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The Effect of Some Immunological Markers (IgM, IgG, CCP, IL1) Associated with RA in a Sample of Iraqi Patients

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Abstract. This work was performed on Iraqi patients with rheumatoid arthritis. It collected blood samples from 60 patients with RA whose ages range between 21-70 years. The study included more females than males, as the former was 55 by 91.7%, while the latter was 5 at 8.3%. To compare, 28 other blood samples were drawn from healthy control participants who range from 21-60 years, and the males was 5, 17.9%. The females were 23 by 82.1%, as the highest was in the ages 41-60 years and 51.7 and the age group (≤20) years (3.3%) was lowest as the disease among non-smokers(90.0%) is the largest in comparison to the rate among smokers (10.0%) with significant differences (P<0.001). The disease among married couples (80.0%) was statistically different (P<0.01) as the largest in reference to the unmarried and (20.0%). A significant rise (P<0.001) in Intreleukin- 1 alpha in patients 2.87 units/ml appeared while in healthy people 1.36 units/ml, and a significant rise "(P<0.001) in cyclic citrullinated peptides antibody in the blood of patients relative to the healthy". Here, the protein in the patients' blood was 406.62 mg/L and the 66.07 mg/L in the healthy. A high significant rise (P<0.001) was seen in immunoglobulin IgG in patients rising to 3.39 (ug/ml) and the healthy 0.72 (ug/ml). A significant rise happened (P<0.001) in the immunoglobulin IgM in patients reaching 8.75 (ug/ml).) compared to the healthy at 5.32 (ug/ml).

Highlights:

1. Demographics: RA affects mostly females (91.7%), non-smokers (90%), and married (80%).
2. Biomarkers: Significant increases in IL-1α, CCP antibody, IgG, and IgM in RA.
3. Controls: Healthy controls show lower biomarker levels compared to RA patients.

Keywords: Marker, IgM, IgG, CCP, IL1.

Introduction

RA is a chronic and common autoimmune disease, mainly influencing the joints. It causes injuries in joints (foot, knee, hip, spine, shoulders) and extraarticular injuries (rheumatoid nodes, eye injuries, vasculitis, pneumonia, muscle weakness and heart disease). It meaningfully influence morbidity [1]. As it is not easy to diagnose, the clinical

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and phenotypic aspect and stability and exacerbation are included and the American Society of Rheumatology has classified it [3]. RA attacking different cell kinds stimulating the immune activating the autoimmune responses and the exacerbated tissue infections [10]. RA reports insistent systemic, synovitis inflammation and autoantibodies (especially rheumatoid and citrulli peptide factors). Its risk is caused by the genetic, environmental factors including smoking, the key environmental risk, as well as microbial infections, bacterial infections including Salmonella bacteria, the age impact, obesity and gender on the disease. It is widespread in females and the elderly as it is uncontrolled damaging the joint, causing disability and deforming the body's joints hindering normal life, cardiovascular diseases and comorbidities. The innate immune system is disturbed partly because of producing autoantibodies like rheumatoid factor (RF) and antibodies to anti-citrulline proteins (ACPAs) having important impacts on the immune system, losing its ability of distinguishing between foreign bodies and body tissues [7].

RA is followed a disruption of the immune regulating the joint synovial membrane, severely damaging and destructing cartilage and bones. It causes many systemic symptoms, fever, osteoporosis, anemia, or muscle weakness and raises cardiovascular issues, pneumonia and cancers at the site of local inflammation, the joint, disturbances in the pathways of cellular motility and chemical attractants causing immune filtration contributing to the higher proliferation of arthroplasts, called synovial cells. It is followed by deficiency hematological disorders or in high number of white and red blood cells, platelets and anemia [2].

