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Research Article

Assessment of the Correlation between Disease Activity and Serum Biomarker Anti-MCV And IL6 in Iraqi Patients with Rheumatoid Arthritis

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Abstract:

Background: Rheumatoid arthritis is an autoimmune disease characterized by autoantibodies against citrullinated antigens. The anti-cyclic citrullinated peptide test is commonly used to diagnose rheumatoid arthritis, whereas the anti-mutated citrullinated vimentin is another anti-citrullinated antibody that reacts with mutated citrullinated vimentin. Anti-mutated citrullinated vimentin antibodies have been suggested as a superior early arthritis diagnostic marker.

Objectives: This study aimed to evaluate the levels of IL6 and anti-mutated citrullinated vimentin biomarkers as well as to determine their potential correlation with disease activity in rheumatoid arthritis Iraqi patients.

Methods: The study included an overall sample of 120 individuals who were recruited from the Department of Rheumatology at Baghdad Teaching Hospital in Baghdad, Iraq, during the period from late August 2023 to early October 2023. They were subdivided into two primary groups. The first group consisted of 60 individuals diagnosed with RA who were further categorized based on disease activity. The second group consisted of 60 healthy individuals as controls. The age range of the participants was between 20 and 79 years. An enzyme-linked immunosorbent test was used to evaluate the blood level of anti-MCV and IL-6.

Results: There was no significant correlation between anti-mutated citrullinated vimentin and disease activity(p-value:0.374) also IL-6 and disease activity(p-value:0.792) but our findings showed there is a statistical accusation between Erythrocytes Sedimentation Rate, and C-reactive protein with disease activity(p-value:0.013 and 0.025) also a high positive correlation between anti-mutated citrullinated vimentin and duration of disease and anti-mutated citrullinated vimentin with Rheumatoid factor and no significant correlation between anti-mutated citrullinated vimentin and IL6.

Conclusion: anti-mutated citrullinated vimentin autoantibody shows a high correlation with the duration of disease and a positive correlation with Rheumatoid factor and has no significant correlation with disease activity.

Keywords: Anti-mutated citrullinated vimentin; Autoantibody; cytokines; IL6**;** Rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that is a polyarticular, inflammatory arthritis of the small joints of the body that are inflamed and symmetrically irrigated. This causes inflammation throughout the joints, which in turn causes morning stiffness, tenderness, pain, swelling, and reduced mobility (1). A patient's mental health and quality of life may be greatly impacted by long-term chronic pain that occurs repeatedly in each joint. This discomfort can also lead to joint deformities (2). Rheumatoid arthritis prevalence is steady at roughly 0.5-1.0% worldwide, while it is greater in particular communities, such as North American Indians.

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Rheumatoid arthritis may strike anyone at any age (3). Having a female-to-male ratio of 3:1, the medical issue is more common among women (4).

Research has shown that the immune system plays a part in developing rheumatoid arthritis (RA) Inflammatory cytokines and enzymes that tear down cartilage and bone are released when immune cells infiltrate synovial joints, which is the first step in developing autoimmune disease (5). Immunoglobulin G's Fc region may be recognized by the Rheumatoid Factors (RF) antibody. In 1987, the ACR included RF in their criteria for classifying RA, and it was one of the first forms of autoantibodies found in the disease (6).Anti-citrullinated protein antibodies ACPA, a novel immunological marker for

RA, are rapidly employed to give improved specificity and better prognostic signs for RA patients (7)**.** It has been suggested that ACPA activates immune cells and up-regulates the production of inflammatory cytokines, which might explain why inflammation is a key factor in the development of RA (8). A study by Mohammed et al. (2023) showed that the diagnostic specificity of anti-CCP antibodies was greater in both early and established RA illness. Nevertheless, anti-MCV Exhibited enhanced sensitivity in detecting early rheumatoid arthritis compared to anti-CCP2. Utilizing anti-MCV antibodies to test for rheumatoid arthritis (RA) may assist in identifying the illness at its early stage. This can help choose the most appropriate first treatment, with more aggressive regimens being reserved for those with elevated levels of anti-MCV antibodies and predicted to have a severe and deforming course of the disease (9). One of the many members of the cytokine family that exhibits pleiotropic and redundant functional activity is the cytokine known as interleukin-6 (IL-6), which is considered to be the prototype kind (10). Interleukin-6 (IL-6) indicates that it has the potential to be a therapeutic target for the treatment of rheumatoid arthritis (RA). Both tocilizumab and sarilumab, which are both IL-6 inhibitors, have shown considerable effectiveness and safety in patients with rheumatoid arthritis who have not responded well to csDMARDs or tumor necrosis factor-alpha inhibitors. It is possible to take these drugs by themselves or in combination (11)**.** Thus the current study aimed to investigate the levels of IL6 and anti-mutated citrullinated vimentin biomarkers as well as to determine their potential correlation with disease activity in rheumatoid arthritis Iraqi patients.

