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Interlukin-35 Gene Polymorphism and Epstein Bar Virus IgG Antibodies against Viral Capsid Antigen Presence in Rheumatoid Arthritis Patients

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Abstract: Rheumatoid arthritis (RA) is a chronic condition of autoimmunological nature marked by inflammation in multiple small joints, known as polyarthritis. The aim of the study that complex illness affected by a numerous of factors involving lifestyle choices, genetics, and environmental elements. The presence of both joint-related and systemic symptoms in RA can ultimately lead to unfavorable long-term consequences, including disability and mortality. The aim of this study was to explore the role of IL-35 gene polymorphism and the presence of Epstein Bar virus (EBV) in RA Iraqi patients. The results revealed that the EBV IgG antibodies index was significant increase in RA patients than healthy control. Such findings suggest that involvement of EBV in RA disease. in addition, all RA patients were sero-positive for ACCP antibodies in this study and receiver operating characteristics (ROC) curve analysis revealed the predictive significance of ACCP in differentiating RA patients from healthy controls (HC). Furthermore, the RA patients revealed sero-negative for ANA-8S antibodies in 57% of total patients and the ROC curve analysis revealed the predictive significance of ANA-8S in differentiating RA patients from HC as well. It was concluded that no correlation found between the presence of EBV IgG antibodies index and ACCP or ANA-8S presence in RA patient's sera.

Keywords: Rheumatoid Arthritis, IL-35, gene polymorphism, EPV, Anti-CCP, ELISA.

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Introduction

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease that causes progressive and painful of multiple joints damage. RA arises when the immune system attacks its own tissues and results in pain, swelling, stiffness, and loss of joint function (1). RA is estimated afflicts nearly 0.62 ± 0.38% of whole population; nonetheless, women are twofold as likely as men to suffer from it (2). While among Eastern Mediterranean populations including Iraqi population; it has been estimated to be 0.37% (3).

In parallel with numerous autoimmune diseases, the causes of RA are multiple and diverse; nevertheless, the RA etiology is unidentified, but extensive indication proposes that the disease is developed next to the interaction between hereditary risk factors and environmental stimulators. Cytokines play a significant role in etiopathogenesis of RA (4). They are probably responsible for inflammatory reactions and joint destruction that occur during the course of disease. Cytokines are low molecular weight (less than 80 kDa) signaling glycoproteins that participate in the

regulation of innate and adaptive immune responses, as well as inflammation and hematopoiesis (5).

The imbalance between pro- and anti-inflammatory cytokines may be an underlying element in RA disease progression via inflammation and the loss of articular cartilage (6). IL-35 is a member of the IL-12 cytokine family have a crucial role in regulating innate and adaptive immunity and also in the management of inflammatory diseases and play significant roles in several autoimmune diseases (7). IL-35 is a heterodimeric cytokine composed of the Epstein-Barr virus-induced 3 (EBI3) and p35 subunits; it belongs to the IL-6/IL-12 cytokine family that includes IL-12, IL-23, IL-27, and IL-35 molecules (8). IL-35 is expressed in different cells, such as T and B cells, dendritic cells, monocytes, endothelial cells, and smooth muscle cells and organs, such as the placental trophoblast, lung, colon, and liver. To date, the effects of IL-35 on immune responses have been widely investigated. However, studies have demonstrated dual effects of IL-35 on immune cell differentiation and function (9). The influence of IL-35 in the pathogenesis of RA is closely related to the immune dysfunction of these immune cells and pathological process. IL-35 is secreted primarily by regulatory T cells (Treg) and has anti-inflammatory and immunosuppressive properties. It can enhance Treg proliferation and inhibit T helper-17 (Th17) cell differentiation. Therefore, by maintaining the balance between Th17 and Tregs cells, IL-35 is crucial to the progression of RA. IL-35 is closely related to the incidence of inflammation in infections and autoimmune diseases (10). Recent findings have shown that IL-35 expression is abnormal in several autoimmune diseases, such as systemic lupus erythematosus (SLE) and Sjogren's syndrome (SS). Functional analysis indicated that this protein may contribute to the development of different disorders. Of note, there are published studies on the serum levels of IL-35 in RA patients (9).

