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Study of Collagenase-3 Levels in Rheumatoid Arthritis Patients with and without Type 2 Diabetes Mellitus

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Abstract

This research sought early signs of type 2 diabetes mellitus (T2DM) in rheumatoid arthritis (RA) patients. The current research's objectives are to identify collagenase-3 (CL-3) in RA patients with and without T2DM as a disease complication, compare these findings to those of a control group, and ascertain whether or not CL-3 correlates with all of the parameters that were examined in each group. In the present research, (150) participants all between the ages of 30 and 50 were divided into three groups: (G1) control (N=50), (G2) RA (N=50), and (G3) RA with T2DM (N=50). Collagenase-3, glycated hemoglobin (HbA1c), erythrocyte sedimentaion (ESR), anti-cyclic citrullinated peptide (anti-CCP), and rheumatoid factor (RF) were all measured in this experiment. Data revealed that (G2) and (G3) had considerably greater RF, anti-CCP, and ESR levels than (G1) in comparison. Compared to G2, there was a very obvious increase in G3. The results of the HbA1c test showed that (G2) increased non-significantly compared to (G1), but (G3) was much more than (G2) and (G1). The levels of CL-3 in (G2) and (G3) were found to be significantly higher than in controls (G1) in these groups. A well-related biomarker with these patients was shown by CL-3, demonstrating a strong positive or negative association with all metrics for all groups. This suggests that the best medicine and therapy will be available for these patients. These results suggest that CL-3 might be a biochemical diagnostic for RA patients' early diagnosis of diabetes.

Keywords: Collagenase-3, rheumatoid arthritis, rheumatoid factor, type 2 diabetes mellitus.

1. Introduction

Rheumatoid arthritis, often known as RA, has been connected to abnormalities in glucose metabolism, most notably insulin resistance, which may lead to type 2 diabetes (T2DM) [1]. This connection has been shown in a few different cases. The research found that patients with RA had a greater risk of acquiring diabetes, but the researchers were unable to determine whether or not those who already had T2DM had an increased risk of developing RA. One of the purposes of the research as a result is to investigate the possibility of an occurrence in patients who have T2DM [2]. The development of tolerance to self-antigens is facilitated by regulatory T cells,

while the pro-inflammatory response to infections is mediated by Th17 cells. The balance between Treg and Th17 cells is disrupted in RA as well as other inflammatory disorders. Because of a rise in the amount of glucose that is consumed and the number of glycolytic pathways, the metabolic state of RA undergoes a transition from a low-energy state to a high-energy state [3].

In human beings, the matrix metalloproteinase-13 (MMP-13) gene [4] is responsible for encoding the collagenase-3 (CL-3) enzyme. It is a member of the MMP family, which stands for matrix metalloproteinases [5]. The MMP-13 has a molecular weight of 54 kDa, according to the calculations [6]. While the catalytic domain of CL-3 is ineffectual on its own, the hemopexin domain of CL-3 is involved in the degradation of collagen. Is expressed in the skeleton during the whole of embryonic development because it serves as a prerequisite for the reorganization of the collagen matrix, which is essential to stimulate bone mineralization. Pathological diseases, such as human carcinomas, RA, and osteoarthritis (OA), are examples of those in which it is significantly overexpressed [7,8]. It is hypothesized that the collagenase subfamily of MMP plays a part in the articular cartilage degradation that is characteristic of RA. Since CL-3 has a preference for collagen type II, it is a feasible candidate in the process of articular cartilage turnover and a prospective therapeutic target [9,10].

2. Materials and Methods

Everyone who took part in this study was between the ages of 30 and 50, and they were randomly assigned to one of three groups: (G1) control (N=50), (G2) RA (N=50), or (G3) RA with T2DM (N=50). There are a total of 150 people taking part in this study. Rheumatologists who have recently diagnosed (before to patients commencing treatment) patients at the teaching hospitals located in Al-Yarmuk and Baghdad during October 2022 and January 2022. The person interviews with each patient were carried out using a recently developed questionnaire format that contained a detailed account of the medical history of each patient. The interviews were conducted in this manner all the way through.

The tests that were varied out of the biochemical examinations were rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), and CL-3 where a sample of serum was used. Also the test erythrocyte sedimentation rate (ESR) and glycated hemoglobin (HbA1c) that a sample of whole blood was used to get an accurate reading.