Methods

This work was performed on Iraqi patients who saw the consultant clinics for diseases at the joints at Baghdad Teaching Hospital of the Medical City. The patients come from Baghdad and all governorates of Iraq for the period between December/2020 February/2021. Specialized doctors diagnosed all 60 cases of rheumatoid arthritis. The study had 28 control healthy samples with no history or any autoimmune diseases. From both groups, 5 ml of venous blood were collected, taking 3 ml of it and placed in plastic gel tubes at room temperature (25 ° C) for 5-10 minutes to form the blood clot which central rejection separates (Centrifuge) at a speed of 4000 cycles / minute for 5 minutes drawing the serum on three parts in Eppendorf Tubes at 500 microliters per test tubes

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freezing at a temperature (38-) m until serological tests. Immunological tests early detect RA for the determination of the severity of injury in those with periodic treatment with (educational) laboratories in Baghdad Medical City including:

1. Test the concentration of the level of Intreleukin- 1 alpha gene -1 IL-1 α

   The IL-1 α IL-1 concentration test using enzyme-linked immunosorption technique is a quantitative method for quantifying and quantifying α IL-1 interleukin by the ELISA sandwich tests in 60 arthritis people.
2. Cyclic citrullinated peptides (CCP) antibody concentration test

   Test Principle

   High purity CCPs are bound to drilling. The determination is according to an indirect enzyme-bound immune reaction, where the antibodies identified in the patient's sample associated with the antigen coated on the surface of the reaction pits. Upon incubation, the washing step is to remove untied and not particularly attached serum components. After that a conjugate enzyme was added that binds to antibody complexes - antigen - frozen. After incubation, the second washing step removes the unbound conjugate enzyme. Upon the addition of the substrate solution, the enzyme associated with the hydrolysis of the substrate that forms a blue product. The addition of acid stops the reaction producing a yellow end thing. The intensity of yellow is related to the antibody-antigen concentration compound and is photomechanized at 450 nm. This test was carried out according to the method prescribed by Demeditec German company and used both materials and solutions prepared by the company.
3. IgG antibody test

   The Enzyme-Related Immunosorption IgG antibody concentration test is a quantitative method of quantifying, quantifying and quantifying IgG antibody antibodies by the ELISA sandwich test for 60 RA and 28 healthy people.
4. IGM antibody test

   "The IgM antibody concentration test with enzyme-linked immunosorption technique is a quantitative method for quantitative measurement and identification of IgM antibody antibodies by the ELISA sandwich test for 60 RA and 28 healthy people., as instructed in the screening group"..

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Result and Discussion

The study sample was 60 RA patients compared with the outwardly 28healthy controls which was a total of people divided based on percentages for sex, ages, marital status, smoking, immune indicators:

1. Distribution of study totals according to percentages for sex:

   This work examined 60 patients with RA at ages 21-70 years, the males were 5 by 8.3% and the females were 55 by 91.7%. The second were 28 the healthy (21-60 years) and the males were 5 by 17.9% with 23 females by 82.1%, as in Table (1).

   More females were infected with the disease than males at statistically significant differences ($P<0.001$) confirming the disease's greater size in females than males because the females suffer the disease due to hormonal discrepancies along with genetic. In addition, environmental factors complexly boost immunity which the autoimmune disease proves and the fluctuations connected to the disease with the changes of hormonal in puberty, hormone replacement therapy (HRT), pregnancy, and menopause and males and females have a different way of coping with with their chronic diseases [5].

2. Distribution of study totals by age groups

   Table 1 shows the rates of disease of the joints of the study groups by age groups and the highest was during 41-60 years and 51.7% for the study patients with significant differences ($P<0.001$). The lowest was for the ages of (≤20) year by (3.3%) between the groups.

   So, this work confirms Littlejohn and Monrad (2018) on those with RA, confirming that the disease rises at the age (55-64) in 65-75 year females. So, this article proves Innala et al. (2014) showing that it is possible to develope the disease at any age.

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Table (1): Comparing criteria among those with rheumatoid arthritis.