Amongst the environmental factors, many pathogens were suspected, among which Epstein-Barr virus (EBV) is the most interesting (10) EBV may function as an adjuvant throughout the autoimmune response initiation, non-specific innate immunity, such as complement receptors mast cells, Toll-like receptors, and dendritic cells (11).

Viral infection might be followed by the clinically development of Polyarthritis resembling RA (12). Hence the global ubiquitous nature of RA would be owing to the exposure to an endemic virus. Nevertheless, this common virus may not be identified even via more sophisticated methods due to a prolonged latency period, alongside RA symptoms appears years after first exposure. Viruses such as human herpesvirus-6 and 8, human endogenous retroviruses-5, Epstein–Barr virus (EBV), HTLV-1, and parvovirus B19 were documented to have a significant role in the RA pathogenesis (13,14) EBV is a gamma herpesvirus 4, responsible for the infection of more than 95% of the adults globally. Mainly, EBV replication takes place in B lymphocytes. The saliva contributes in the spread of infection (15, 16).

The involvement of EBV in RA were studied for more than 15 years. Even though solid evidence is absent, a cumulative body of inconclusive evidence ideas suggests an association linked them. In peripheral blood, Ollier, (17) showed that RA patients developed reduced count of T cell precursor to

EBV gp110 (a late-stage regulatory protein) that correlates with the severity and activity of RA. This decline in the immune response to such significant regulatory protein may result in weak regulation of EBV infection (or even the re-infection), prolonged contact with other antigens of EBV, in addition to the development of chronic immune response in RA. According to previous facts, this study aims to detected the role of IL-35 gene polymorphism and presence of EBV Ab in serum as a risk factor of RA in Iraqi patients.

Materials and methods

Patients and control

A cross-sectional study was conducted, which included a total of 150 participants, consisting of 100 RA patients and 50 individuals who were deemed to be apparently healthy controls (HC). The patients were referred for diagnosis and treatment to the Baghdad Teaching Hospital and Al-Kadhimiya educational Hospital in Baghdad during the period from December 2021 to March 2022. The diagnosis was confirmed by the consultant physician in previous hospitals and for HC the results of RF, ESR and CRP tests were negative. Demographic data was collected for each patient through a special form. Patients' exclusion criteria consisted of absence of informed consent, have other disease with RA, and unavailability of blood samples. Five ml blood specimens were withdrawn from the study groups. then, transferred into three tubes; firstly, 2ml transferred to the gel tubes; thereafter, allow to settle at 18-25°C to clot. Centrifugation was performed at 3000 xg for a duration of 5 minutes to separate the sera and distributed into Eppendorf tubes in duplicate and kept at -20ºC until assayed. secondly 1.5 ml was transferred to EDTA tube for DNA extraction, whereas the third one, 1.5 ml of blood was transferred ESR tube for the ESR estimation.

ESR, RF and CRP estimation

For ESR estimation, the conventional Westergren assay was used. In the case of CRP and RF, a qualitative test (positive or negative) was carried out using a slide-agglutination kit, and instructions recommended by the manufacture were followed.

Measurement of serum Anti-Cyclic citrullinated peptide antibodies, anti-nuclear antibodies-8S and epstein bar virus IgG antibodies

AESKULISA-CCP ELISA kit and ANA-8S ELISA Kit (AESKU / Germany) were used to determination of A-CCP and ANA-8S serum levels, while EPSTETEIN-BARR VCA ELISA IgG Kit (Vircell/ Spain) was used to detected EBV IgG in serum of RA patients and control.