To analyze the data, the statistical program known as Statistical Packages for Social Sciences-version 21 was used. The mean value as well as the standard deviation were utilized to present a condensed overview of the data. The student's t-test, which is used to determine the degree of separation between two independent means, was used to explore the significance of the difference in mean between a variety of "quantitative data" kinds. In cases where the *P*-value was less than 0.05 or was otherwise comparable to that value, it was decided that statistical significance existed. Highly significant (HS) expressed at $p \leq 0.01$.

3. Results and Discussion

Rheumatoid arthritis may be a chronic, multisystemic, autoimmune, and inflammatory condition that can produce substantial functional impairment and depressive feelings. The performance of routine duties and activities at work may be adversely affected as a result of these changes, which may eventually influence life quality [11].

Serum levels of anti-CCP and CL-3 were tested in three groups: G1 represented the control group, G2 represented patients with RA, and G3 represented patients with RA who also had T2DM as a consequence.

When compared to group (G1), the serum RF levels in groups (G2) and (G3) were significantly greater than those seen in group (G1), as shown by the data in **Table 1**. In addition, a statistically significant higher quantity was recorded in (G3) in comparison to what was seen in (G2).

Table 1. Levels of RF, ESR, and HbA1c in the studied groups.

Parameter	mean± SD			*p*-value		
	G_1 (n=50)	G_2 (n=50)	G_3 (n=50)	G_1& G_2	G_1& G_3	G_2& G_3
RF (IU/mL)	0.95±0,60	3.57±0,76	3.69±0,73	HS	HS	NS
ESR (mm/hr)	12.33±4,21	34.37±8,36	45.10±7,43	HS	HS	HS
HbA1c (%)	4.87±0.46	5.14±0.59	7.82±0.97	NS	HS	HS

**HS: High significantly at the 0.01 level.

The RF autoantibodies are generated locally by B cells in lymphoid follicles and germinal center-like structures that arise in inflamed RA synovial. These autoantibodies directly bind to the "fraction crystallizable" (Fc) component of accumulated IgG. Studies have shown that RF testing in individuals with RA has a sensitivity of up to 90% and an accuracy ranging from 48 to 92%.

According to **Table 1**, groups (G2) and (G3) revealed a significant increase in ESR levels compared to group (G1). Results agree with another study [12]. When examining a variety of clinical diseases, including RA, an elevated ESR might be of assistance [13]. In RA patients, the ESR is often use as a monitoring tool for inflammation even though it is not a diagnostic tool for the underlying cause of the disease. It is the result of the ESR strategy, which is uncomplicated, achievable, applicable, and inexpensive in addition to having great therapeutic significance. An elevated ESR may be a result of inflammatory responses or the breakdown of tissue in the body, described in previous study [14]. The levels of HbA1c were also established as a part of this inquiry that was carried out. The results of the HbA1c test showed that the rise in (G2) was not statistically significant when compared to the increase in (G1), but the increase in (G3) was statistically significant when compared to both (G2) and (G1). The results of the vast majority of high-quality research point to a relationship between RA and the onset of T2DM. The potential relationship between RA and incident T2DM may be cause by the inflammatory responses that are triggered when both diseases occur. Both T2DM and RA are strongly linked to persistent inflammation throughout the body. Moreover, RA has been linked to the production of pro-inflammatory cytokines such as TNF-α and IL-6, which lead to systemic inflammation and insulin resistance. Insulin resistance, in turn, contributes to T2DM by blocking insulin action [15]. In addition, the immune cells in the liver produce proinflammatory cytokines in response to the inflammatory mediators that are generated by the adipose tissue. The development of RA has been linked to a wide variety of cytokines that promote inflammation [16]. In individuals with RA, impaired glucose metabolism has been linked to both clinical symptoms that are difficult to control and a considerably increased risk of developing T2DM [17].

The CL-3 and anti-CCP levels are presented for all of the groups that are the focus of this inquiry in **Table 2**.

In **Table 1**, patients with RA (G2) and patients with T2DM (G3) exhibited anti-CCP levels that were significantly greater than those of the controls (G1). In addition, in comparison to G2, it was found that G3 had a substantially large increase.

