Identification serum CXCL13 chemokine as a novel biomarker in diagnosis of rheumatoid arthritis

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Abstract:
Background: Rheumatoid arthritis is a systemic inflammatory autoimmune disease, characterized by chronic polyarthritis. The determination of novel biomarkers to RA patients very necessary to facilitate quick diagnosis and management of the disease. Rheumatoid arthritis researchers has a recently discovered biomarker, CXCL13, which can be regarded as part of pathogenesis of RA. Objective: Comparison diagnostic value of CXCL13 Chemokine with Anti-cyclic citrullinated peptide (CCP) antibody in RA patients. Methods: A case control study was carried out during the months of November 2021 and March 2022. 60 patients were obtained from the Rheumatology Unit at Al-Sadr Medical City, and an additional 60 individuals were used as the control group. Laboratory examinations such as ESR and rheumatoid factor (RF) were obtained as routine work for RA patient's. CXCL13 and Anti-CCP antibodies were measured by enzyme-linked immune-sorbent assay. Results: Most of the patients were women 46(76.7%), making the female-to-male patient ratio (3.2:1). Patients have a greater CXCL-13 concentration (0.913±0.52) compared to healthy controls (0.417±0.28) (P value = 0.000). Moreover, anti-CCP had highest AUC (0.94%) than CXCL13 (0.88%) and RF (0.76%) at significant value is 0.000. The sensitivity of CXCL13 (93%) was the highest and was comparable to anti-CCP (83%) and RF (68%). As regard the specificity, anti-CCP (94%) was the highest and was comparable to that of CXCL13 (83%) and RF (83%). Conclusions: The sensitivity of CXCL13 was higher than that of anti-CCP antibodies for determining RA patients but specificity for CXCL13 was less. With its outcomes, CXCL13 can play an additive role in establishing the diagnosis of RA.

Keyword: Rheumatoid arthritis (RA), Anti-cyclic citrullinated peptide (CCP) antibody, serum CXCL13 Chemokine

1. Introduction

Rheumatoid arthritis is a chronic inflammatory condition that affects more than one organ. The most important clinical sign is polyarthritis that affects mostly the small joints and is spread evenly. RA can affect other parts of the musculoskeletal system (bursitis, muscle atrophy, and osteoporosis) and almost every organ in the body. [1] RA disease that affects around 1% of adults globally.[2] Different autoimmune and inflammatory processes are involved in RA, causing the disease entity clinically and pathobiologically diverse.[3] However, the complicated interaction of immune modulators (cytokines and effector cells) results in joint destruction that starts at the synovial membrane. Synovitis is characterized by the inflow and/or local activation of mononuclear cells (including macrophages and T, B, plasma, dendritic, and mast cells) as well as the development of new blood vessels. [4] Chronic inflammation in the synovium of people with RA is caused by the release of chemokines, cytokines, matrix metalloproteinases (MMPs), and growth factors, which stay the innate and adaptive immune systems working all the time. In addition, chemokine receptors and ligands have been linked to different parts of the development of RA, such as inflammation and the growth of new blood vessels. [5]Chemokines are made in different amounts at different stages of RA. CCL4, CXCL4, CXCL7, and CXCL13 were released at an early stage, while CCL3 and CCL9 were released at a later stage. [6] The chemokine CXCL13 works a crucial function in the development of an adaptive immune response. Follicle growth depends on C-X-C motif chemokine 13 (CXCL13). Furthermore, both tumor necrosis factor alpha (TNF) and T cell receptor activation raise CXCL13 expression.

C-X-C chemokine receptor type 5, often known as (CXCR5), is the sole known receptor for CXCL13. Naive B cells and TFH cells both express this receptor. By attracting B cells to the secondary lymphoid tissue, it stimulates the production of antibodies and causes inflammation in the local area. [6] Patients with RA have ACPA and rheumatoid factor (RF) in their blood years before they are diagnosed with RA. While these autoantibodies, particularly anticyclic citrullinatedpeptide (anti-CCP), are highly specific (~95–99%) for RA, the sensitivity in those who later develop RA is notably lower. Yet sensitivity of these combinations is still limited. Additional biomarkers that improve sensitivity for RA while maintaining high specificity would be useful diagnostic and prediction tools. [7] The aim of this study was to be measuring CXCL13 diagnostic value

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by compare it with Anti-CCP. For accuracy

2. Methods

Subjects

A case control study carried out in Al-Sadr Teaching City in Al-Najaf province, Iraq for periods from November 2021 and March 2022.A convenient sample of RA Patients were selected from patients seen in the rheumatology clinic .60 healthy voluntary controls. Patients and controls range in age from 20 to 70 years.