Detection of *IL-35* gene polymorphism

The polymorphism of IL-35 SNP rs9807813 was identified in 75 RA and 40 HC via polymerase chain reaction (PCR) technique as follows; DNA was extracted by Favorprep^{TM} Blood/Culture cells Genomic DNA Extraction mini-Kit (FAVORGEN/ Austria) according to manufacture instructions manual. IL-35 SNP rs9807813 polymorphisms were examined by PCR assays and performed in a 25 µl volume containing 12.5 µl of 2X GoTaq Green Master Mix (Promega, USA), 1 µl of each of 10 pmol forward (TGTAAAACGACGGCCAGTGTTGG TACTTGCCTGTAGTC) and reverse (CAGGAAACAGCTATGACGGATG TCAGCATTCATTCATTC) primers, 8.5 µl nuclease-free water, and 2 µl of template DNA. PCR cycling was performed using BioRad (USA)

thermocycler for 32 cycles under the following condition; initial denaturation at 95 ^{o}C for 5 min., denaturation 95 ^{o}C for 30 sec., annealing at 60 ^{o}C for 30 sec., extinction at 72 ^{o}C for 30 sec. and final extinction at 72 ^{o}C for 7min. (11).

PCR products were visualized by 2% agarose gel electrophoresis stained by Ethidium Bromide Solution.Then, the PCR amplicons were sequenced by the Sanger method using an ABI3730XL automated DNA sequencer and the results were downloaded through Geneious software version 10.2.2.

detection of EBV by PCR

Epstein Barr virus REAL-TIME PCR Detection Kit (DNA-Technology/Russia) was used to detected EBV nucleic acids in human blood after DNA extraction by PREP-NA extraction kit (DNA-Technology/Russia).

Statistical analysis

The statistical analysis was done by IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.). The categorical variables were expressed as percentage and number, moreover, the significant differences were evaluated using a two-tailed Fisher exact test. The BMI was calculated as weight in kg divided by height in m squared (kg/m2). area under the curve (AUC) was via performing the receiver operating curve (ROC) analysis, 95% confidence interval (CI), cut-off value, sensitivity and specificity. For the purpose of cut-off value optimization, the Youden index was employed. Correlations between variables were assessed by correlation coefficient (r) analysis.

Results and discussion

1. demographic parameters

The demographic parameters of Rheumatoid arthritis (RA) patients in this study were included their age, gender, rheumatoid factors (RFs), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), Body mass index (BMI), and undergoing therapy. However, in controls, their sera were negative for RF and CRP, and their ESR values were within a normal range (less than 20 mm), as shown Table (1).

2. Anti-Cyclic Citrullinated Peptide Antibodies

All RA patients were sero-positive for ACCP antibodies. The serum level was classified as weak level (18.0 - 99 U/ml), moderate level (100 - 199 U/ml) and high level (≥ 200.0 U/ml), which showed different frequencies in total (15, 13 and 46%, respectively). The normal value for less than 18 U/ml. However, the differences between the frequencies percentage of ACCP results in RA patients attend a significant level, Table(2).

The analysis of ROC curve demonstrated the ACCP predictive significance (AUE =0.9231; 95% confidence interval= 0.8638 to 0.9824; p<0.0001; Youden index =0.7647; cut-off= 291.832; Sensitivity= 0.7647%; specificity= 1%) in discriminating between RA patients and HC, Figure(1).

3. Anti-nuclear antibodies-8S

Rheumatoid arthritis patients were divided for ANA-8S antibodies sero- negative (0.45>) which consist 57% of total patients and sero-positive (0.45<). The positive reaction was classified as weak (0.45-1.0), moderate (1.1-2.0) and strong (>2.0), which showed different frequencies in total (25, 6 and 12%, respectively). However, the differences between the frequencies percentage of ANA-8S results in RA patients attend a significant level, Table(3).

ROC curve analysis indicated the ANA-8S predictive significance (AUE =0.7989; 95% confidence interval= 0.6897 to 0.9081; p<0.0001; Youden index =0.4992; cut-off= 0.423;

Sensitivity= 0.6757%; specificity= 0.8235%) in discriminating between RA patients and HC Figure (2).

