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Association of Interleukin-17 and ACCP levels with Rheumatoid arthritis patients and Control Groups

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Abstract

Background: Rheumatoid arthritis (RA) represents the prevailing form of chronic inflammatory polyarthritis, characterized by an autoimmune response directed against citrullinated antigens and subsequent synovial joint destruction. The susceptibility to RA appears to be influenced by a complex interplay between a specific immune response to various environmental factors and a favorable genetic predisposition. IL-17 Association with Diseases found to be elevated in various chronic inflammatory conditions including RA especially in cases resistant to anti-TNF therapy.
Aim : To investigate the value of IL-17 and ACCP levels with study groups as well as the association of chronic periodontal disease as an environmental risk factor of RA.
Methods : This case-control study involved a total of 140 participants were enrolled, with 70 individuals meeting specific criteria and serological testing confirming their RA diagnosis, while the remaining 70 served as healthy controls.The study involved the collection of blood samples from the participants. Various measurements and tests were conducted, including the assessment of disease activity using the Disease Activity Score (DAS28-ESR) and erythrocyte sedimentation rate (ESR), detection of RF through latex agglutination, quantification of anti-cyclic citrullinated peptide (ACCP) and interleukin-17 (IL-17) levels via enzyme-linked immunosorbent assay (ELISA).
Results : RA patients exhibited substantially higher values for ESR, RF, ACCP compared to the control group. Also IL-17 substantially higher values for RA patients Notably, the p-values for ESR, ACCP, and IL-17 were 0.0001, 0.02, and 0.0001, respectively, indicating strong statistical significance. patients who had gum problems were 43(61.4 %) while the control group was 9 (12.5%) had a gum problem Statistically, parameter had significant differences (p.value=0.0001).

Keywords: Anti-cyclic citrullinated peptide (ACCP), Disease Activity Score 28 (DAS28), Interleukin-17 (IL-17), periodontal disease, Rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is the most common type of re-inflammatory polyarthritis in clinical practice, it is an autoimmune disease that develops gradually, and this autoimmune disease with no known cause, characterized by antibodies against citrullinated antigens and immune-mediated destruction of the synovial joints [(1)].About 1% of people have RA, with a

female to male ratio of 2.5/1, the disease can strike anyone at any time, but its age-related increase in incidence ,those in their 40 to 70 at higher risk[2]. Although RA can affect any joint, it is typical to detect involvement of the metacarpophalangeal (MCP), proximal interphalangeal (PIP), interphalangeal, wrist, knee, and metatarsophalangeal (MTP) joints of the toes [3]. Joints that are impacted are swollen and painful, the cervical spine is the only part of the axial skeleton that is often spared, it's typical to feel lousy, stiff in the morning, and worn out[4]. The susceptibility to rheumatoid arthritis (RA) appears to be influenced by a complex interplay between a specific immune response to various environmental factors and a favorable genetic predisposition, infections are believed to play a significant role in triggering autoimmune diseases, often associated with specific microorganisms among the identified environmental factors[5]. RA is characterized by synovial inflammation, swelling (hyperplasia), the production of autoantibodies such as RF and aACPA, as well as the destruction of cartilage and bone (leading to deformity). It also presents with systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders[6]. Rheumatoid factor (RF) and antibodies against citrullinated proteins (ACPA) are well-recognized autoantibodies that serve as defining features of RA. In individuals with RA, testing positive for RF and/or ACPA (referred to as "seropositive") often indicates a distinct etiology and disease progression compared to those classified as "seronegative [7]. ACPAs are antibodies that recognize antigens containing the non-coding amino acid citrulline, IgG is commonly used in diagnostic tests to evaluate ACPA, and there are also other isotypes of ACPAs, including IgM, IgA, IgG1, IgG2, IgG3, and IgG4, second-generation assays are utilized to detect ACPA, with IgG1 subclass being predominantly involved and responsible for immunological activities through FcR-mediated cell activation. Immune complexes containing ACPA can stimulate the release of TNF by macrophages. Furthermore, ACPA has been shown to directly activate monocytes, leading to cytokine production and complement system activation [8,9] . Around 80% to 90% of RA patients with established disease exhibit ACPAs in their blood when using standard tests, these tests have a specificity close to 90% and are more accurate than RF in diagnosing RA [10]. Function of IL-17 encompasses a group of pro-inflammatory cytokines, IL-17A/F is responsible for inducing neutrophilia, promoting the mobilization of granulocytes through granulopoiesis, facilitating their migration via CXC chemokines, and prolonging their survival in target tissues. It also triggers the production of cyclo-oxygenase-2 (COX-2), nitric oxide (NO), and IL-6 in certain target cells[11,12].IL-17 Association with Diseases, IL-17 has been found to be elevated in various chronic inflammatory conditions including rheumatoid arthritis especially in cases resistant to anti-TNF therapy, psoriasis, cutaneous T lymphoma and multiple sclerosis,polymorphisms in the IL17RB gene which encodes a receptor for IL-17E now known as IL-25 have been linked to asthma [13] . A Role of IL-17 in RA Patients Complicated With Atherosclerosis[14] .