Methods

Study population: A total of 120 individuals participated in this case-control study; 60 of them were diagnosed with rheumatoid arthritis (RA), while the other 60 served as healthy controls. The controls were selected to closely match the patients in terms of age and sex. The rheumatology outpatient clinic at Baghdad Teaching Hospital was the location where the patients were enrolled in the study between late August 2023 and early October 2023. A total of 60 Iraqi patients with RA were diagnosed by a rheumatologist using either the criteria established by the American College of Rheumatology (ACR) in 1987 or the criteria established by the European Alliance of Associations for Rheumatology (ACR-EULAR) in 2010. All patients were on treatment with different type of treatment. The inclusion criteria were adults over 18 years of age with either early or established rheumatoid arthritis, while exclusion criteria encompassed children and individuals with other autoimmune disorders such as systemic lupus erythematosus (SLE) and multiple sclerosis. A comprehensive medical history was collected for each participant, documenting basic demographic and clinical data, including age, gender, weight, height, family medical history and the duration of the disease. Body mass index (BMI) was calculated for all participants. The disease activity (SDAI score) was used to classify the patients into three different categories; mild, moderate, and severe, according to the disease activity of their condition. The SDAI is based on four variables: the patient's whole health condition on a visual analog scale from 0 to 10, the number of painful joints (0-28), the number of swollen joints (0-28), and C-reactive protein these four variable was used to elevate the disease activity. A control group of 60 persons who were in apparently good health and had no familial history of autoimmune illness were randomly selected.

Statistical analysis

For numerical variables that follow a normal distribution, the descriptive statistical analysis used the Mean±SD, whereas for variables that do not, the median (interquartile range) was employed. For the categorical data, percentages, and rates were computed. Using the Chi-squared test, we looked at the correlation between anti-MCV and several socio-demographic variables. To compare the means of the samples, we used the independent student *t*-test. Calculated the area under the curve (AUC) using the ROC curve to calculate the sensitivity and specificity of Ant-MCV. To find the factors that affect or are associated with IL6, multiple linear regression analysis using the enter approach. All statistical analyses were conducted using SPSS version 26. Using 0.05 threshold of significance to accept the findings.

Results

The demographic information of the cases and controls was broken out by age, gender, and age group, there were seven males and fifty-three females, with ages ranging from twenty to seventy-nine years old. The results demonstrated that the first two variables showed a good match between controls and patients (P = 0.732 and P = 0.676, respectively). There was a discernible disparity between the two sets of data. Table 1 showed the correlation between the disease activity and other parameters When the variables were compared with respect to disease activity using ANOVA, the mean Anti-MCV level were increased in the blood of RA patients with Low disease activity, i.e. 31.866 ± 54.141 (P= 0.424), compared to those with high disease activity (26.764 ± 56.739) (P=0.851) or moderate disease activity (23.008 ± 44.480) (P = 0.176). there was no statistical relation observed between Anti-MCV and disease activity P-value =0.374. The mean of IL6 values was increased in the blood of RA patients with moderate disease activity, i.e. 306.537 ± 59.450 (P=0.508), compared to those with low disease activity (301.505 ± 78.532) (P=0.552) or high disease activity (294.276 ± 86.155) (P=0.936) there was no significant relation was observed between IL6 and the disease activity P-

value =0.792. While the mean of Anti-CCP values were increased in the blood of RA patients with moderate disease activity, i.e. 112.396 ± 179.584 (*P*=0.863), compared to those with low disease activity (83.902 ± 65.150) (*P*=0.888) or high disease activity (63.880 ± 72.146) (*P*=0.987) there was no significant relation was observed between Anti-CCP and the disease activity, P-value =0.981.