Table 2. Levels of anti-CCP and CL-3 in the studied groups.

parameter	mean± SD			*p*-value		
	G_1 (n=50)	G_2 (n=50)	G3 (n=50)	G_1& G_2	G_1& G_3	G_2& G_3
Anti-CCP (U/mL)	0.31±0.09	0.61±0.52	0.74±0.15	HS	HS	HS
CL-3 (ρg/mL)	3.99±149.49	3037.75±1452.64	4013.49±2181.23	HS	HS	HS

**HS: High significantly at the 0.01 level.

The peculiar amino acid citrulline is one of the antigenic determinants that may be bound by autoantibodies that are directed against anti-CCP. Although it is possible to praduce amino acids by post-translational modification, the amino acid is not incorporated into the protein during the synthesis process itself. This is because post-translational modification is a process that occurs after translation has taken place. This is because the amino acid is not produced until after the synthesis of the protein has taken place. Citrullination is a process that involves the hydrolysis of the positively charged NH_2– group that is present in the amino acid arginine into a neutral oxygen group. This process is catalyzed by enzymes. This method is also known as deamination in certain circles. People who suffer from RA are more likely to develop autoantibodies because these antibodies can detect a particular oxygen group included inside peptidyl citrulline. Citrulline residues, which are a key component of antigenic determinants such as these, are one of the antigenic determinants that may be identified by RA antibodies. Anti-CCP testing is particularly helpful in the diagnosis of RA [18] due to its high specificity early on in the progression of the disease as well as its ability to identify those who are likely to suffer from significant sickness and damage that cannot be repaired. Both of these qualities are important in determining who will ultimately be affected by RA. Both of these symptoms may be seen in a patient who is at an early stage of the disease's development [19].

The data that are presented in **Table 2** for CL-3 illustrate that G3 has risen in a manner that is statistically significant when compared to the prior value. This is shown by the comparison of G3 to G2 and G1, which are both displayed in the Table. In addition to this, it was discovered that G2 had significantly improved in contrast to G1's performance. People who have T2DM have an increased risk of experiencing a rapid deterioration of the knee cartilage [20], which is now the issue that attracts the most attention. In particular, MMPs are anticipated to play a significant role in the process of cartilage disintegration during the induction of degenerative cartilage alterations that occur as a result of OA [21]. This is because MMPs are known to have a significant role in the progression of OA. This is because osteoarthritis is known to produce changes to the cartilage, which may result in discomfort and stiffness in the joints. The MMPs are the primary proteolytic enzymes of these proteins; they can destroy type II fibrillar collagen, in addition to collagen types IX, XI, and VI, as well as extra secondary collagens [22,23]. They also have the

potential to cleave additional secondary collagens. It is plausible to infer that this is the case given that the majority of cartilage's extracellular matrix is made up of proteoglycans, which are made up of collagen. In addition to this, MMP-13 was shown to be present in the synovial tissue of persons who had either OA or RA [24]. It has been shown that CL-3 is capable of degrading types I, II, and III of collagen, in addition to cartilage proteoglycan aggrecans. The characterization of CL-3 by the use of biochemical methods showed that it participates in a wide range of actions that are aimed at the components of connective tissue. Because CL-3 prefers to degrade collagen type II of hyaline cartilage rather than CL-3, which degrades this substrate more efficiently, one is tempted to speculate that CL-3 is an essential component of the cellular machinery that is responsible for the turnover of articular cartilage. This speculation is based on the fact that CL-3 prefers to degrade collagen type II of hyaline cartilage. This hypothesis is supported by the observation that CL-3 shows a strong preference for degrading type II collagen in hyaline cartilage [25]. As a direct consequence of this finding, this molecule would subsequently be brought to light as a potential therapeutic target for the treatment of cartilage degradation. A new glimmer of hope for the treatment of OA emerged not too long ago in the shape of an inhibition of CL-3, which was reported by Li and colleagues [26]. In the antibody-induced arthritis paradigm, the pharmacological lowering of CL-3 did not result in decreased arthritis; however, it did result in decreased arthritis in the collagen-induced arthritis model and the severe mixed immunodeficiency mouse implantation model [27].