4. IL-35 gene polymorphism

IL-35 rs9807813 PCR product was shown in figure (3). Analysis of Hardy-Weinberg equilibrium (HWE) revealed that the genotypes of rs9807813 polymorphism were consistent with HWE in HC. The association of IL-35 SNP rs9807813 polymorphism with RA was evaluated in RA patients versus HC using alleles and genotypes of SNP. The frequency of T alleles and TT genotypes showed a significant increased frequency in patients compared to controls (54 vs. 32.5%; OR=4.04; p<0.001), Table (4).

5. Epstein Bar virus IgG antibodies against viral capsid antigen Presence in RA patients

Rheumatoid arthritis patients and HC were divided for sero- negative (< 9) which consist 49 (49%) and 46 (98%), respectively and sero-positive (>11) for EBV IgG antibodies index which consist 51 (51%) and 4 (2%), respectively of total patients (100) and control (50). However, the differences between the frequencies percentage of EBV IgG antibodies index results in RA patients attend a significant level (*P*<0.001), Figure (4).

6. Correlation Coefficient (r) between EBV IgG antibodies index and ACCP and ANA-8S.

In this study**,** there is unsignificant positive correlation (r =0.093, r =0.075, p>0.05) between EBV IgG antibodies index with ACCP and EBV IgG antibodies index with ANA-8S, respectively.

7. EBV nucleic acids (NA) detection by RT-PCR

From 51 EBV IgG antibodies positive rheumatoid arthritis patients; 4 (8%) showed positive for EBV nucleic acid (NA) and 47(92%) observed negative results. However, the differences between the frequencies percentage attend a significant level (P<0.001), (Figure 5).

The exact origin of RA, a severe autoimmune disease affecting the entire body, is still uncertain. However, research involving family studies and comprehensive genetic investigations has revealed a significant genetic component in determining susceptibility to RA, such as IL-35 rs9807813 with T allele or TT genotype.

Moreover, evidence suggests that a viral infection, such as EBV, may potentially function as a catalyst in individuals who are genetically predisposed to the disease. The EBV IgG antibodies index was significant increase in RA patients than control in this study. Such findings suggest that involvement of EBV in RA disease, and this result was in agreement with several studies (18, 19, 20) and few numbers of cases have EBV NA positive which mean acute infection.

Considerable number of researches have focused on investigating the role of EBV in triggering RA by exploring the concept of molecular mimicry. The early association between RA and EBV was documented by Alspaugh and Tan (21) as they indicated that sera collected from RA patients reacted with a nuclear antigen presented in transformed lymphocytes of EBV. This antigen, which is known as 'RA nuclear antigen' was identified as a glycine/alanine-rich repeat within EBNA-1. Antibodies targeting this repeat showed cross-reactivity with a 62 kDa protein existed in the RA patient synovium rather than in healthy individuals (18). Additionally, there are sequence similarities in antigenic structure between RA-specific proteins and other proteins of EBV, among which antibodies against EBV peptide p107

and the EBV-encoded protein gp110 (12). EBV induces the expansion of polyclonal lymphocytes and persistence inside B lymphocytes throughout the host's lifetime, remaining suppressed by the immunity. In its latent and replicating forms, EBV displays immunomodulatory reactions, which may potentially participate in developing such autoimmune disease (22, 23 and 24).

In the other hand, all RA patients were sero-positive for ACCP antibodies in this study and ROC curve analysis revealed the predictive significance of ACCP in recognizing RA patients from HC. This result was accordance with numerous studies reported that Anti-CCP elevated in RA and strong evidence supports the connection between anti-CCP and radiographic alterations in RA. Anti-CCP serves as a reliable indicator of both radiographic damage and its advancement, independent of other factors [15, 25,26, and 27). However, the RA patients revealed sero- negative for ANA-8S antibodies in 57% of total patients and the ROC curve analysis revealed the predictive significance of ANA-8S in recognizing RA patients from HC also. This result was in agreement with others who reported that Antinuclear antibodies are found in approximately 25- 50% of RA patients. In seropositive patients and ANA-positive, there was a prolonged time to fulfill the criteria of RA as well as the treatment (28, 29, 30, 31 and 32).