Patient's selection

Inclusion criteria

Patients in the age range of 20–70 years old who have been diagnosed with rheumatoid arthritis by a rheumatologist utilizing the ACR/EULAR Criteria from 2010 and who have a score of six or higher on this criterion.

Exclusion criteria: - People with a history of severe allergies, other autoimmune illnesses, or malignant tumors will not be allow to participate.

Ethical approval

Before the beginning of the research project, the ethical committee of the Faculty of Medicine at the University of Kufa gave this study their approval, and informed consent was obtained from participants in the study. The research was explained to all of the patients, and they all consented to having their blood drawn and to having a questionnaire filled out about them.

Sample collection and processing:

Each patient and control participant used to have five to ten millilitres of blood. The serum sample from each patient and control were placed in an Eppendorf tube. The tubes were kept at -20 to -45 °C until they were used. During the ESR test, blood is put into a Westergren tube until it reaches the 200 mm mark. Inflammatory markers (RF and ESR) were measured. Use of an ELISA kit (Bioassay technology Lab Company) for determining the presence of Anti-Cyclic Citrullinated Peptide and serum CXCL13 chemokine in the serum of patients and control groups.

3. Data analysis

The results of this study were processed by using SPSS program version 20. Categorical parameters were represented by frequencies and percentages, whereas continuous variables were represented by mean and standard deviation. The Mann Whitney U test was utilized to compare the study groups, duration of disease and treatment response. The probable utility of serum CXCL13 chemokine as RA diagnostic indicator in comparison to RF and anti-CCP antibodies were measured by performance ROC curve.

Results

This study comprised a total of 60 individuals who were diagnosed with RA as well as 60 healthy individuals who served as controls. Demographic characteristics of the studied groups were demonstrated in table 1. Most of the patients were women 46(76.7%), making the female-to-male patient ratio (3.2:1).In addition, 16 patients (26% of the total) were living in rural areas, whereas 44 patients (73.3% of the total) were living in urban areas. According to age, the majority of RA patients are within the age category of age >= 40 years old (45; 75%). The percentage of people suffering from RA who also has hypertension was 26.7%, while the percentage of people suffering from diabetes was 20%.

According to age, the majority of controls were found within the age >=40 years old. The control group had a mean age of 42.8±1.5. There were 41 women and 19 men who served as controls. The majority of healthy individuals were found to be live in urban centres 36(60%). The control group's percentages of with hypertension and diabetes were 20% and 15% respectively.

Table 1: Demographic and clinical characteristics of the study population

Variable		RA Patients No. (%)	Control No. (%)
Age group	<40	15(25)	21(35)
	>=40	45(75)	39(65)
Gender	Male	14(23.3)	19(31.7)
	Female	46(76.7)	41(68.3)
Residency	Urban	44(73.3)	36(60)
	Rural	16(26.7)	24(40)
Hypertension	Yes	16(26.7)	12(20)
	No	44(73.3)	48(80)
Diabetes Mellitus	Yes	12(20)	9(15)
	No	48(80)	51(85)

The averages of Anti-CCP and CXCL-13 are shown in the table 2. Patients have a greater CXCL-13 concentration (0.913±0.52) compared to healthy controls (0.417±0.28) (P value = 0.000). Patients had a greater Anti-CCP concentration (73.7±7.9) than controls have (15.3±1.2), and this difference is statistically significant (P value = 0.000). The table below (table 2) shows the correlation between serum RF level in healthy controls and RA patients. 43(71.7%) RA patients had a positive RF, compared to only 7(11.7%) of controls. There were 17 (28.3%) individuals with RA who had a negative RF, or and 53(88.3%) patients in the control group. There is a significant alteration between the study

groups (controls and patients) when measure up positive and negative RF (P value = 0.000).