Recent study has focused on using a model of arthritis called the K/BxN sera-transfer model to explore the part that CL-3 plays in the illness. This model is known as the K/BxN sera-transfer model. In the K/BxN paradigm, arthritis develops on its own in mice that express both the transgene-encoded KRN T-cell receptor and the IAg7 major histocompatibility complex class II allele [28,29]. This occurs because the KRN T-cell receptor acts as a trigger for the development of arthritis in these animals. This is because it is believed that the KRN T-cell receptor is involved in the development of arthritis. This is because the KRN T-cell receptor is important for the development of arthritis. Animals that are deficient in CL-3 have a reduced risk of inflammatory conditions and joint damage [30, 31]. This occurs as a result of an increase in the expression of CL-3 in C57BL/6 mice throughout the K/BxN serum-induced arthritis. There is an increase in the expression of CL-3 as the K/BxN serum-induced arthritis progresses in C57BL/6 mice. This increase in expression may be seen throughout the arthritis. Collagenase3 is the most effective collagenase when it comes to type II collagen, which is the primary structural collagen that is present in articular cartilage. This is the case since type II collagen is the primary constituent of articular cartilage. A diverse network of signal transduction pathways is activated when powerful pro-inflammatory stimuli such as IL-1 and OSM are present [32].

Research has demonstrated that a broad array of pro-inflammatory stimuli all use the same pathways to cause inflammation. These routes, when activated in conjunction with one another, have a synergistic effect that, when applied to chondrocytes, significantly boosts the synthesis of collagenase-3 [33]. Although we have just recently reported a role for the AP-1-binding factor activating transcription factor 3 (ATF3) in selectively mediating CL-3 expression that was nonetheless dependent on AP-1 [34]. It is essential to keep in mind that the cFos/cJun activator protein-1 (AP-1) transcription factor complex is required for the expression of CL-3. In addition, we have shown in the past that the signaling pathways for signal transducer and activator of transcription (STAT)3, phosphatidylinositol-3' kinase (PI3K/Akt), and protein kinase C (PKC) are of the utmost significance [35]. However, the identification of the downstream signaling components that it influences is unknown, as are the likely sites of cross-talk. Although it has

been shown that the atypical PKC isoform PKC may alter the induction of CL-3 in response to IL-1+OSM stimulation, it is unclear which components of the signaling pathway it impacts. This is even though it has been shown that the identification of the downstream signaling components that it affects is unknown, as proven by the fact that this is the case [36].

In **Table 3**, the findings of an analysis that was carried out on each of the groups that were examined to estimate the coefficient of association (r) between CL-3 and the other parameters are shown. This analysis was carried out to identify the relationship between CL-3 and the other parameters.

Table 3. Relationship coefficient between CL-3 and other parameters for the studied groups.

Parameters	Relationship coefficient-r		
	G_1	G_2	G_3
RF (IU/mL)	0.04	-0.11	0.12
ESR (mm/hr)	0.33	-0.11	0.19
HbA1c (%)	0.02	0.22	0.33
Anti-CCP (U/mL)	-0.07	0.35	0.17

The present study showed a negative relationship between CL-3 levels and RF in G2 (r2= -0.11). A positive relationship was found in G1 and G3 (r1=0.04, r3= 0.12) as shown in **Figure 1**. Collagenase-3 levels and ESR in G2 had a poor connection, according to the research (r2=0.11). While G1 and G3 showed a positive association (r1=0.33, r3=0.19), which is seen in **Figure 2**.