Table 1: Mean, and Std. deviation distributions of study parameters and disease activity of patients' group

Disease activity		Mean	Std. deviations	P-value (LSD)	
ESR mm/h	Low activity	27.550	10.709	A	0.015
	Moderate Activity	33.350	15.776	B	0.015
	High activity	45.730	28.894	C	0.507
	One-way ANOVA test (P-value): P-value=0.013				
CRP Mg/L	Low activity	25.910	26.300	A	0.047
	Moderate Activity	29.480	32.345	B	0.015
	High activity	31.150	31.847	C	0.842
	One-way ANOVA test (P-value): P-value=0.025				
Anti-CCP Ng/ml	Low activity	83.902	65.150	A	0.888
	Moderate Activity	112.396	179.584	B	0.863
	High activity	63.880	72.146	C	0.987
	One-way ANOVA test (P-value): P-value=0.981				
RF U/ml	Low activity	105.907	78.746	A	0.709
	Moderate Activity	102.856	82.334	B	0.610
	High activity	114.772	69.872	C	0.999
	One-way ANOVA test (P-value): P-value=0.858				
Anti-MCV U/ml	Low activity	31.866	54.141	A	0.424
	Moderate Activity	23.008	44.480	B	0.176
	High activity	26.764	56.739	C	0.851
	One-way ANOVA test (P-value): P-value=0.374				
IL6 Pg/ml	Low activity	301.505	78.532	A	0.552
	Moderate Activity	306.537	59.450	B	0.508
	High activity	294.276	86.155	C	0.936
	One-way ANOVA test (P-value): P-value=0.792				

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, Anti-CCP: Anti-cyclic citrullinated protein, RF: rheumatoid factor, Anti-MCV: anti-mutated citrullinated vimentin, LSD: Least significant difference, ANOVA: One-way analysis of variance.

The mean of ESR values were higher in RA patients with high disease activity (45.730 ± 28.894 mm/h) *P*= 0.507 compared to those with moderate activity (33.350 ± 15.776 mm/h) P=0.015 or low disease activity (27.550 ± 10.709 mm/h) *P* = 0.015. A statistical relationship was found between ESR and moderate and low disease activity The *P*-value is 0.013. Mean CRP values were increased in the blood of RA patients with high activity of the disease, i.e. 31.150± 31.847 (*P*=0.842), compared to those with moderate activity (29.480 ± 32.345) (*P*=0.015) or Low disease activity (25.910 ± 26.300) (*P*=0.047) there was statistical relation was observed between ESR and moderate and low disease activity *P*-value =0.025**.** Mean RF values were increased in the blood of RA patients with high activity of the disease, i.e. 114.772 ± 69.872 (*P*= 0.999), compared to those with moderate activity (102.856 ± 82.334) (*P*=0.610) or Low disease activity (105.907 ± 78.746) (*P* = 0.709). there was no statistical relation observed between RF and disease activity because autoantibody plays a weak role in disease activity. One-way ANOVA test (*P*-value): *P*-value =0.858**.** Table 2 showed the correlation between Anti-MCV and other parameters there were positive significant relation between Anti-MCV and RF (r:0.229, *P*= 0.039), highly positive correlation with Duration of disease and Anti-MCV (r:0.381, *P*=0.001), Inverse significant relation were identified between Anti-MCV and ESR (r:-0.228, P:0.040) and there was no significant correlation was found between Anti-MCV and Anti-CCP (r:-.0.099, *P*=0.226), CRP (r:-0.152, *P*=0.123, Smoking(r:0.047, *P*=0.361), BMI (r:-0.144, *P*=0.137), Treatment Type (r:0.154, *P*=0.121). Table 3 showed the correlation between IL6 and other parameters there were no significant identified between IL6 and other parameters like Age (r: -0.032, *P*=0.405), BMI (r:0.030, *P*: 0.409), Duration (r:-0.058, *P*: 0.330), ESR (r: 0.034, *P*= 0.397), CRP (r: -0.087, *P*: 0.254), Anti-CCP (r: 0.051, *P*: 0.350), RF (r: -0.117, *P*: 0.187), Anti-MCV (r: -0.150, *P*: 0.126), Smoking (r: -0.049, *P*: 0.354), Treatment type (r: -0.110, *P*= 0.201) .

