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Relationship of Rheumatoid Arthritis with Microbial Pathogens (TORCH Test), Case-control Study in Iraq

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects the joints and many factors are involved in the emergence and progression of the disease, including genetics and environmental factors, among the latter factors are the infectious agents, which have not been proven accurately to date, which necessitated this study to identify the role of some different pathogens in the occurrence of the disease. Seventy-five serum samples (62 females, 13 males) were taken for patients suffering from rheumatoid arthritis, 30 of whom were receiving methotrexate (MTX) therapy, in addition to some auxiliary medications, and 45 others who had not received any treatment except pain relievers, from Al-Sadr Teaching Hospital in Najaf Governorate / Iraq. The ages of patients ranged 26 -73 years, in addition to 23 healthy people (15 females, 13 males) within the control group, collected from separate areas of the same governorate and the ages ranged 32-64 years. Serological investigation of IgM and IgG antibody was performed using TORCH Panel Rapid Test which includes Toxoplasma gondii, Rubella virus (RV) Cytomegalovirus (CMV), Herpes simplex virus 1 (HSV-1), and Herpes simplex virus 2 (HSV-2). The results showed that there was significant increase in the prevalence of IgG antibody for both RV and CMV in rheumatoid arthritis patients, and the proportion was 70.6% and 48%, respectively, compared to the healthy group which was 34.7% and 0%, respectively. The prevalence rate of IgM seropositive antibody was 0% for all above pathogens except for one case for HSV-1 in RA patients. Statistical analysis of Chi-Square also showed significant differences in the prevalence of IgG antibody for only both the RV and CMV between the three study groups (the group of patients receiving MTX treatment and the group of patients without treatment and the control group). The IgG positive antibody ratio for both Rv and CMV was higher for females than males, while no significant differences were seen in other pathogens. As a final outcome of our study, it found a positive relationship between presence of serum IgG, which represents chronic infection of rubella, and Cytomegalovirus with rheumatoid arthritis, which proves the role of infection factors in the emergence of autoimmune diseases.

Keywords: Rheumatoid Arthritis, Torch Test, Microbial Pathogens, Cytomegalovirus, Methotrexate.

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INTRODUCTION

Rheumatoid arthritis is one of 100 types of arthritis that infect human, it is characterized by synovial lining inflammation of the joints and erosion in the ends of the bones, which impedes movement and reduces the efficiency and quality of a patient's life (1). It is an autoimmune disease caused by a mixture of the causative factors, as genetic factors which constitute a high incidence of disease, and environmental factors, which include microbial infections, exposure to silica dust, smoking, food type, and economic and social status, or may be caused by the interaction of genetic and the environment factors (2). It is clear that infection with microbials leads to the development of rheumatoid arthritis, also RA patients are more at risk of infection than other healthy people (3). Experimental animal studies provide clear proof of the causal association between infection and rheumatoid arthritis. In human, it has been shown that *Porphyromonas gingivalis* infection is associated with RA patients (4, 5). Other local and global studies have found a significant relationship between *Toxoplasma gondii* infection and rheumatoid arthritis (6-8). In a retrospective study conducted by Widdifield *et al*.(9) on a number of people with rheumatoid arthritis which receiving corticosteroid treatment, it was found that those with a high risk of infection with the herpes zoster virus. Also, a significant relationship was recorded for increased cases of IgG antibody to cytomegalovirus in RA patients and it is believed that this virus has a role in triggering and stimulating RA factors (10). Early studies shed light on the impact of rubella virus infection on the emergence of rheumatoid arthritis, as well as it is demonstrated the relationship of rubella vaccine with this disease (11-13).

There are two opinions, or there are two possibilities that explain the relationship of increased infection with microbes or parasites in patients with rheumatoid arthritis. The first is that infection with the pathogen may provoke inflammatory factors leading to arthritis, or perhaps the second possibility is that the immune imbalance in patients gave an opportunity to the pathogen to enhance its presence in the host with the disease (8). Sultan et al. (14) identified a significant correlation between the use of antibiotics and the development of rheumatoid arthritis. They attributed this to the effect of antibiotics on the microbial group that naturally present in the human body, or to the induction of the disease that emerged as a result of the microbes themselves that the antibiotics were used against it, or the two causes are compatible.

Methotrexate is a drug has been used since the early eighties of the last century in the treatment of RA and now it is the first line of treatment for this disease, this drug has several mechanisms supposed to reduce rheumatoid arthritis, the adenosine signaling mechanism is maybe the best broadly accepted elucidation for the methotrexate mechanism against RA (15).