Table (1): Demographic parameters of rheumatoid arthritis (RA) patients and controls

Parameters		RA (n = 100)	Control (n = 50)
Age (years)	**30>**	7(7)	16 (32)
	30-50	57 (57)	27(54)
	50<	36 (36)	7 (14)
Sex	**Male**	15 (15)	6 (12)
	Female	85 (85)	44 (88)
ESR (mm/hr)	**higher the normal value**	(76)	0 (0)
CRP	**Positive**	65 (65)	–
RF	**Positive**	68 (68)	–
BMI	**overweight/obese**	72 (72)	30(60)
Therapy	**No therapy**	24 (24)	–
	With therapy	76 (76)	–

Table (2): Percentage frequency of rheumatoid arthritis patients sero-positive for anti-cyclic citrullinated peptide (ACCP) antibodies

Groups	Number of Cases for ACCP Antibodies (%)			
	Negative	**Weak positive**	**Moderate positive**	**Strong positive**
Normal value	(<18 U/ml)	(18.0-99 U/ml)	(100-199 U/ml)	(≥200 U/ml)
RA Patients (N:100)	26 (26)	15 (15%)	13 (13%)	46 (46%)
HC (N:50)	50(100)	0	0	0
***P*-value**	<0.001 Significant			

Table (3): Percentage frequency of rheumatoid arthritis patients sero-negative and sero-positive for anti-nuclear antibodies (ANA)-8S.

Groups	Number of Cases for ANA antibodies (%)			
	Negative	**Weak positive**	**Moderate positive**	**Strong positive**
Negative value	<0.45	0.45-1.0	1.1-2.0	> 2.0
RA Patients(N:100)	57 (57)	25(25%)	6 (6%)	12 (12%)
HC (N:50)	50(100)	0	0	0
***p*- value**	< 0.05 significant			

Table (4): Statistical analysis of association between genotypes and alleles of IL-35 gene (rs9807813) and rheumatoid arthritis.

IL-35 SNP	Allele/ Genotype	RA patients (N= 75)		HC (N= 40)		OR	95% CI	*ρ-value*
		N	%	N	%			
rs9807813 (C/T)	*C*	69	46	62	77.5	Reference		
	T	81	54	18	32.5	4.04	2.19 to 7.46	<0.001
	C/C	15	20	24	60	Reference		
	C/T	39	52	14	35	2.01	0.92 to 4.40	0.116
	T/T	21	28	2	5	7.39	1.66 to 32.81	<0.001

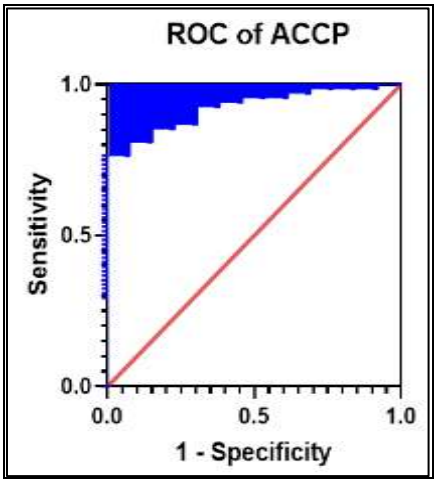

Figure (1): ROC curve analysis of ACCP in RA patients and HC.

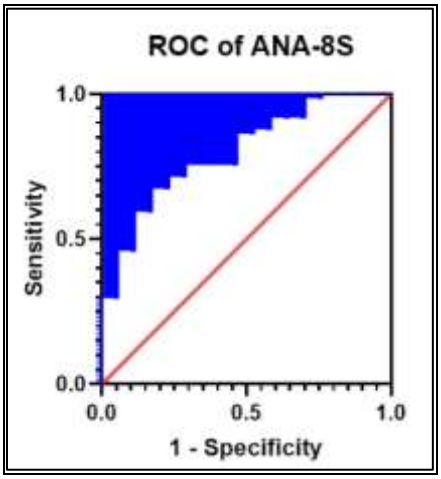

Figure (2): ROC curve analysis of ANA-8S in RA patients and HC.

