided to the volumes are equal and then sterilized by autoclave then save in temperature (4 C) for use.

Table (1): Materials needed to prepare a balanced salt solution Hank.

Symbol	Wight / gram
NaCL	8.00
KCLl	0.40
CaCl2	0.14
MgSo4,2H2o	0.10
Na2HPo4,2H2o	0.06
KH2Po4	0.06
MgCl2,6H20	0.10
C6H12O6.H2o	1.00
Red Phenol	0.20
NaHco3	0.35

E. Coli Suspension
It was prepared for the purpose of studying the process of phagocytosis and as the following: Brought petri dish contain growth of E .coli, some colonies were taken by sterilizer loop and put them in plane tube contain 5 ml of normal saline to make suspension when the turbidity was happened the suspension was ready to use in the test phagocytosis.

Wright's stain
The testing equipment (kit) was used for Wright's stain which consist of fixative solution and Eosine Stain and solution of methylene blue which is produced by syrbio company form republic of Arabia Syria.

Phagocytosis Procedure
The procedure carried out according (Met-Calf., et al 1986) as follow: 0.025 ml of the collected blood was put

in plane tube, then added for it 0.05 ml from bacterial suspension, 0.025 ml of HBSS were added to the mixture and incubated at 37 C for 30 minutes. One drop of the mixture was placed on a slid and smeared, then left to dry, fixed by methyle alcohol (99%) for min and stained for 20 min with Wright stain. then, examined under oil immersion.

$$\text{Phagocytosis index} = \frac{\text{No. of phagocytic cells}}{\text{Total number of cells}} \times 100$$

Hematological assay

The hematological tests that included (differential WBCs counts) were done by using Genux Auto Hematology Analyzer in which the results read and printed automatically. Statistical analysis: The analysis of data were expressed as mean ± SD. The comparisons between each RA patients group with matched healthy control were performed with T-test by using computerized Minitab 14 program. P<0.01 was considered to be the least limit of significance, the statistical analysis were done by using Pentium-4 computer through the Statistical Package For Social Sciences (SPSS program version-20).

RESULTS

Hb, PCV, WBC total, Neutrophil, Monocytes, lymphocytes and Platelets count:

The results of the current study show in table (2) ,lake significant differences in hemoglobin concentration (Hb) and packed cell volume (PCV) percentage in RA patients compared with the healthy control group while the hemoglobin concentration were (12.53+1.87) g/dl and (12.78+0.84) g /dl for patients and healthy control group respectively. The PCV percentage was (37.79+2.0)% for patients and (37.21+0.82)% for healthy control group this result agree with, on other hand current study show 0high significant differences in WBCs count between patients and healthy control group, where the WBCs mean were (10.07+0.35) $X10^3$cell/ml for patients and (5.3+0.50) $X10^3$cell/ml for healthy control group respectively.

As well as current study show high significant differences in count between patients and healthy control group in each of Neutrophil, monocytes, lymphocytes and platelets count as describe in (table 2). The results of the current study showed high significant difference ($p \leq 0.001$) in the rate of phagocytosis, as it increased the rate of phagocytosis in RA patients to (**62.23±107**) compared to the healthy controls group, and that the rate of phagocytosis (26.38± 1.34), as the (table 2). The results of the current study show,high significant difference($p \leq 0.001$)in the concentration of immunoglobulins (IgM, IgG) in a patients group with RA compared a healthy control group, the concentration of IgG (1859.38)of the patients, while the healthy control (1074.93)and IgM (171.98) for patients and (126.235) for healthy control as in(table 3).

Table (2): some hematological parameters and phagocytosis of RA patients and healthy control

Parameter	Subject	No.	Mean±SD	T-value	Df	P-value
HB(g /dl)	Pateints	100	12.53+1.87	0.89	148	0.37
	Control	50	12.78+0.84			
PCV	Pateints	100	37.79 ± 2.0	0.88	148	0.39
	Control	50	37.21± 0.82			
WBC	Pateints	100	10.07 ± 0.35	66.9	148	0.000HS
	Control	50	5.3 ± 0.50			
Neutrophil	Patients	100	5.11 ± 0.59	15.5	148	0.000HS
	Control	50	2.58 ± 1.38			
Monocytes	Patients	100	4.91±1.66	6.04	148	0.000HS
	Control	50	3.36±1.03			
Lymphocytes	Patients	100	4.02±0.17	5.3	148	0.000HS
	Control	50	1.46±0.39			
Platelets	Patients	100	281.91+6.7	72.82	148	0.000HS
	Control	50	212.18±0.75			
Phagocytosis	Patients	100	62.23±11762.23+107	1.86	148	0.000HS
	Control	50	26.38±1.34 24.38± 1.34			