In a retrospective population study on rheumatoid arthritis patients from 1999-2006 included 1993 patients who were found to be more at risk of infection with hospitalized infections compared to control, as well as this risk is more in people receiving oral corticosteroids and depending on the dose, while people receiving treatment with methotrexate

and hydroxychloroquine have a lower risk of hospitalized infections (16). Ibrahim *et al*. (17) conducted a meta-analysis study on rheumatoid arthritis patients showing that there was no risk of developing inflammatory diseases when patients received MTX treatment. Favalli *et al*. (18) concluded through their study that patients with rheumatoid arthritis are more susceptible to viral infections than others, including COVID-19 infection as a possible result of immune impairment generated in patients, but this risk increases when patients stop treatment with anti-arthritis drugs, so they recommend patients to continue treatment despite the risk of spreading corona virus 2019.

MATERIALS AND METHODS

The study design consisted of collecting 3 ml blood samples from 75 patients (62 women and 13 men) their ages ranged 26 -73 years with pre-diagnosed rheumatoid arthritis, also this group was divided into two sub-groups, namely, the 30 patients are receiving a Methotrexate drug, and other the 45 patients no receiving any treatment, except for pain relievers. The control group included 23 healthy people (15 women and 8 men) their ages ranged 32-64 years, the period of samples collection were between October 2019 and December 2019. The patients and healthy people were from Al-Sadr Teaching Hospital in the Al- Najaf province located 160 km south of the capital, Baghdad, Iraq. The control group was chosen from those who did not have any complaints and illness collected from separate areas of Al-Najaf province. Pre-approval was obtained from patients and controls. The study was approved by the Ethical Committee at the University of Babylon. A diagnostic kit (TORCH Panel Rapid Test) for CTKBIOTECH Company, USA was used. The principle of kit work depends on chromatographic and through it, IgG and IgM antibody are determined for the pathogens *Toxoplasma gondii*, Rubella virus (RV) Cytomegalovirus (CMV), Herpes simplex virus 1 (HSV-1) , and Herpes simplex virus 2 (HSV-2). The work steps were performed as described in the kit manual. Some supporting information for the study was obtained from patients and specialist physicians. The results values were expressed as the mean ± standard deviation (M±SD), and the Chi-square (X^2) statistical analysis was used by Microsoft Excel, values were significant when P was less than 0.05.

RESULTS

The current study included taking blood samples from 98 people, including 75 samples for patients with rheumatoid arthritis (13 male and 62 female), ages ranging from 26-73 years, of them 30 patients took a main drug for treating rheumatoid arthritis, methotrexate with other support drugs, while 45 of them did not take any medication other than analgesics, and 23 samples (8 male and 15 female) ages ranging from 32-64 years for healthy people as a control group.

The serological investigation of the microbial etiology that related to the TORCH test in patients with rheumatoid arthritis was conducted, the statistical analysis showed a significant increase ($P \leq 0.05$) in the rates of chronic infection (IgG) for both CMV and Rubella in the patient's group compared to the control group. While other pathogens (*Toxoplasma*, HSV-1 and HSV-2) did not show significant differences ($P \geq 0.05$) between the two groups, the positive incidence of IgM antibody for all pathogens was zero except for one case for HSV-1 in RA group Table (1).

Table 1: Microbial infections of TORCH test in rheumatoid arthritis patients and healthy people

Pathogens (TORCH)		RA (n=75)		Control (n=23)		P value Chi-square (X^2)	
		Positive %(n)	negative %(n)	Positive %(n)	negative %(n)		
Toxoplasma	IgM	0 (0)	100 (75)	0(0)	100 (23)	-	-
	IgG	33.3 (25)	66.6 (50)	43.4(10)	56.5(13)	0.374	NS
Rubella	IgM	0 (0)	100 (75)	0 (0)	100 (23)	-	-
	IgG	70.6 (53)	29.3 (22)	34.7 (8)	65.2(15)	0.0019	S
CMV	IgM	0 (0)	100 (75)	0 (0)	100 (23)	-	-
	IgG	48 (36)	52 (39)	0 (0)	100 (23)	0.000029	S
HSV-1	IgM	1.3 (1)	98.6 (74)	0 (0)	100 (23)	0.57	NS
	IgG	96 (72)	4 (3)	95.6(22)	4.3 (1)	0.94	NS
HSV-2	IgM	0 (0)	100 (75)	0 (0)	100(23)	-	-
	IgG	1.3 (1)	98.6 (74)	0 (0)	100(23)	0.57	NS

S = significant, NS = Non-significant

When comparing infection rates (IgG and IgM positive antibodies) with microbial causes (TORCH Test) in groups of patients with rheumatoid arthritis those are taking methotrexate treatment and those do not use this drug in addition to the control group, it was found that the significant increase in the rates of chronic infection (IgG) for both CMV and Rubella in patients groups compared to control, There was a significant decrease in CMV chronic infection in the group of patients taking the drug than the group of patients who did not take treatment, and the opposite Rubella chronic infection (seropositive IgG) have a significant decrease in the patient without drug group compared to the patient with drug group, and other pathogens did not appear Significant differences between the three study groups, Table (2).