Table 2: Comparisons biomarkers with studied groups			
parameters	controls (n=60) Mean ±SE	patients (n=60) Mean ±SE	p. value*
CXCL13	15.3±1.2	73.7±7.9	0.000
Anti-CCP	0.417±0.28	0.913±0.52	0.000
RF +ve N (%)	7(11.7)	43(71.7)	0.000
RF -ve N (%)	53(88.3)	17(28.3)	0.000

The potential value of CXCL13 as diagnostic markers for RA compared to RF and anti-CCP antibodies were presented by performance ROC curve (figure 1). ROC curve is the plotting of sensitivity versus 1-specifity and plays a central role in comparing two alternative diagnostic tests when each test is performed on the same disease. The area under the curve (AUC) was used as an effective measure of accuracy of biomarkers in diagnosis of disease. Moreover, anti-CCP had highest AUC (0.94%) than CXCL13 (0.88%) (Show figure1) and RF (0.76%) at significant value is 0.000 for overall. The RF antibodies had low sensitivity (68%) of diagnostic potential compared to CXCL13 (93%) anti-CCP (83%) and. As regard the specificity, anti-CCP (94%) was the highest and was comparable to that of CXCL13 (83%) and RF (83%). PPV and NPV for CXCL13 were calculated as 83%, 93% respectively, while PPV and NPV of anti-CCP were 94%, 84% respectively (table 3).

Table 3: Sensitivity, specificity, PPV, NPV and AUC of study biomarkers						
Biomarkers	sensitivity	specificity	PPV	NPV	AUC	P value*
CXCL13	93	83	83	93	0.88	0.000
Anti-CCP	83	95	94	84	0.94	0.000
RF	68	83	78	74	0.76	0.000

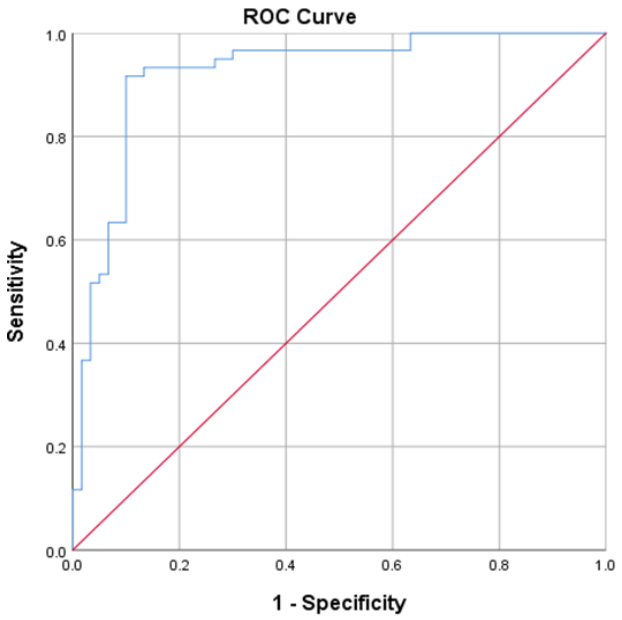

Figure 1: Receiver operating characteristic (ROC) curve of CXCL13.

The RA patients who participated in this study were classified into two groups, G1 (=<12) month with 23 patients and G2 (>12) month with 37 patients, based on the length of time they had been living with the disease. The table (4) presents the averages of CXCL-13 and demonstrates that there is a statistically significant difference between G1 and G2 (0.7860.03 and 0.991±0.08 correspondingly with a P value of 0.05).

The means of ACCP in Group 1 were (59.4± 4.4), while the means of ACCP in Group 2 were (82.6 ±12.4) at (P value =0.1). The means of ESR in Group 1 were (29.1± 2.8), and the means of ESR in Group 2 were (36.2 ±4.4) at (P value =0.2); the means of DAS-28ESR in Group 1 were (4.3± 0.2), while the means of G2 were (4.7±0.2) at (p value=0.2).