PATIENTS AND METHODS

This study was a case-control investigation conducted on a total of 140 participants were involved, with 70 of them diagnosed with RA, confirmed by rheumatologists using the ACR/EULAR 2010 Criteria and serological testing. The remaining 70 participants served as healthy controls.The data were collected from October 2022 to December 2022. Out of the 70 RA patients, 64 were female and 6 were male, and their ages ranged from 20 to 70. The healthy control group also consisted. A comprehensive questionnaire was used to collect this information.

Inclusion criteria (patients and healthy group)

***Patients group*:** Patients who will be enrolled in the research are thoroughly examined to ensure that they satisfy the precise diagnostic standards necessary for RA all patients between the ages of 20 and 70 who have a rheumatologist-diagnosed inflammatory arthritic condition and get a score of >or =6 on the 2010 ACR/EULAR Criteria and without Alzheimer`s disease or any other auto immmune disease.

Controls group: The number-age-gender was matched with patients apparently healthy individuals without joint discomfort or issues or Alzheimer`s disease or any auto immune disease and those without a family history of RA.

Exclusion criteria (patients and healthy group)

- Patients with other autoimmune diseases, systemic lupus erythematosus, Bachet disease, ankylosing spondylitis and multiple sclerosis
- Exclusion of Alzheimer's disease.
- patients over the age of 70 and those under the age of 20.

Samples Collection

Blood samples

In this study venipuncture was used to obtain 5 ml of blood from each patient and healthy control, 3 ml of this blood were then separated by centrifugation and a serum sample was divided into 2 aliquots in an Eppendorf tube after that isolated and stored at (-20°C) until it was needed (ELISA test) mean while 1.6 ml of blood was added to 0.4 sodium citrate to measure the erythrocyte sedimentation rate (ESR).

The diagnostic kits and their companies used in this study list in table No.1

Table No.1: The diagnostic kits and their companies

No.	Kits	Catalogue number	Company	Origin
1	Human anti-cyclic citrullinated peptide (ccp) ELISA kit	SL0154Hu	Sun long	China
2	Human interleukin 17 (IL -17) ELISA kit	SL 0978Hu	Sun long	China
3	Rheumatoid factor titer (ichroma II kit)		Spinreact	Spain

Ethical approval

The University of Kufa's College of Medicine's ethics committee gave its approval to this work. Additionally, approval from the Rheumatology Unit of Al-Sadr Medical City was received prior to the research project's start, as well as informed consent from each participant. All patients were informed about the study and gave their consent for researchers to collect a blood sample and complete a questionnaire for them.

Statistical analysis: Data was processed using Statistical Package for the Social Sciences (SPSS) version 26 for Windows (GraphPad Software, San Diego, California, USA) for statistical analysis.Used to compare the means between two groups, a T-test , while ANOVA was used for comparisons among multiple means. Statistical significance was defined as a p-value of <0.05, and high significance was considered if the p-value was <0.001. Chi-square (χ^2) test was utilized to compare two categorical variables.

RESULTS

In comparison of parameters between patients and controls, all inflammatory indicators were found to be higher in patients compared to the controls group these included heightened values for ESR (31.01), anti CCP (7.76) and IL-17(13.05) versus the ESR in control group was (12.13), Anti CCP was (5.57) and IL-17 was (6.25) these results demonstrate a statistically significant difference ($p < 0.05$).