			Groups		Total	Statistics
			patients (N=60)	healthy (N=28)		
Gender	Female	N	55	23	78	p>0.05
		%	91.7%	82.1%	88.6%	0.89 (0.44-1.28)
	Male	N	5	5	10	p>0.05
		%	8.3%	17.9%	11.4%	2.21 (1.29-5.22)
P value			P<0.001***	P<0.001***	P<0.001***	
Age groups (years)	≤20	N	2	0	2	p>0.05
		%	3.3%	0.0%	2.3%	0.42 (0.12-1.11)
	21-40	N	18	6	24	p>0.05
		%	30.0%	21.4%	27.3%	0.71 (0.23-1.43)
	41-60	N	31	22	53	p>0.05
		%	51.7%	78.6%	60.2%	1.52 (0.89-2.81)
	>60	N	9	0	9	**P<0.05***
		%	15.0%	0.0%	10.2%	**0.11 (0.09-0.81)**
P value			P<0.001***	P<0.01**	P<0.01**	

3. Smoking

RA in the sample of smokers and non-smokers is studied. According to table (2), (P<0.001) among non-smokers were significantly different and the largest than the 60 smoker with the disease. The former were 54 patients (90.0%) with 6 smoker patients (10.0%). Also, non-smokers are susceptible than the smokers contradicting Vittecoq et al. (2018) showing that smoking is a risk to avoid rheumatoid arthritis. According Söderlin et al., (2013), no effect of secondhand smoke was found and use Pre-tobacco on RA activity confirmed our results.

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4. Marital status

This work studied rheumatoid joints in married and unmarried couples in the Iraqi sample of patients. Table (2) shows a statistically significant difference (P<0.01) with the disease in the married larger than in the unmarried in the 60 patients. 48 married patients accounted for (80.0%) and the 12 unmarried (20.0%). Also, the married develop the disease more than the unmarried, maybe because female or male hormonal disorders as risk factors (Risk Factor) to get sick. Jennifer et al., (2010) stated that the the married suffer more from the disease because RA strains the marital relationship because of more limitations on activities, responsibilities, and emotional anxiety. According to Ward and Leigh, (1993), unmarried patients with Inflammation Rheumatoid joints have a greater progress of disease and greater disability than their married counterparts, contradicting our study.

Table (2): Comparison of some studied criteria in RA patients.

			Groups		Total	Statistics
			patients (N=60)	healthy (N=28)		
Smoking	No	N	54	23	77	p>0.05
		%	90.0%	82.1%	87.5%	0.91 (0.44-2.01)
	Yes	N	6	5	11	p>0.05
		%	10.0%	17.9%	12.5%	1.78 (1.23-3.29)
	P value		P<0.001***	P<0.001***	P<0.001***	
Social status	Married	N	48	20	68	p>0.05
		%	80.0%	71.4%	77.3%	0.89 (0.21-1.92)
	Unmarrid	N	12	8	20	p>0.05
		%	20.0%	28.6%	22.7%	1.42 (1.21-4.24)
	P value		P<0.001***	P<0.001***	P<0.001***	

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Intreleukin - 1 alpha concentration

Table (3) shows significant rise (P<0.001) in Intreleukin- 1 alpha in the blood serum of the patients than in the serum of the healthy. These antibodies in the serum of the healthy was 1.36 units/ml and to 2.87 units/ml in those with the disease.

So Ruscitti et al., (2018) is confirmed indicating a significant increase in leukopenia 1 in blood serum of patients. Also, there is a positive correlation of level of these antibodies increases with the severity of the disease secreting cellular kinetics, and in Tiao et al's (2016) pro-inflammatory signs with cellular kinetics, including leukopenia -1, 6, 7 and 8 and tumor necrosis factor (TNF-α) risen values in patients. Selmi et al. (2014) studied RA people patients suffering worsening symptoms of RA with a rise in cytokinetics secreted as albino albino 1. The high increase cellular kinetics causes secondary complications including cardiovascular disease, causing of death in the patients confirming the need for inhibiting it with leukocytosis 1treatment increasing stress because of nitrogen oxidation.

Anti cyclic citrullinated peptides) antibody 1

Table (3) reports statistically significant increases (P<0.001) in the protein of cyclic citrullinated peptides antibody in the patients' blood than the healthy. The protein in the patients accounted for 406.62 mg/L than 66.07 mg/L in the healthy.