Whereas the means of CDAI in Group-1 (17.7±1.7) and Group-2 (21.3±1.6) at (P value = 0.1). This table (4) demonstrates that, with the exception of CXCL-13, there are no statistically significant differences between any of the research parameters and the duration of disease

Table 4: Comparison between study parameter according to the disease duration			
Parameters	Disease duration		P value
	<=12 (n=23) early patients Mean± SE	>12 (n=37) established patients Mean± SE	
CXCL-13	0.786±0.03	0.991±0.08	0.05
ACCP	59.4±4.4	82.6±12.4	0.1
ESR	29.1±2.8	36.2±4.4	0.2
DAS	4.3±0.2	4.7±0.2	0.2
CDAI	17.7±1.7	21.3±1.6	0.1

The RA patients who participated in this research were gave either a good or a poor rating based on how they responded to treatment. Table (5) displays the average CXC13 for good 0.899±0.60 and poor 0.940±0.1 at P value = 0.7 and the average ACCP for good is 73.89.2 and for poor is 73.615.2 at P value = 0.5. Mean ESR values included good (27.1±2.4) and poor (46.3±6.6), with a significant difference at (P 0.009); mean DAS28-ESR values reported good (4.1±0.1) and poor (5.4±0.2), with a significant

alteration at (P 0.0001). While the mean CDAI for good was 16.61.2 and for bad was 26.22, there was a big difference between the two (P value = 0.0001). Except for the ESR, DAS28-ERS and CDAI, none of the study parameters shows a significant difference in how well a treatment works.

Table (5): Comparisons of treatment response and study parameters

Parameter	Response		P
	Good (n=40) Mean±SE	Poor (n=20) Mean±SE	
CXCL-13	0.899.5±0.60	0.940±0.1	0.7
ACCP	73.8±9.2	73.6±15.2	0.5
ESR	27.1±2.4	46.3±6.6	0.009
DAS	4.1±0.1	5.4±0.2	0.0001
CDAI	16.6±1.2	26.2±2	0.0001

4. Discussion

According to the results of this research, the majority of RA patients are in the age category of more than 40 years old. This result is almost identical to that found by Neovius, which revealed a significant incidence of RA in people between the ages of 40 and 60.[8]

Based on the findings of this study, the prevalence of RA is significantly higher in females than it is in males (ratio=3.2:1) (23.3 % in males vs. 76.7% in females). According to Hussein study, the female to male ratio was 4.5:1,[9] which is different from the findings of Al-Bedri and Mohamed, who reported that the ratios were 7.6:1 and 10.2:1 in their studies, respectively.[10] [11]

In concepts of where they lived, most of the findings (73.3% of them) came from urban centers. Liu supports this result by study was done in China, and the results showed that 72.8% of the patients lived in cities or towns. This is because the research was carried out in a metro region, which explains why these results were found. [12]

According to the clinical parameters, the findings of the research show that the proportion of patients who suffered from hypertension was 26.7%, and the percentage of patients who suffered from diabetes mellitus was 20.0%. This outcome was agreed with the results of Labitigan, which discovered that the proportion of people with RA who also suffered from hypertension and diabetes mellitus was, respectively, 40% and 11%.[13]

The findings of this research show that CXCL-13 levels of RA patients are significantly higher than those of the control group (p<0.000). This finding was confirmed by Bechman, who found that the CXCL13 levels were significantly more frequency in RA patients than those of healthy control.[14]

The CXCL13 biomarker had high sensitivity (93%) of diagnostic potential compared to RF (68%) and anti-CCP (83%). As regard the specificity, CXCL13 had, the same specificity with RF was 83% and it is low comparable to that of anti-CCP antibodies (95%), while PPV of CXCL13 was good (83%). Moreover, anti-CCP (0.94%) had highest AUC than both CXCL13 (0.88 %) and RF (0.76%).In results of other study conducted by Rioja, which has been reported that CXCL13 sensitivity, specificity and PPV were 68%, 81% and 79% respectively.[15]

The current investigation discovered a statistically significant relationship as well as a slightly positive correlation between CXCL13 and the duration of disease (p = 0.05, R = 0.431). Sherif observed that there was significant connection between CXCL13 level and disease duration (r = 0.41), which added credence to the findings presented here.[16]

In point of fact, the study found no significant link between CXCL13 and treatment (P=0.7), a finding that is in same with the results of Bugatti et al., (2014), who found that there was no change in CXCL13 levels following treatment. In contrast to the findings of a study that was carried out by Meeuwisee, it was revealed that serum CXCL13 levels responded effectively to therapeutic intervention in the form of anti-TNF medication.[17]

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