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A Study of the Relation between Human Cytomegalovirus and IL-35 in Rheumatoid Arthritis Disease Patients in Iraq

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ABSTRACT

Background: An autoimmune and inflammatory illness called rheumatoid arthritis (RA) occurs when your immune system mistakenly attacks normal cells in your body. Interleukin-35 is a brand-new cytokine that belongs to the immunosuppressive and anti-inflammatory IL-12 family. β -herpesvirus that produces inflammation and stays dormant in its host for life is the human cytomegalovirus. Human herpesvirus (HCMV) has been at the core of several RA-related theories.

Objective: The current study looked at the association between RA and serum IL-35 levels as well as the association between RA and CMV.

Patients and methods: Blood samples were taken in the Baghdad Teaching Hospital and Typical Rheumatology Unit from January 2022 to Mars 2022 for the current study, which comprised 70 Iraqi patients with RA illnesses and 22 Iraqi controls. ELISA was utilized to measure the biomarkers in this investigation, including the levels of IL-35 and anti-CMV lgG**.**

Results: In comparison to control group, RA patients had increased levels of IL-35, but there was no statistical significant difference between the two groups. Because all participants in our research had anti-CMV IgG, it was determined that both sick and healthy individuals had previously been infected with the virus.

Conclusion: Our results showed the role of IL-35 as a cytokine against inflammation and contributed to the control of RA pathogenesis, as well as the association between IL-35 and HCMV and whether the illness is active or not.

Keywords: Rheumatoid arthritis, IL-35, CMV, Case control study, University of Diyala.

INTRODUCTION

Rheumatoid arthritis (RA), whose etiology is unknown, is characterized by inflammatory changes in cartilage and bone, as well as in the synovial tissue of joint [(1)]. It is an inflammatory condition that is constantly progressing and involves systemic, extra-articular, and articular symptoms. Its origin is unclear [(2)].

It has been obvious in recent years that genetic and epigenetic variables affect how RA manifests itself, but environmental factors like exposure to dust and cigarette smoke must also have a substantial impact. In addition to T and B cells entering the synovium, there appears to be extensive interaction between the innate immune system's components and the immune system's adaptive components. Autoantibodies are created as a result of anomalies in the cellular and humoral immune response (RF), including the innate immune system and the system, most notably rheumatoid factors, antibodies against proteins that have undergone post-translational modifications, including acetylation, carbamylation, and citrullination, as well as abnormalities in the cellular and humoral immune systems, known as anti-modified protein antibodies (AMPAs) [(3)].

RA, which can severely destroy joints and leave sufferers disabled, affects about 5 out of every 1000 people. In the last 20 years, there has been a significant advancement in our understanding of illness pathophysiology, optimum outcome measures, and effective treatment modalities, including the recognition given the significance of RA early detection and treatment [(4)].

RA often attacks numerous joints at once if it predominantly affects the joints. The joint tissue in a RA-affected joint is harmed by the inflammation of the joint lining. This tissue injury may result in deformity, unsteadiness (loss of balance), and long-lasting or severe discomfort (misshapenness) When the body's immune system attacks its healthy cells, RA results., RA frequently affects the knee, wrist, and hand joints. Although the specific cause of RA is unknown, there are a few things you may do to lower your chance of getting the disease [(5)].

Regulatory T cells, such as peripheral and thymic Tregs, directly release IL-35, a novel type of cytokine, according to research done in 2007 by Collison et al. and Niedbala et al. Additionally, some non-immune cells, such as tumor cells, have the capacity to produce IL-35 [(6)].

Both T regulatory (Treg) and B regulatory cells express the pro-inflammatory cytokine interleukin-35 (IL-35). AML blast growth is encouraged by IL-35, which also inhibits apoptosis [(7).] Infections, inflammation, malignancies, and autoimmune disorders such systemic sclerosis, RA, Crohn's disease, and primary biliary cirrhosis are all strongly correlated with IL-35 [(8)].

HHV5 is categorized as a beta-herpesvirus and is also known as human herpesvirus (HCMV). Seroprevalences vary with socioeconomic class. The virus is spread by body fluids like as saliva, blood, breast milk, and others. The incidence of serum positive for the infection in the general population has been found to be 83% [(9).]

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PATIENTS AND METHODS

Based on information based on medical conditions and test results obtained from Baghdad Teaching Hospital and Typical Rheumatology Unit, samples of blood were drawn from 70 individuals with a confirmed diagnosis of RA, and 22 healthy participants without RA were furthermore a part of the control group. Ages of cases and controls ranged between 20 and 67 years old.

Measurement of anti-CMV lgG and serum IL-35 levels: Blood samples were taken from both RA patients and healthy volunteers. ELISA kits (Al-shkairate, Jordan) were used in line with the manufacturer's instructions to measure the levels of IL-35 in the blood. An automated ELISA reader was used to determine the optical density at 450 nm. Enzyme-linked immunosorbent test is also used to measure anti-CMV IgG. (Human, Germany).

Ethical Consideration:

The Ethical Committee at the University of Diyala's College of Education for Pure Sciences approved the study. This study was conducted in compliance with the code of ethics of the world medical association (Declaration of Helsinki) for human subjects.