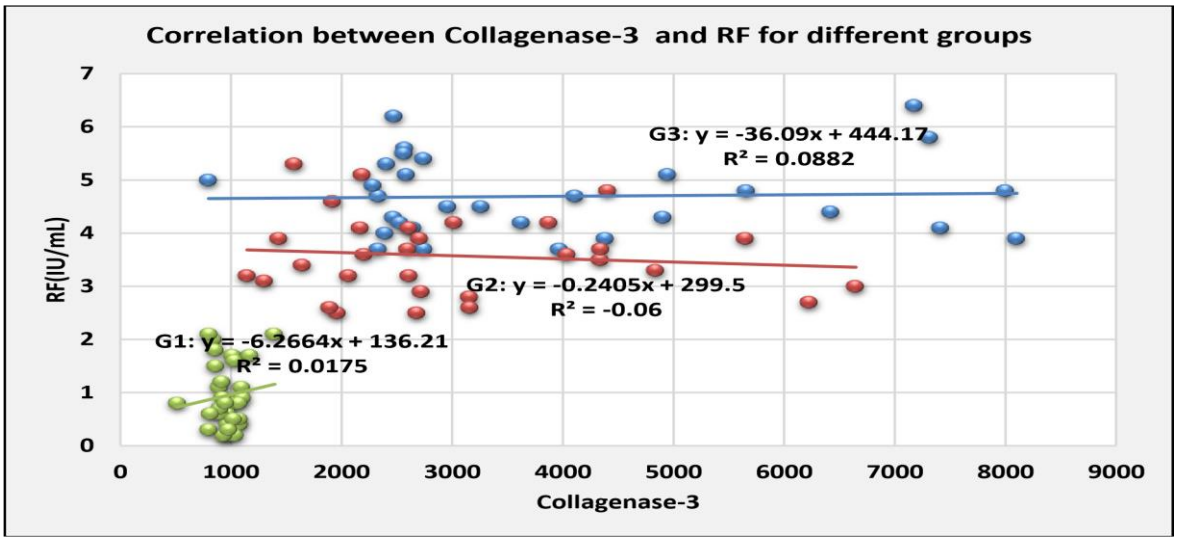

Figure 1. Relationship between collagenase-3 and RF.

Study revealed a positive relationship between CL-3 levels and HbA1c in all studied groups as shown in **Figure 3**. The results also showed an inversely correlated relationship between collagenase-3 levels and anti-CCP in G2 and G3 (r2= 0.35, r3= 0.17). In contrast, G1 showed a negative relationship (r1= -0.07), as seen in **Figure 4**. The suppression of cartilage development and increased chondrocyte death may be the underlying mechanism for CL-3's impacts on diabetic RA articular cartilage. However, further research is needed to fully understand the processes.

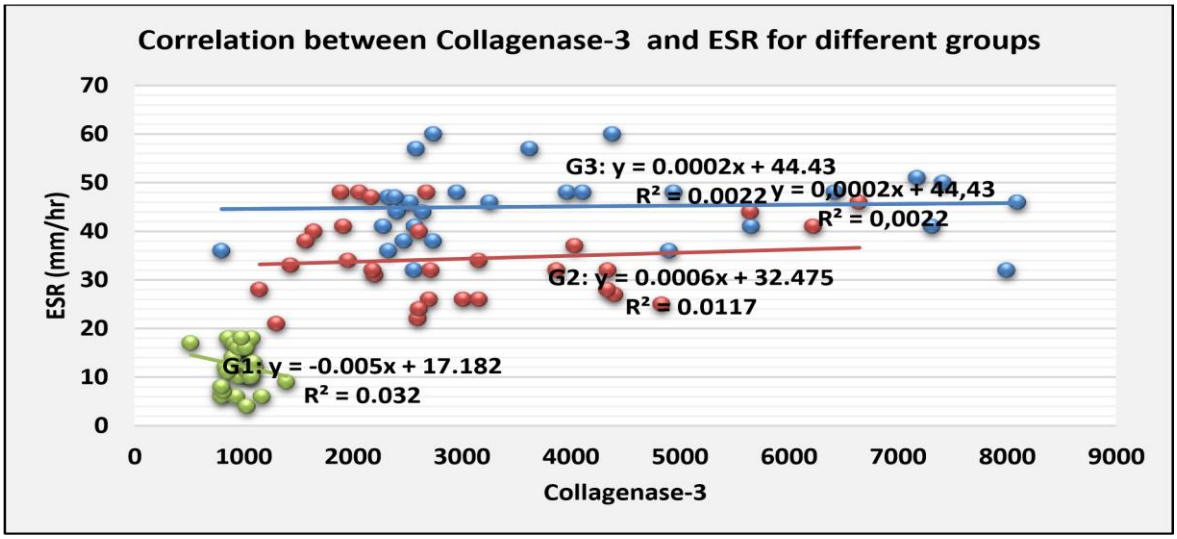

Figure 2. Relationship between collagenase-3 and ESR.