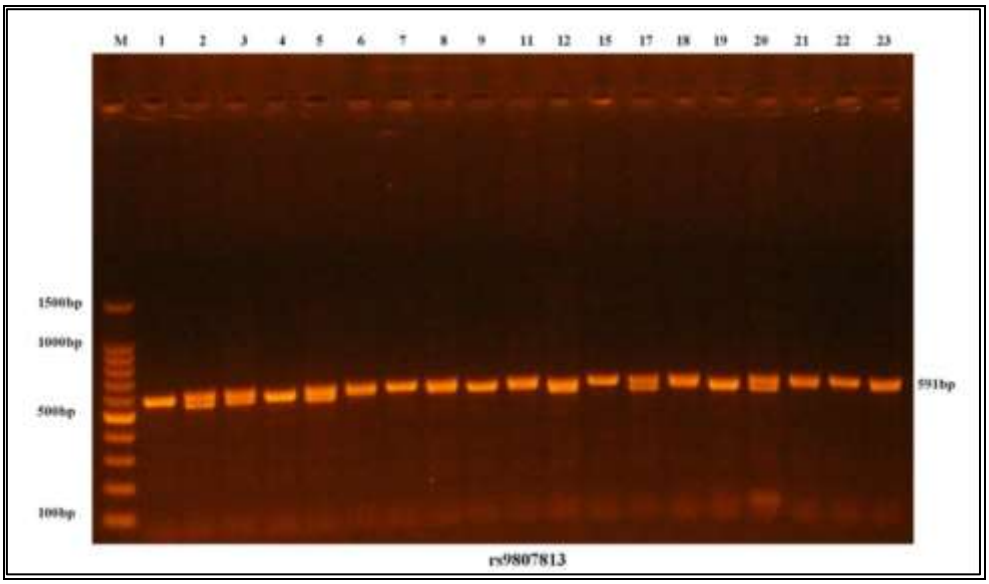

Figure (3): Results of the amplification of rs9807813 region of Human samples were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-23 resemble 591bp PCR products.

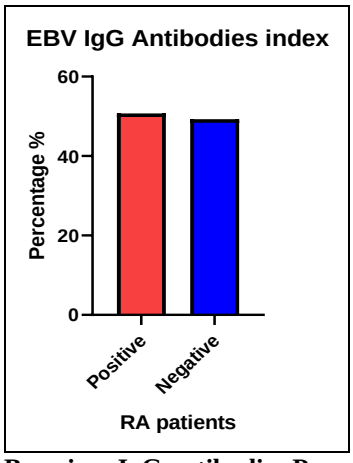

Figure (4): Epstein Bar virus IgG antibodies Presence in RA patients.

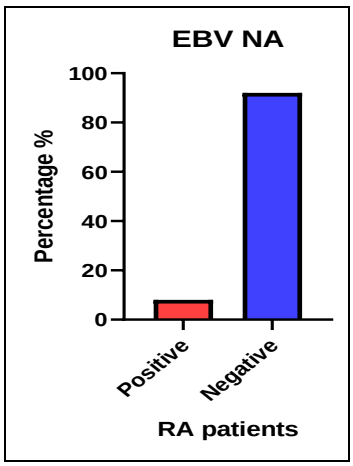

Figure (5): Epstein Bar virus nucleic acids Presence in RA patients.

Conclusion

In conclusion, the outcomes reached by this work hypothesized that Il-35 of rs9807813 T allele and TT genotypes (risk factors) showed susceptibility to RA, moreover,the molecular mimicry between EBV and RA, either through influencing recognition of T cell receptor of the HLA 'shared epitope' or by the synthesis of joint protein autoantibodies, may has a significant participation throughout the pathogenesis of RA.

Acknowledgement

We would like to thank all participants in this study.

Conflict of Interest

No conflict of interests to declare.

Funding Statement

There is no funding to report.

Ethical Clearance

The ethics committee of the College of Science, University of Baghdad permitted this research (Ref.: CSEC/0122/0156). The patients as well as the controls were enrolled through a written informed consent.