Table 2. Correlation between Anti-MCV and RA parameters in studied patients

Patients with Rheumatoid arthritis (N=60)		
Pearson correlation		Anti-MCV
RF	r	0.229
	P-value	0.039
Anti-CCP	r	-0.099
	P-value	0.226
CRP	r	-0.152
	P-value	0.123
ESR	r	-0.228
	P-value	0.040
Smoking	r	0.047
	P-value	0.361
Duration	r	0.381
	P-value	0.001
BMI	r	-0.144
	P-value	0.137
Treatment Type	r	0.154
	P-value	0.121

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, Ani-CCP: Anti-cyclic citrullinated protein, RF: rheumatoid factor, and BMI: body mass index.

Table 3 Correlation between IL-6 and RA parameters in studied patients

Patients with Rheumatoid arthritis (N=60)		
Pearson correlation		IL6
Age	r	-0.032
	P-value	0.405
BMI	r	0.030
	P-value	0.409
Duration (yrs)	r	-0.058
	P-value	0.330
ESR	r	0.034
	P-value	0.397
CRP	r	-0.087
	P-value	0.254
Anti-CCP	r	0.051
	P-value	0.350
RF	r	-0.117
	P-value	0.187
Anti-MCV	r	-0.150
	P-value	0.126
Smoking	r	-0.049
	P-value	0.354
Treatment type	r	-0.110
	P-value	0.201

ESR: Erythrocyte sedimentation rate, CRP:C-reactive protein, Ani-CCP: Anti-cyclic citrullinated protein, RF: rheumatoid factor, Anti-MCV: mutated citrullinated vimentin and BMI: body mass index

Discussion

This study found the mean± standard deviation of the age of patients diagnosed with rheumatoid arthritis (RA) was 50,02±12.907 years. This finding was consistent with previous research conducted on Iraqi RA patients by Mohammed AM et al(2023) and Rashid MK et al(2023) (12, 13). and other international studies (14-16). These studies mentioned that the onset of RA typically occurs during the middle years of life and that the disease is most commonly affects individuals who are over the age of 40 years**.** The present study indicated that RA is more common in females than in men, according to the gender difference in susceptibility at a ratio of 4:1 autoimmune disease is more common in females due to hormonal factors. This finding is roughly to the findings of a local study that was conducted by Albarzinji et al. (2023)(17, 18). This study proposes that even in treating RA patients with different types of treatments, the biomarker Anti-MCV is still detected in sera of these patients. This Anti-MCV autoantibody showed high sensitivity and less specificity (82.7%,72.1%) respectively. Similarly, a study conducted by Lee et al. (year) demonstrates that anti-MCV is more sensitive, but it is less specific, and it has worse diagnostic accuracy than anti-CCP in RA patients (19).Moreover, there is no significant association between anti-MCV and IL6, as well as between anti-MCV and anti-CCP, CRP, disease activity, smoking, body mass index, and the type of therapy. Their findings contradict the findings of previous studies, which demonstrate a high correlation between anti-MCV and anti-CCP (20, 21) The findings of this investigation were comparable to those of Nigm et al. (Year) who found no association between anti-MCV and disease activity (20, 22) and disagree with other study show correlation Anti-MCV with disease activity(23) Because Anti-MCV shows high positive correlation with duration of disease. In addition, a study by Nigm et al. (2022) showed that the Anti-MCV used to diagnose RA is independent of other marker and shows higher level in newly diagnosed RA. In this study most patient have duration of disease lasting more than 5 years also all patients were on treatment. No association was found between anti-MCV and CRP, according to research conducted by Nigm et al. (22).According to this research, there was a substantial correlation between Anti-MCV, ESR and RF, as well as a very high correlation with the duration of disease has been found. In agreement with the findings of the research carried out by Al-Shukaili et al (year) which demonstrated a substantial positive connection between anti-MCV and RF (24). Also another study similar to our study showed a significant association between Anti-MCV, ESR, and duration of disease (25).This study showed no significant correlation between IL6 and Anti-CCP, RF, ESR, CRP, and Anti-MCV similar to the study conducted by Abeer et al. (year) showed no significant correlation between IL6 and ESR, CRP (12). Also it was similar to a study conducted by Matsumoto et al. (year) who showed no correlation between IL6 and Anti-CCP(26). All patients in this study were on treatment with many types of anti-inflammatory drug. IL6 shows high sensitivity and specificity (98.7% - 91.3%) respectively. This study was similar to a study conducted by Abeer et al. (year) showed high sensitivity (92.50%) but this study disagreed with our study by showing very low specificity (42.50%)(12). The patients in this study suffered from the disease for a long period and type of treatment may influence. Thus, the current study aimed to investigate the levels of IL6 and anti-mutated citrullinated vimentin biomarkers as well as to determine their potential correlation with disease activity in rheumatoid arthritis Iraqi patients.