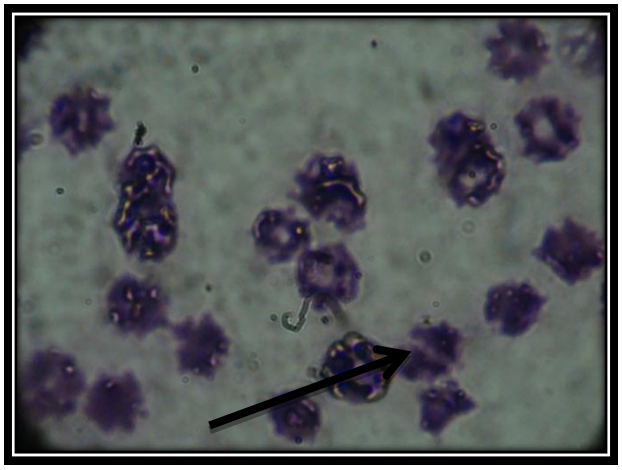

Figure (1): Phagocytosis of E.coli by Phagocytic cell 1000 x.

Table (3): concentration of IgM and IgG in serum Patients RA

Parameter	subjucts	Mean	N	P-value
IgM	Patients RA	170.985	20	0.000 H s
	control	126.235	10	
IgG	Patients RA	1790.38	20	0.000 H s
	control	1074.93	10	

DISCUSSION

The results of the current study showed a lack of significant difference (P > 0.05) of the concentration of hemoglobin between the two groups of patients (12.53±1.87) and healthy control group (12.78±0.84) this finding agree with (Graudal.,et al .2000; Rubin., et al 2005). Also this study show no of significant difference (P > 0.05) of the concentration of Pcv between the two groups of patients (37.79 ± 2.0) and healthy control group (37.21±0.82) this finding agree with (Zaid., et al 2011). On the other hand there was a significant variation($p<0.05$) in the WBCs count between RA patients and the control (10.07±0.35) $X10^3$cell/ml , (5.3±0.50) $X10^3$cell/ml respectively the increase in WBCs may be due to the response of the immune system to face the inflammatory effects in the tissues (Arvidson,2003) .Neutrophilalso show high significant between RA patients and the control (5.11±0.59) $X10^3$cell/ml, (2.58 ± 1.38) $X10^3$cell/ml respectivelythis increase may be due to the fact that this cells migrate and increase through the inflammation .Monocyt cells also show high significant between RA patients and the control (4.93±0.09) $X10^3$cell/ml, (4.09±0.05) $X10^3$cell/ml respectively. Lymphocyte too show high significant between RA patients and the control (27.66±0.37) $X10^3$cell/ml, (22.38±0.69) $X10^3$cell/ml respectively .Platelets too show high significant between RA patients and the control (281.91 ± 6.7) $X10^3$cell/ml, (212.18±0.75) $X10^3$cell/ml respectively,these results wich show high significant between RA patients and the control for monocytes, neurophils, lymphocytes because these cells are crucial for defense against invading organism, cellular debris and injury damage of collagen tissue. (Davidson 2010) this finding agree with (AL-Salih, 2014). Phagocytosis considered the first line of defense against injury and carried out by Neutrophil and Macrophage and Dendritic cells (Doan., et al 2008), which should migrate to the places of injuries or infections in order to do its job and be the migration process in response to some of the attraction of chemical (Marhoffer, 1992) and phagocytosis one of Non-specific immune mechanism and motivated by the entry of foreign bodies (Abid,.2009). The current results showed the significant difference ($P < 0.001$) as the percentage of phagocytosis in patients with RA (62.23±107) compared to a healthy control (26.38±1.341).

Showed the results of the current study, high significant difference ($p \leq 0.001$) in the concentration of immunoglobulins (IgM, IgG) in a patients group with RA compared a healthy control group, where the concentration of IgG (1859.38)of the patients, while the healthy control is (1074.93) and IgM (171.98) for patients and (126.235) for healthy control. It is important to point out that marked increase in IgG and IgM level is a common feature in collagen diseases like RA (Marina., et al 2001) The results of this study agree with (Al-salh., 2014)

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