These findings prove Cheng et al. (2021) examining patients with RA with oral microbial infections with more protein levels than healthy control samples confirming [8] indicating significant increases in the anticyclic citrullinated peptides antibody protein. The RA patients with microbial infections showed a severe disease and bacteria in the mouth due to the high anticyclic citrullinated peptides antibody protein and aggravating the disease. On the other hand, According to Schwenzer et al. (2017), patients with RA show higher level of peptide anti-citrulline antibodies as good RA biomarkers commonly used in diagnosing the disease because of problems of immunity with uncertain triggers of how they are produced. Akiyama and Kaneko (2022) studied arthritis patients of patients exacerbating the disease to the external part of the joint affecting the lung as meniscus pneumonia increasing the cyclic citrullinated peptides antibody confirming our work.

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Table 3: IL-1 alpha and anti-CCP antibody measurement rates among study groups than the healthy

Groups		N	Mean	SD	P value
IL-1 alpha (ng/l)	patients	60	2.87	0.96	P<0.001***
	healthy	28	1.36	0.49	
Anti-CCP antibody (U/ml)	patients	60	406.62	178.28	P<0.001***
	healthy	28	66.07	12.06	

IgG antibodies

Table (8) shows Statistically significant increase (P<0.001) in immunoglobulin IgG rates in the serum for patient samples Arthritis Rheumatoid reaching 3.39 (ug/ml) than the healthy controls 0.72 (ug/ml).

These results were identical to his findings Lin and Li, 2010 in the study Conducted on 72 patients with RA and divided on two categories. The first are high disease and the other disease activities lower and high immunoglobulin found IgG in their blood serum and for both groups [14]. Patients with Arthritis Rheumatoid and injured Necrosis of the femoral head reported an elevation in immunoglobulin and IgG was a strong indicator of disease activity, immunoglobulins and antibodies were identified to Globulin Immunoglobulin (autoimmune globulin) as a major factor in causing Arthritis Rheumatoid. It can be specific autoimmune globulins called rheumatoid factors (RFs) and are prevalent in patients Inflammation Rheumatoid joints long before the outbreak and may also reflect the progression of the disease and is often used as diagnostic tools.

4- IgM antibodies

Table (8) reports statistically significant rises (P<0.001) in immunoglobulin IgM in the RA patients' serum of at 8.75 (ug/ml) in reference the healthy control sample- 5.32 (ug/ml).

Nicolò et al. (2022) showed a rise in most patients with a high profile of IgM antibodies explicitly linked to IgG. These self-propelled antibodies are referred to as RF

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suggesting that IgG-aware active IgM antibodies are important in the regulation of IgG equilibrium as an imbalance between IgG-mediated IgG degradation and stability influencing the autoimmune disease onset and development. So, restoring this balance with a low-affinity IgG antibody is an active therapeutic method for autoimmune diseases including IgG. Self-active.

Table 4: Measurement of IgG and IgM Immunoglobulin Rates among Study Groups of Iraqi Patients with RA than the healthy

Groups		N	Mean	SD	P value
IgG (ug/ml)	patients	60	3.39	1.21	P<0.001***
	healthy	28	0.72	0.21	
IgM (ug/ml)	patients	60	8.75	1.87	P<0.001***
	healthy	28	5.32	1.31	

Conclusion

The study has come up with the following conclusions:

1. The disease incidence is higher in women than in men.
2. The 41-60 year patients with RA were the highest.
3. Married people are more vulnerable the diseases than the unmarried.
4. Non-smokers are more vulnerable the diseases than the smokers.
5. A high level of Intreleukin- 1 alpha in the serum of the patients than that of the blood serum of a healthy controls, and thus these indicators are immunobiochemical to diagnose the disease.
6. Increase inCCP, IgG and IgM in serum of the patients than the healthy controls. Thus, these indicators are biochemical immunomodulators to diagnose the disease.

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