References

1. Al Ghuraibawi, Z.; Sharquie, I. and Gorial, F. (2023). A novel Link of Serum IL-39 Levels in Patients with Rheumatoid Arthritis. Iraqi Journal of Sciences, 64 (4): 1651-1661.
2. Al-Ani, N.; Gorial, F.; Yasiry, D.; Al Derwibee, F.; Abbas, Y.; Sunna, N., *et al*. (2021). Clinical Outcomes in Iraqi Patients with Rheumatoid Arthritis Following Earlier or Later Treatment with Etanercept. Open Access Rheumatology., 13: 57-62.
3. Rudan, I.; Sidhu, S.; Papana, A.; Meng, S.J.; Xin-Wei, Y.; Wang, W., *et al*. (2015). Global Health Epidemiology Reference Group (GHERG). Prevalence of rheumatoid arthritis in low- and middle-income countries: A systematic review and analysis. Journal of Global Health, 5(1): 010409.
4. Nazar, L. and Abass, E. (2019). Study of IL-33 and IL-1R4 in Iraqi Rheumatoid Arthritis Female Patient's with and without Dyslipidemia Prone to Atherosclerosis. IHJPAS**,** *32* (1): 48-56.
5. Gillespie, S.; Kadiyala, S. and Greener, R. (2007). Is poverty or wealth driving HIV transmission. AIDS, 7: S5-S16.
6. Manhal, F.S. (2010). Cytokine Profile in Patients with Rheumatoid Arthritis. Journal of the Faculty of Medicine Baghdad, 51(4), 433-436.
7. Vignali, D.A. and Kuchroo, V.K. (2012). IL-12 family cytokines: immunological playmakers. Nature Immunology, *13*(8): 722-728.
8. Xie, Q.; Xu, W.D.; Pan, M.; Lan, Y.; Liu, X.; Su, L. C., *et al*. (2021). Association of IL-35 expression and gene polymorphisms in rheumatoid arthritis. International Immunopharmacology, 90: 107231.
9. Al-Saffar, E. A., & Al-Saadi, B. Q. (2022). Study the association of IRAK1 gene polymorphism and some immunological markers with the risk of rheumatoid arthritis incidence in sample of Iraqi patients. Iraqi Journal of Biotechnology, 21(2), 46-60.
10. Taha, M. A., & Da'ad, A. H. (2022). Role investigation of interleukin-IL-17 rs763780 T/C gene polymorphism with Iraqi rheumatoid arthritis patients. Iraqi Journal of Biotechnology, 21(2), 103-114.
11. Costenbader, K.H. and Karlson, E.W. (2006). Epstein-Barr virus and rheumatoid arthritis: is there a link. Arthritis Research and Therapy, 8(1): 1-7.
12. Fairweather, D.; Frisancho-Kiss, S. and Rose, N.R. (2005). Viruses as adjuvants for autoimmunity: evidence from Coxsackievirus-induced myocarditis. Reviews in Medical Virology, *15*(1), 17-27.
13. Umekita, K. (2022). Effect of HTLV-1 Infection on the Clinical Course of Patients with Rheumatoid Arthritis. Viruses, 14(7): 1460.
14. Carty, S.M.; Snowden, N. and Silman, AJ. (2003). Should infection still be considered as the most likely triggering factor for rheumatoid arthritis. The Journal of Rheumatology, 30: 425–429.
15. Abd, N.; Al-Ahmer, S. and Ghafour K. (2021). Detection of Epstein Barr Virus in Some Iraqi Women Patients with Invasive Ductal Carcinoma Using Immunohistochemistry Technique. Iraqi Journal of Biotechnology, 1(20).
16. Jassim, N.; Aboud, R.; Joda, A.; Fadil, H.; Al-Humadani, F.; Ahmed, D. M., *et al*. (2015). Detection of Epstein -Barrvirus

Capsid antigen (EBV CA) in Sera of Rheumatoid Arthritis, Reactive Arthritis and Ankylosing Spondylitis Patients. Iraqi Journal of Science, 56 (4B): 3130-3134.