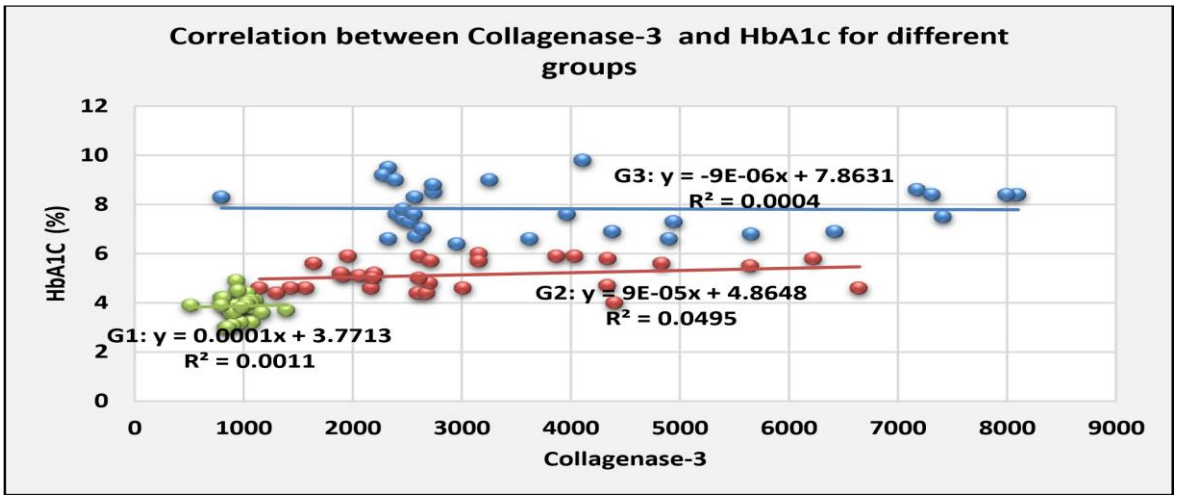

Figure 3. Relationship between collagenase-3& HbA1C.

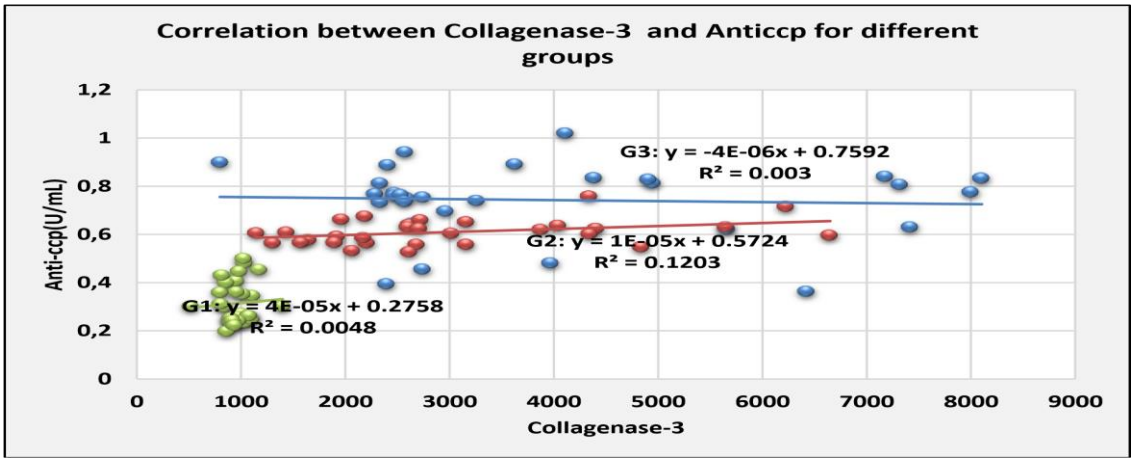

Figure 4. Relationship between collagenase-3 and Anti-CCP.

4. Conclusion

This study provides solid evidence that CL-3 contributes to an increase in inflammation and joint degradation in RA models by demonstrating a significant shift (either an increase or a decrease) in CL-3 and anti-CCP levels. According to evidence that demonstrates it is essential

for a protracted inflammatory response that takes place in the effector phase of arthritis, CL-3 may have a job in managing the inflammatory response in addition to damaging cartilage. This information suggests that CL-3 may have a role in controlling the inflammatory response. These results showed that these features have the potential to be use as a biochemical diagnostic for the early diagnosis of diabetes in RA patients. In addition to this, a strong positive or negative association between CL-3 and all parameters for all groups revealed a good connected biomarker with these patients, suggesting that it would be possible to employ the most effective medicine and treatment.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

No founding.

Ethical Clearance

This work has been approved by the Scientific Committee at the University of Baghdad/ College of Education for Pure Science (Ibn Al–Haitham), which is consistent with the instructions of the Iraqi Ministry of Health and Environment (No. 15765 on 4/10/2022).

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