Based on the disease activity score (DAS) 70 participants patients were divided into four groups remission (25 patients), mild (20 patients), moderate (20 patients) and severe (5 patients) the results of the correlation of parameters are shown in Table No.5. There are significant differences (p value 0. 001) of the concentration of ESR according to DAS-ERS the means of ESR are in remission (21.24 ± 2.92 mm/hr) in mild (29.90 ± 3.20), moderate (40.25 ± 5.70) and sever (47.40 ± 4.50), on the other hand RF titers, ACCP levels and IL-17 concentrations did not show any significant differences between the groups the p values of these parameters are 0.4, 0.6 and 0.1 respectively.

From other point shows patients who had gum problems were 43(61.4 %) while the control group was 9 (12.5%) had a gum problem Statistically, parameter had significant differences (p.value=0.0001), a total of 43 RA patients with gum problems and RA patients without gum problems were included in the study among the RA patients with gum problems 14 (20.0%) had experienced gum problems before the onset of RA 19 patients (27.1%) developed gum problems after the onset of RA and 10 patients (14.3%) had gum problems that were unknown either before or after the onset of RA.

In terms of completely tooth loss five RA patients experienced tooth loss with four patients in the age group less than45 years and one patient in the age group more than 45 years, the distribution of tooth loss indicated that 80.0% of the patients who lost their teeth were in the younger age group while the remaining 20.0% were in the older age group. Statistical analysis revealed a p-value of 0.09 suggesting a trend towards a higher occurrence of gum problems among RA patients, although the difference between RA patients with and without gum problems was not statistically significant.

Table No.2 The clinical parameters of patients and Healthy control

Parameters	Category	Patients		Controls		P.Value*
		No.	Percent %	No.	Percent %	
Hypertensive	Yes	29	41.4	7	9.7	0.000
	No	41	58.6	63	87.5	
Diabetics	Yes	13	18.6	2	2.8	0.000
	No	57	81.4	68	94.4	
Smoking	Yes	9	12.9	4	5.6	0.1
	No	61	87.1	66	91.7	
Gum problems & periodontitis	Yes	43	61.4	9	12.5	0.000
	No	27	38.6	61	84.7	

*Significant at p.value 0.05 or less

Table No.3 Patients' Clinical Parameters

Parameters	Category	No.	Percent %
Family history RA	Yes	25	35.7
	No	45	64.3
Diagnosis Disease duration	Early	21	30.0
	Established	49	70.0
DAS_ESR	Remission	25	35.7
	Mild	20	28.6
	Moderate	20	28.6
	Severe	5	7.1
Mean DAS_ESR ± SD	3.03 ± 1.18		

Table No.4 Parameters comparisons in patients and controls

Parameters	Groups		p. value*
	Patient (N = 70) Mean ± SE	Control (N = 70) Mean ± SE	
ESR mm/hr	31.01 ± 2.37	12.13 ± 0.88	0.0001
ACCP ng/ml	7.76 ± 0.65	5.57 ± 0.72	0.02
IL-17 Pg/ml	13.05 ± 1.25	6.25 ± 1.14	0.0001

Table No.5 parameters of RA patients according to DAS28-ESR

Parameters	DAS-ESR				p. value
	Remission(N=25) Mean ± SE	Mild (N=20) Mean ± SE	Moderate (N=20) Mean ± SE	Severe (N=5) Mean ± SE	
ESR mm/hr	21.24 ± 2.92	29.90 ± 3.20	40.25 ± 5.70	47.40 ± 4.50	0.001*
RF (Titer)	30.10 ± 5.28	42.27 ± 6.93	47.96 ± 10.76	52.65 ± 27.35	0.4
ACCP ng/ml	8.20 ± 1.25	6.80 ± 1.23	7.58 ±0.94	10.26 ± 2.98	0.6
IL-17 Pg/ml	12.09 ± 1.42	12.29 ± 1.94	16.93 ± 3.20	5.34 ± 4.36	0.1

Table No.6 Distribution of periodontal problems in patients with Rheumatoid arthritis