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 26.0 for windows. Qualitative data were defined as numbers and percentages. Pearson Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and standard deviation (SD), and independent sample t-test was used for comparison between groups. P value ≤0.05 was considered to be statistically significant.

RESULTS

Table 1 shows there are no discernible age differences between the control group and RA patients.

Table 1: Comparison of research samples based on patient and control age information.

Groups	Age Mean ± SD (Years)	P-value
Patients group	48.79 ± 1.29	0.302
Control group	47.37 ± 2.56	NS

Table 2 shows no significant differences in IL-35 levels between RA patients and the control group.

Table 2: Comparison of samples study according to IL-35 level data of the patients and control

Groups	IL-35 Mean ± SD	P-value
Patients group	14.63 ± 2.85	0.171
Control group	8.81 ± 0.31	NS

Table 3 shows no significant differences in IL-35 level and age.

Table 3: Correlation between IL-35 level and age in the studied sample.

Variable		IL35
Age	R	0.010
	Sig. (2-tailed)	0.924

As shown in **Table 4** there was a significant difference in anti-CMV IgG serum levels among the studied groups

Table 4: Comparison between healthy control and RA patients in anti-CMV IgG level in serum

Groups	IgG (Mean ± SD) anti-CMV	P-value
Patients group	2.44 ± 0.02	1.73 x 10^{-13}
Control group	2.06 ± 0.05	

As shown in **Table 5** there was no significant difference in anti-CMV IgG serum level and IL-35

Table 5: Correlation between anti-CMV lgG serum level and IL-35 in the studied sample.

Variable		IL-35
Anti-CMV IgG	r	0.134
	Sig. (2-tailed)	0.213

Table 6 shows there is a positive relationship between age and anti-CMV lgG.

Table 6: Correlation between anti-CMV lgG and age in the studied sample.

Variable		anti-CMV IgG
Age	r	0.378**
	Sig. (2-tailed)	0.000

DISCUSSION

Our study investigated the association between RA and serum IL-35 levels as well as the association between RA and CMV. No statistically significant (P >0.05) differences between the age means of the RA group and the control group were found in our study.

We discovered that blood IL-35 levels were greater in RA patients, which closely mirrors the experimental findings of **Li *et al.*,** based on a small but remarkably homogenous sample of recruited individuals [10].

Iraqi research found that RA patient groups had significantly higher levels of IL-35 than did control groups. On the other hand, albeit not significantly, IL-35 levels were somewhat higher in RA patients [11].

Another study agreed with the current study that there was no association between age and IL-35 level that could be seen [9]. According to another study, RA patients had higher levels of IL-35 [12]. A less recent study found that those with inactive RA had notably higher blood IL-35 levels compared to those with active RA [13].

Studies have shown a connection between the RA etiology and Th17/IL-17(13). The growth of Th17/IL-17 cells may be prevented by IL-35. This led to the hypothesis that IL-35 might affect RA by inhibiting the Th17/IL-17-related pathway [10]. IL-35's significance RA pathogenesis is intimately correlated with the pathogenic process and immunological malfunction of these immune cells. IL-35 promotes the proliferation of Tregs while suppressing the development of Th17 cells. Tregs and Th17 cells are critical for inflaming the synovium and harming joints when it comes to infiltrating immune cells. Therefore, by maintaining the proportion of Tregs to Th17 cells, IL-35 greatly contributes to the pathological development of RA [15].

Another study, a level was found Patients with inactive RA had considerably greater serum IL-35 levels than those with active RA [13].

Studies have demonstrated a link between Th17/IL-17 and the pathophysiology of RA [3]. The differentiation of Th17/IL-17 cells may be inhibited by IL-35. This led to the hypothesis that IL-35 could influence RA by inhibiting the Th17/IL-17-related pathway [10].

The pathogenic processes and immunological dysfunction of these immune cells are intimately connected to the role of IL-35 in the pathogenesis of RA. IL-35 inhibits the development of Th17 cells while encouraging the growth of Tregs. Numerous immune cells, including as Tregs and Th17 cells, invade the joint and play a key role in the destruction of the joint and synovium. As a result, IL-35 contributes significantly to the pathological development of RA by maintaining the balance between Tregs and Th17 cells [15].

A study indicated that IgG antibody was present in 76% of RA patients, and IgM antibody was found in 3% of patients, as well as IgG antibody was found in 76% of healthy subjects, and lgM antibody was not found in healthy subjects. Neutrophils, which pick the virus up in endosomes and spread it to infect other cells, can get the virus from both endothelium and epithelial cells [16].

Latent HCMV is implicated in the etiology of inflammatory illnesses because it can reactivate in inflamed tissues and exacerbate inflammatory processes. Although HCMV DNA has been found in RA synovial tissue, it is unknown if HCMV plays a pathogenic function in the disease [16].

CONCLUSION

IL-35 as a cytokine against inflammation could contribute to the regulation of RA pathogenesis, and determine whether the disease is active or inactive and its relationship with IL-35 and HCMV.

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Author contribution: Authors contributed equally in the study.

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