Etiopathogenesis of CMV in Rheumatoid Arthritis Patients

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Abstract
Rheumatoid arthritis (RA) is one of the autoimmune diseases characterized by the synovial inflammation which causes organs and tissues damage especially synovial tissues and joints. The study included 50 serum samples from patients with rheumatoid arthritis (RA) when compared with 50 serum samples from healthy individuals as control with age range 35 – 60 years (41.3 ± 2.4 years vs. 41.0 ± 2.0 years, respectively). ELISA technique was used to assess the Anti-cyclic citrullinated peptide IgG antibody (anti-CCP IgG Ab) level, anti-rheumatoid factor IgG antibody (anti-RF IgG) and anti-Cytomegalovirus (anti-CMV IgG) antibodies frequencies in the studied groups.

The present findings demonstrated that all RA patients have 100% seropositive frequencies for each anti-CCP IgG and anti-RF IgG antibodies compared to 100% seronegative frequency in controls. There was a significantly increased frequency and level of anti-CMV IgG antibody in RA patients compared to control. The present findings suggested that CMV may one of the triggering factors for RA in cooperation with the incidence of anti-CCP and anti-RF antibodies.

Keywords: Cytomegalovirus, Rheumatoid arthritis, anti-CCP IgG antibody, anti-RF IgG antibody, infectious agents.

Introduction

Rheumatoid arthritis (RA) is one of systemic autoimmune inflammatory diseases which occurs in the synovial tissues characterized by polyarticular inflammation which causes progressive joint damage. It is associated with substantial functional disability, morbidity and accelerated mortality that led to huge societal burden[10].

The disease may also affect other organs of the body such as lungs, heart, eyes, liver and skin, nervous system and other parts. Also, its prevalence in women appears to be more common than men[21]. According to the American College of Rheumatology (ACR), RA is defined as the most common autoimmune inflammatory arthritis in adults[12]. It has a significant negative impact on the ability to achieve regular activities and it increases mortality rates[17]. The mortality rates are more twice in RA patients than the general population[11]. In addition, RA is considered as a multifactorial disease that arises from many causes as a result to the interactions between genetic and environmental factors, some personal and lifestyle factors also influenced the development and/or course of RA that included age and gender, socioeconomic factor, hormonal factors and ethnicity[19]. Genetic factors contribute about 50% to 60% of the developing RA risk[14]. The HLA-DRB-1gene in the major histocompatibility complex is a strongly associated gene in RA. DRB1*04 and *01 clusters encode the "shared-epitope" sequences[8].

In contrast, the environmental factors play a critical role in the etiopathogenesis and triggering RA. Cytomegalovirus (CMV) is member of the Herpesviridae family of viruses which includes herpes simplex virus type 1 and type 2, Varicella Zoster Virus, Epstein–Barr virus, one of these factors that triggers RA through driving the development of large expansions of CD8+ CMV-specific T cells, which increased further during ageing. Also, CMV infection is associated with vascular disease and increased risk of mortality in older people which may be related to damage from this CMV-specific immune response[22]. In addition, Human CMV is a prevalent herpesvirus that is related to autoimmunity, especially in genetically predisposed persons[9].

The infection with CMV is generally asymptomatic in immunocompetent people, although clinical symptoms of primary infection can include a glandular fever (mononucleosis) syndrome characterized by flu-like symptoms, or occasionally persistent fever[1,2]. Laboratory tests may show elevated lymphocyte counts (lymphocytosis) and/or elevated liver transaminase levels[15]. The current study aimed to evaluate the anti-CMV IgG antibody in rheumatoid arthritis patients in addition to the estimation of anti-CCP antibody and anti-rheumatoid factor IgG antibody in the studied groups.

Material and Methods

Samples collection: The current study included 50 RA female patients with age ranging from 35 to 60 years in Iraq enrolled to the Rheumatology Consultant Clinic at Rheumatology Department, Baghdad Teaching Hospital, Ministry of Health, Baghdad, Iraq during the period January – May 2018 for diagnosis and treatment, which they compared with healthy persons as control group that matched the patients' group in the number, age and sex. The patients were diagnosed by the rheumatologists according to the 2010 American College of Rheumatology/ European League Against Rheumatology for RA[3]. The study was permitted by the local ethics committee of the Health Ministry, Baghdad Teaching Hospital in addition to the

informed permission that was obtained from the study volunteers. Five milliliters of venous blood were dropped from all the studied groups, the dropped blood samples were left to clot at room temperature (25^0C), then centrifuged at 5000 rpm for 5 min to collect the serum in a sterilized Eppendorf tubes that stored at -20 until use.

Methods: Anti-CCP IgG antibody was detected by using Sandwich ELISA technique according to the manufacturer instructions [EUROIMMUN company (Germany)]. Such anti-CMV IgG antibody was evaluated by Sandwich ELISA technique according to the manufacturer instructions (HUMAN Company, Germany).