Variables	Category	RA patients with gum problems N=43 patients			RA patients without gum problems	P.value
		Patients ≤ 45 year N= 11	Patients >45 year N= 32	Total		
RA Patients with Gum Problems	**Before RA**	1	13	14(20.0%)	27 (38.6)	0.09
	After RA	7	12	19(27.1%)		
	Un known	3	7	10(14.3%)		
RA patients with completely tooth loss	No.	4	1	5	0	
	%	80.0	20.0	100%	0.0	

DISCUSSION

The gender distribution in this study revealed a significant difference in the incidence of RA between males and females, females showed a much higher prevalence of RA (91.4%) compared to males (8.6%) with a female to male ratio of 11.3:1, nearly findings was reported by Mohamed *et al.*[15] with ratios of 10.2:1. Increased prevalence of RA among women suggests that hormonal factors in women are involved in the onset and progression of the disease, the peak incidence of RA usually occurs during the fifth decade of life which coincides with the of menopause hormones such as estrogen for instance have been suggested to have a pro-inflammatory impact [16] . The statistical significance of the differences between the patient group and the control group for each measured parameter, in the case of the ESR (erythrocyte sedimentation rate) parameter, the p-value was determined to be 0.0001, the p-value for the ACCP (anti-cyclic citrullinated peptide) parameter was determined to be 0.02, indicating a statistically significant difference between the two groups, p-value for the IL-17 (interleukin-17) parameter was determined to be 0.0001.

Our results agree with Jafat [16] highlighted the significance of ACCP testing in predicting the prognosis of rheumatoid arthritis as it can detect ACCP production even before the onset of clinical symptoms, also our results align with the research conducted by Li *et al.* [17]. which observed a significant increase in serum IL-17 levels in patients diagnosed with rheumatoid arthritis (RA) compared to healthy controls.

IL-17 has emerged as a potential therapeutic target for RA due to its involvement in promoting the development of osteoclasts leading to bone destruction and

joint damage, IL-17-producing cells were initially identified within the synovial tissue of individuals with rheumatoid arthritis (RA). Several investigations have indicated elevated levels of both IL-17 and Th17 cells in the blood serum and synovial fluid of inflamed joints among RA patients, Furthermore, a large amount of IL-17 was synthesized in peripheral blood mononuclear cells (PBMC), with concentrations surpassing those found in the blood of healthy individuals[14]. the results indicate significant differences in ESR values among the four groups (p = 0.001*), the remission group had the lowest mean ESR value while progressively higher ESR values were observed in the mild, moderate and severe groups, this finding suggests a positive correlation between ESR levels and disease severity in RA patients.On the contrary the RF titer, ACCP level and IL-17 concentration did not exhibit statistically significant differences among the groups Regarding the assessment of DAS-ESR to RF this study found no significant difference with a p-value of (0.4)

The results of the study reveal that a majority of patients, around 61.4% of the sampled individuals with rheumatoid arthritis (RA), exhibited issues related to gum health and periodontitis, these results align closely with the studies conducted by Castellar-Mendoza *et al*,[18] which reported a prevalence of 70.5% for gum problems and periodontitis in RA patients, the increased occurrence of periodontal disease in RA patients may be attributed to the pro-inflammatory characteristics of bacteria which can act as a triggering factor in mucosal sites especially in individuals with genetic susceptibility or it may result from impaired periodontal healing in RA patients.

Conclusions:

the association between these biomarkers and disease severity in the study may not be clear, these results provide insights into the relationship between ESR and disease severity in RA patients. The rise in ESR levels as disease severity escalates underscores the potential practicality of using ESR as an indicator to evaluate disease activity in RA. Conversely, the absence of noteworthy correlations between RF titer, ACCP level, IL-17 concentration, and disease severity suggests that these biomarkers might hold greater utility for diagnosis rather than serving as dependable gauges of disease severity, The study findings demonstrate that a significant portion of patients, approximately 61.4% of those sampled who have rheumatoid arthritis (RA), experienced challenges concerning gum health and periodontitis, a noteworthy observation is that a higher proportion developed gum problems after the onset of RA compared to those who had pre-existing gum issues before the onset of RA.

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