Table 2: Distribution of IgG and IgM antibodies for microbial infection (TORCH) depending on receiving Methotrexate in patients with RA

Pathogens (TORCH)		RA with MTX 30		RA without MTX 45		Control 23		P value Chi-square (X^2)
		Positive %(n)	Negative %(n)	Positive %(n)	Negative %(n)	Positive %(n)	Negative %(n)	
Toxoplasma	IgM	0(0)	100(30)	0(0)	100(45)	0(0)	100(23)	-
	IgG	33.3 (10)	66.6(20)	33.3 (15)	66.6(30)	43.4 (10)	56.5(13)	0.64
Rubella	IgM	0(0)	100(30)	0(0)	100(45)	0(0)	100(23)	-
	IgG	80 (24)	20 (6)	64.4 (29)	35.6(16)	34.7 (8)	65.2(15)	0.0031*
CMV	IgM	0(0)	100(30)	0(0)	100(45)	0(0)	100(23)	-
	IgG	30 (9)	70 (21)	60 (27)	40 (18)	0(0)	100(23)	4.9 E-06*
HSV-1	IgM	3.3 (1)	96.6(29)	0(0)	100(45)	0(0)	100(23)	0.31
	IgG	90 (27)	10(3)	100(45)	0(0)	95.6 (22)	4.3(1)	0.10
HSV-2	IgM	0(0)	100(30)	0(0)	100(45)	0(0)	100(23)	-
	IgG	3.3 (1)	96.6(29)	0(0)	100(45)	0(0)	100(23)	0.31

* Significant, P <0.05

Table (3) indicate to the role of the gender factor on the acquisition of infection with microbial pathogens under study with the TORCH test through chronic infection ratios, statistical analysis using the Chi-square test appears that females are more susceptible to infection than males in both RV and CMV (P≤0.05), but the statistical analysis did not prove the presence of differences Significant in other pathogens.

Table 3: Relationship of pathogens seropositive IgG antibody ratio and gender in patients with rheumatoid arthritis

Pathogens / Gender	Toxoplasma IgG Positive %(n)	Rubella IgG Positive %(n)	CMV IgG Positive %(n)	HSV-1 IgG Positive %(n)	HSV-2 IgG Positive %(n)
Male (n=13)	15.3 (2)	38.5 (5)	23.1 (3)	92.3 (12)	0 (0)
Female (n=62)	37.1 (23)	77.4 (48)	53.2 (33)	96.7 (60)	1.6 (1)
P value Chi-square (X^2)	0.13	0.00503*	0.048*	0.455	0.64

* Significant, P <0.05

DISCUSSION

The main causes responsible for rheumatoid arthritis are not specifically identified, but in general the causes are divided into two main factors, the genetic factor and the environmental factor, Environmental factors include biological stimuli, such as viral infections and hormonal changes, as well as chemical and physical environmental factors (19). The relationship between infectious agents and rheumatoid arthritis is complicated and may follow two paths. As for the pathogen settling directly in the joint area and urging the occurrence of the arthritis disease or by urging the immune system to generate an anomalous state of the immune response as a reaction to the infection (17).

We examined the relationship of the biological aspect (microbial infections) with rheumatoid arthritis using the torch test, which includes five different pathogens are parasitic (*Toxoplasma gondii* IgG and IgM) and viral (Rubella, Cytomegalovirus and Herpes1 and Herpes2 IgG and IgM). The results showed a significant increase in chronic infection rates (IgG) for CMV and rubella in patients with rheumatoid arthritis compared to healthy controls. Whereas, there was no significant difference with other pathogens (*Toxoplasma*, HSV-1 and HSV-2) between the patient and healthy groups. Perhaps the reason for the high rate of infection with some pathogens such as rubella and CMV in patients compared to healthy people is due to the deterioration of the immune system in patients with rheumatoid arthritis due to disability and inhibition of the patient's immunity as a result of taking anti-disease drugs. Doran et al. (3) found that people with RA were more likely to have infections than the control group.