Statistical analysis: Statistical differences in anti-CCP IgG and anti-CMV IgG antibodies frequencies between the RA patient's group and controls were determined by using Chi-square and Fisher's exact probability tests and the chi-squared test. Unconditional logistic regression was used to calculate the odds ratios (OR) with 95% confidence intervals (95% CI) and the attributable or protective fraction between the anti-CCP IgG antibody and anti-CMV IgG antibody frequencies with RA group[7]. Cutoffs were based on anti-CCP IgG and anti-CMV IgG antibodies level among controls. Mann Whitney *U* test was used to analyze the age, anti-CCP IgG antibody of the studied groups and expressed as mean ± SE, independent t-test and Duncan test. The statistical analyses were performed by using the IBM SPSS version 25. *P* value <0.05 was considered statistically significant[18].

Results

A total of 50 RA female patients and 50 healthy individuals as controls were involved in the present study, the sociodemographic appearances matched between the studied groups (table 1). The present results showed a significant difference between RA group and controls in each of anti-CCP IgG and anti-RF IgG (OR: 10201.0, 95% CI: 206.39 – 504193.42, *P*: $2.0x10^{-29}$, attributable fraction: 0.99) (table 2). The high OR value of each of CCP, RF referred to the risk fraction of them in inducing RA. In contrast the findings of anti-CMV IgG antibody showed that 84% of RA group and 15% of controls have seropositive for CMV, such the OR value referred to the risk role of CMV in inducing the disease through several mechanisms (OR:12.25, 95% CI: 4.70 – 31.95, *P*: $6.9x10^{-8}$, attributable fraction: 0.77) (table 2).

Table 1
Sociodemographic appearances data of RA patients and controls

Sociodemographic	Patients (n= 50)	Controls (n= 50)	*P*
Age (Y)	41.3 ± 2.4	41.0 ± 2.0	1.0
Height (Cm)	159.4 ± 29.9	164.0 ± 39.1	0.789
Weight (Kg)	72.9 ± 17.9	70.5 ± 16.5	0.912
BMI (Kg/m^2)	28.69 ± 7.7	26.21 ± 5.5	0.895
Educational status			
Universal	15 (30)	18 (36)	0.671
Secondary	5 (10)	7 (14)	0.760
Intermediate	20 (40)	15 (30)	0.402
Primary	10 (20)	10 (20)	1.0
Social status			
Married	43 (86)	45 (90)	0.760
Single	7 (14)	5 (10)	
Job status			
Employed	35	33	0.830
Unemployed	15	17	

The data were presented as mean ± SD or %, *P*: Fischer's exact probability (Two tailed)

Table 2
Anti-CCP antibody IgG and anti-CMV IgG seroprevalence status in RA patients and controls.

	Patients (n= 50)		Controls (n= 50)		OR	Attributable fraction	*P*	95% Confidence interval
	No.	%	No.	%				
Anti-CCP IgG	50	100	0	0.0	10201.0	0.99	$2.0x10^{-29}$	206.39 – 504193.42
Anti-RF IgG	50	100	0	0.0	10201.0	0.99	$2.0x10^{-29}$	206.39 – 504193.42
Anti-CMV IgG	42	84	15	30.0	12.25	0.77	$6.9x10^{-8}$	4.70 – 31.95

OR: Odds ratio; *p*: Fischer's exact probability (Two tailed fisher exact probability)

Table 3
Anti-CCP value in the studied groups

Anti-CCP antibody level RU/ml (mean ± SD)	
RA patients	**Healthy control**
51.5±17.7 [a]	3.9±0.85 [b]

Similar letters referred to non-significant differences (P > 0.05) (Duncan test).
Different letters referred to significant differences (P < 0.05) (Duncan test)

Also, the results in table 3 showed the level of anti-CCP IgG antibody in the sera of RA patients and healthy control groups. There was a significant increased level of anti-CCP IgG antibody in RA group compared to controls.

Discussion

RA is a multifactorial disease that arises from many causes through the interactions between the genetic and environmental factors. Some personal and lifestyle factors also influenced the development and/or course of RA including age and gender, socio-economic factor, sociodemographic factors, hormonal factors and ethnicity[4,12].

The high OR value of each of CCP, RF and CMV referred to the risk fraction of them in inducing RA through several mechanisms. Other researchers reported that the high level of anti-CCP antibodies in the serum increased the disease activity[5,23]. Also, any change in CCP molecule might induce the immune system through several pathways[13]. The anti-CCP antibody can activate the complement system through the classical and the alternative pathways, but not through the lectin pathway. These findings indicated that anti-CCP antibody is able to activate major immune effect or mechanisms that caused tissues damage[20].

The anti-RF antibody can enhance anti-CCP antibody to stimulate the macrophage cytokines production in addition to its role in RA pathogenesis through the proinflammatory cytokines effects in increasing disease activity[16,23]. CMV has a critical role in the etiopathogenesis of RA, there were several mechanisms inducing the immune cells as a result to damaging the cells and tissues, leading to pro-inflammatory cytokines production and the mimicry to some self-antigens in the human body. The immunization with a small cytomegalovirus-specific peptide leads to multiple autoreactive antibodies, possibly through the molecular mimicry and the epitope spreading, especially in the genetically predisposed persons[2].

Conclusion

The present findings indicated that CMV may be one of the triggering factors for RA and might had a role in pathogenesis of RA with the incidence of anti-CCP and anti-RF antibodies.

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