Limitations and recommendations

Sample size is always a challenge in such studies, but it is recommended that, where possible, future studies elucidate the anti-MCV antibodies how contributions to immunopathogenic mechanisms, as these may enhance disease diagnosis, management, and therapeutic options to confirm the present findings.

Conclusion

Anti-MCV autoantibody shows a high correlation with the duration of disease and a positive correlation with RF, but has no significant correlation with disease activity.

Authors' declaration

Adnan Al-Rubaee is an editorial board member but did not participate in the peer review process other than as an author.

We here by confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.

Ethical Clearance: As part of an MSC degree in microbiology, the study received approval from the Ethical Committee of the Research and Ethics Committee of the Immunology, Department of Microbiology College of Medicine Baghdad University, Baghdad, Iraq (October 2023).

Conflicts of interest: None

Funding: None.

Authors' contributions

Study conception & design: (Mohammed .M.Al-Ani, Nizar. A.L.Al-Ani). Literature search: (Dania.A.K.Ali). Data acquisition: (Dania.A.K.Ali). Data analysis & interpretation: (Dania.A.K.Ali). Manuscript preparation: (Dania.A.K.Ali). Manuscript editing & review: (Adnan Al-Rubaee, Mohammed .M.Al-Ani& Nizar. A.L.Al-Ani).

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تقييم العلاقة بين نشاط المرض والمؤشرات الحيوية العراقيين المصابين بالالتهاب المفاصل الرثوي في مصل المرضى IL6 ,Anti-MCV

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عدنان الربيعي / قسم علوم الدواجن جامعة اركنساس/فايتفيل/ الولايات المتحدة الامريكية

الخلاصة

خلفية البحث: التهاب المفاصل الرثياني أو الداء الرثياني أو التهاب المفاصل الروماتويدي هو أحد أمراض المناعة الذاتية التي تتميز بوجود الأجسام المضادة الذاتية ضد المستضدات السيترولينية. يُستخدم اختبار الأجسام المضادة المقاومة للببتيد السيترولييني الحلقي (AntiCCP)بشكل شائع لتشخيص التهاب المفاصل الروماتويدي، في حين أن الفيمتين السيترولييني المضاد للتحور (Anti-MCV)هو جسم مضاد آخر مضاد للسيترولينات يتفاعل مع الفيمتين السيترولييني المتحور. تم اقتراح الأجسام المضادة (MCV)كعلامة تشخيصية مبكرة لالتهاب المفاصل.

الاهداف: يهدف هذا البحث إلى تقييم مستويات المؤشرات الحيوية لـ IL6 وAnti MCV مع نشاط المرض، وتحديد العلاقة المحتملة بين Anti-MCV وIL6 في المرضى الذين يعانون من التهاب المفاصل الرثوي.

المواد وطرق العمل: مئة وعشرون شخصا تم تقسيمهم الى مجموعتين، المجموعة الأولى 60 شخصا مصاب بالتهاب المفاصل الرثوي تم تقسيمهم بالاعتماد على نشاط المرض , و المجموعة الثانية 60 شخص سليم تتراوح أعمارهم (20-79) تم استخدام مقايسة الممتز المناعي المرتبط بالأنزيم (ELISA) لتحليل مستويات Anti-MCV و IL6في الدم

النتائج: لم يكن هناك ارتباط كبير بين مضادات MCV ونشاط المرض وكذلك IL6 ونشاط المرض ولكن النتائج التي توصلنا إليها تظهر أن هناك ارتباط إحصائي بين ESR وCRP مع نشاط المرض، كما توجد علاقة إيجابية قوية بين مضادات MCV ومدة المرض ومضادات MCV معRF,النتائج التي توصلنا إليها لا تظهر أي علاقة ذات دلالة إحصائية بين Anti-MCV و IL6.

الاستنتاجات: تُظهر الأجسام المضادة لـ MCV ارتباطًا عاليًا مع مدة المرض وارتباطًا إيجابيًا مع RF، وقد تؤدي هذه النتيجة إلى جعلها أداة تشخيصية جديدة لتشخيص RA بالإضافة الى استخدام RF و.Anti-CCP.

الكلمات المفتاحية: التهاب المفاصل الرثوي، الأجسام المضادة الذاتية، مضادات MCV، الحركيات الخلوية ، IL6.