The study of Bassyouni et al. (20) supported the results of the current study, they determined serum DNA by RT-PCR technique for both Cytomegalovirus and Epstein-Barr virus in 68% and 40%, respectively, and the common incidence of both viruses was 28% for 50 patients with RA in Egypt, while the presence of DAN of these two viruses was 0% in Healthy people who numbered 32. This study recorded a significant increase in the presence of IgG antibody against rubella virus in RA patients compared to the control group, whereas IgM antibody was not recorded in both groups, several studies support the role of rubella virus in inducing arthritis disease (11-13). Other studies do not converge with the current result, the study of Secmeer and Kanra (21) did not obtain a direct relationship between rubella virus and rheumatoid arthritis when identified of the antibody of virus in synovial and serum fluid for patients and control. Bosma *et al*. (22) did not find a significant relationship between infection with RV and various chronic inflammatory joint diseases, but they confirmed that the RV is present in the synovial fluid of the joints and can be activate when suppressing immunity, after they took samples of synovial fluid for patients with various conditions of joint problems and investigating the RV using RT-PCR.

One of the strategies or mechanisms by which autoimmune diseases are generated is the great similarity between the determinants or viral sites and the sites of the host's cells. When the immune system is provoke by infection with these viruses, this immunity will later attack the human cells themselves and thus an autoimmune disease will arise (23).

Previous studies have given opposite results from the current study in the case of *Toxoplasma* infection in RA patients, Al kalaby et al. (6) found that there was a significant correlation between the incidence of chronic and acute infection with toxoplasmosis in patients with rheumatoid arthritis, and attributed this to the fact that infection with this parasite induces a change in the immune response to enhance autoimmune diseases. The study of Al-Oqaily & Al-Ubaidi (7) in Baghdad demonstrated that there was a significant correlation between chronic infections of *Toxoplasma* with rheumatoid arthritis patients, while statistical analysis did not record a significant correlation of acute parasite infection with the disease. Salman and Mohammed (24) recorded a significant relationship between *T. gondii* infection and polyarthritis in Kirkuk city, northern Iraq. In China, a study by Tian et al. (8) showed that the serum presence of IgG antibody to the parasite *Toxoplasma* has a close association with many different arthritis diseases. Rheumatoid arthritis is ranked first by 28.4% and other types of this disease came after it. In a Turkish study to investigate the prevalence of latent toxoplasmosis in rheumatoid arthritis patients, İnal and Tas (25) found that the prevalence of IgG antibody to the parasite was high in patients treated with biological therapy and then treated with DMARD (87.9% and 80.8%, respectively) while, the antibody prevalence rate in healthy people was 21.1%.

The study of the distribution of pathological infections based on whether or not MTX drug received, it was showed a significant differences between study groups of IgG antibodies for both Rubella and CMV only. The highest incidence 80% of Rubella was in the group of patients receiving treatment, while the highest incidence 60% of CMV in the group of patients without MTX treatment. These results may be explained by the selective effect of MTX on the various pathogens under study, as it may have a fatal effect or an obstacle to the proliferation of the virus in the case of CMV. On the other hand, it plays another role by increasing the incidence of rubella, perhaps through its effect on the immune system. One of the mechanisms that support the function of methotrexate in its anti-inflammatory action is by increasing the production of reactive oxygen species that activate the process of apoptosis of T cells (26). Tian *et al*.(8) strongly recommended to conduct a survey of the latent infection of *Toxoplasma* in rheumatoid arthritis patients before they are given the biological therapy (TNF-α inhibitor) used to treat rheumatoid arthritis.

The results of the influence of the gender factor on the rates of microbial infection indicate that females are more susceptible to infection with all pathogens, however the significant increase in RA females appeared only in the case of infection with rubella and cytomegalovirus. This coincidence came with an established fact that women are more susceptible to rheumatoid arthritis than men (27). This supports the role of infection in causing the disease, and this may be due to hormonal effects. The response to infection varies between males and females, depending on the type of pathogen. The female may be more sensitive to certain pathogens than males and vice versa, due to the difference of hormones according to gender and their effect on the immune system (28). Robinson et al. (29) confirmed estradiol's role in reducing the severity of symptoms with influenza A virus in female mice by regulating inflammatory factors but did not affect the reproduction of the virus. This study concluded that viral etiology (CMV and RV) has a great role in the possibility of developing rheumatoid arthritis.

CONFLICT OF INTEREST

None

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