17. Ollier, W. (2000). Rheumatoid arthritis and Epstein-Barr virus: a case of living with the enemy? Annals of the Rheumatic Diseases, 59: 497-499.
18. Al- Bayati, O.; Abood, W. and Salloom, D. (2020). Detection of Epstein Barr Virus Infection inReactive Arthritis Patients. Iraqi Journal of Science, 61(10): 2479-2485.
19. Fechtner, S.; Berens, H.; Bemis, E.; Johnson, R.L.; Guthridge, C.J.; Carlson, N. E., *et al*. (2022). Antibody Responses to Epstein-Barr Virus in the Preclinical Period of Rheumatoid Arthritis Suggest the Presence of Increased Viral Reactivation Cycles. Arthritis and Rheumatology, 74(4): 597-603.
20. Al-Derzi, A.R.; Alani, M.M. and AL-Hadithi, H.S. (2014). Epstein-Barr Virus Antibodies in Rheumatoid Arthritis Iraqi patients. Journal of the Faculty of Medicine Baghdad, 55(4):358-61.
21. Alspaugh, M.A.; Jensen, F.C.; Rabin, H. and Tan, EM. (1978). Lymphocytes transformed by Epstein-Barr virus. Induction of nuclear antigen reactive with antibody in rheumatoid arthritis. Journal of Experimental Medicine,147(4): 1018-1027.
22. Kouri, T.; Petersen, J.; Rhodes, G.; Aho, K.; Palosuo, T.; Heliövaara, M., *et al*. (1990). Antibodies to synthetic peptides from Epstein-Barr nuclear antigen-1 in sera of patients with early rheumatoid arthritis and in preillness sera. The Journal of rheumatology, 17(11): 1442–1449.
23. Nagata, K. and Hayashi, K. (2020). Epstein-Barr Virus Reactivation-Induced Immunoglobulin Production: Significance on Autoimmunity. Microorganisms, 8(12): 1875.
24. Wistinghausen, B.; Dave, H. and Bollard, C. (2022). Adoptive cellular immunotherapy for Epstein-Barr virus-associated lymphoproliferative disease. Ann Lymphoma, 6, 5.
25. Jog, N. and James, J. (2021). Epstein Barr Virus and Autoimmune Responses in Systemic Lupus Erythematosus. Frontiers in Immunology, 11: 623944.
26. Forslind, K.; Ahlmén, M.; Eberhardt, K.; Hafström, I. and Svensson, B. (2004). Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Annals of the Rheumatic Diseases, 63(9): 1090-1095.
27. Radwan, A.; Allam, A. and Radwan, A. (2021). The relationship of serum calprotectin with disease activity, functional status, ultrasonographic findings and radiological damage in rheumatoid arthritis patients. The Egyptian Rheumatologist, 43(2): 147-151.
28. Braschi, E.; Shojania, K. and Allan, G.M. (2016). Anti-CCP: a truly helpful rheumatoid arthritis test? Canadian Family Physician., 62(3): 234.
29. Steiner, G. and Smolen, J. (2002). Autoantibodies in rheumatoid arthritis and their clinical significance. Arthritis research, 4 (2): 1-5.
30. Paknikar, S.S.; Crowson, C.S. and Davis, J.M. (2021). Thanarajasingam U. Exploring the Role of Antinuclear Antibody Positivity in the Diagnosis, Treatment, and Health Outcomes of Patients with Rheumatoid Arthritis. ACR Open Rheumatology, 3(6): 422-426.
31. Guruparan, T.; Hutchinson, M.; Hayward, R.; Penn, H. and Kalia, P. (2021). The relationship between antinuclear antibodies and erosive disease in patients with rheumatoid arthritis. Rheumatology, 60 (1): 247.125.
32. Rönnelid, J.; Turesson, C. and Kastbom, A. (2021). Autoantibodies in Rheumatoid Arthritis – Laboratory and Clinical Perspectives. Frontiers in Immunology**,** 12: 685312.