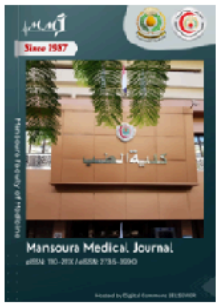
MANSOURA MEDICAL JOURNAL

ISSN - Print: 1110-211X - Online: 2735-3990

journal homepage: mmj.mans.edu.eg



Volume 52 | Issue 1 Article 4

Evaluation of CXCL2 and autophagy genes expression in rheumatoid arthritis patients and its relation to cardiovascular diseases

Mona Elhelaly

Medical Biochemistry and Molecular Biology department, Faculty of Medicine, Mansoura University, Mansoura, Egypt,

Dr_mona_elhelali@mans.edu.eg

Dalia Kamal Nassar

Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Mohammed Kamal Nassar

Mansoura Nephrology & Dialysis Unit (MNDU), Department of Internal Medicine, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Khaled Tarek Abdelalim

Mansoura Manchester Medical Programme, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Samar Tharwat

Rheumatology & Immunology Unit, Department of Internal Medicine, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Recommended Citation

Elhelaly, Mona; Nassar, Dalia Kamal; Nassar, Mohammed Kamal; Abdelalim, Khaled Tarek; and Tharwat, Samar (2023) "Evaluation of CXCL2 and autophagy genes expression in rheumatoid arthritis patients and its relation to cardiovascular diseases," *Mansoura Medical Journal*: Vol. 52 : Iss. 1 , Article 4.
Available at: https://doi.org/10.58775/2735-3990.1373

ORIGINAL STUDY

Evaluation of CXCL2 and Autophagy Gene Expression in Patients with Rheumatoid Arthritis and its Relation to Cardiovascular Diseases

Mona Elhelaly Elsherbeny [a,*], Dalia K. Nassar [b], Mohammed K. Nassar [c], Khaled T. Abdelalim [d], Samar Tharwat [e]

[a] Department of Medical Biochemistry and Molecular Biology, Mansoura University, Mansoura, Egypt
[b] Departments of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[c] Department of Mansoura Nephrology & Dialysis Unit (MNDU), Department of Internal Medicine, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[d] Mansoura Manchester Medical Programme, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[e] Department of Rheumatology & Immunology Unit, Department of Internal Medicine, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Abstract

Background: **Cardiovascular disease is among the most common comorbidity and the leading cause of mortality in patients with rheumatoid arthritis (RA). The aim of this study was to assess the levels of CXCL2 and autophagy genes such light chain (LC3) and beclin1 in patients with RA and how they relate to the clinical manifestations and carotid intimal−medial thickness.**

Patients and methods: **This cross-sectional study included patients with RA. Sociodemographic characteristics and clinical and therapeutic data were recorded. Thorough medical and clinical examination was conducted to evaluate the disease status, including the number of swollen and tender joints, visual analog scale, and disease activity score 28. Approximately 5 ml of whole blood was collected from each participant, and CXCL2, LC3, and beclin1 expression levels were evaluated. The carotid intima−media thickness (CIMT) was assessed by a duplex ultrasound system. As a control group, 79 healthy individuals of matching age and sex were included.**

Results: **A total of 79 patients with RA were included in the study, with mean ± SD age of 45.24 ± 10.07 years. Most of them were females (87.3%), and the median duration of RA was 7 years. Approximately 85% had positive rheumatoid factor, whereas 70.9% had positive anti-CCP antibody. CXCL2 and LC3 had a statistically significant positive correlation of medium strength ($P = 0.026$). CXCL2 showed a significant positive correlation with presence of subcutaneous rheumatoid nodules, whereas there was a positive correlation of low strength between CXCL2 and systolic blood pressure, diastolic blood pressure, and health assessment questionnaire score. For LC3 gene expression, it was significantly correlated positively with presence of joint deformities and negatively with hypercholesterolemia. There was a statistically significant positive correlation of medium strength between LC3 and CIMT in those with disease duration more than or equal to 7 years after controlling (adjusting) for patient's age.**

Conclusions: **CXCL2 and autophagy gene expression levels (LC3 and beclin1) are positively correlated with the clinical manifestations and CIMT in patients with RA. These genes may serve as predictors for cardiovascular disease in patients with RA.**

Keywords: Autophagy gene, Carotid intimal−medial thickness, CXCL2, Rheumatoid arthritis

Received 13 January 2023; revised 19 February 2023; accepted 20 February 2023.
Available online 1 June 2023

* Corresponding author at: Mona Elhelaly Elsherbeny PhD, Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Mansoura University, Mansoura, 35511, Egypt.
E-mail address: dr_mona_elhelali@mans.edu.eg (M.E. Elsherbeny).

https://doi.org/10.58775/2735-3990.1373

1. Background

Rheumatoid arthritis (RA) is a chronic, systemic, and autoimmune disease characterized by irreversible articular damage and permanent disability (Smolen et al., 2018; Miyabe et al., 2020). In RA, cardiovascular disease (CVD) is the major comorbidity and the leading cause of death (Karpouzas et al., 2021). Although inflammation is a key contributor to RA and CVD, the pathophysiological link between these diseases remains unclear (Jamthikar et al., 2020).

It was predicted that variables linked with RA and persistent systemic inflammation may contribute to the acceleration of atherosclerosis, hence increasing the risk of CVD (Karpouzas et al., 2014). Multiple chemokines have been found to be overexpressed in patients with RA relative to healthy controls (Miyabe et al., 2020). Chemokines are secretory proteins with a low molecular weight that act as essential mediators in immune cell recruitment, maturation, and activation (Chistiakov et al., 2018).

C-X-C motif chemokine ligand 2 (CXCL2) is a key proinflammatory chemokine that acts as an inflammatory mediator and is produced by macrophages exposed to endotoxins (Wolpe and Cerami, 1989). CXCL2 is a significant chemokine that binds to particular receptors expressed on macrophages, neutrophils, and epithelial cells (De Filippo et al., 2013). It is involved in the chemotaxis of neutrophils, the control of their migration, and the deposition of leukocytes in extravascular tissue (Li et al., 2004). As a result, it plays critical roles in the onset and progression of chronic inflammation (Yang et al., 2020). Furthermore, it may have a crucial role in the progression of atherosclerosis (Mohanty et al., 2010; McPherson and Davies, 2012).

Autophagy primarily performs an adaptive function in protecting cells from nutrient shortage and other harmful causes, and it is important in the etiology and development of a wide range of diseases (Levine et al., 2011; Lotz and Carames, 2011). Several regulatory factors that may play important roles in autophagy processes have been encountered in recent years, including beclin1, which is the key regulatory factor in the autophagy startup process, and autophagosome components microtubule-associated protein light chain 3 (LC3) and autophagy-related gene 5 (Atg5). Throughout the autophagy process, LC3-I accumulates in autophagosomes and converts to LC3-II. Before being distributed among the membrane fractions, LC3-II becomes a structural component of the double-membraned autophagosome. As a result, LC3-II may be regarded as a protein marker, indicating the degree of autophagy (Kroemer and Jäättelä, 2005; Kraft and Kenworthy, 2012).

Currently, there are no CVD biomarkers for patients with RA, and most research is retrospective and postmortem (Ormseth et al., 2021). As a result, developing biomarkers for assessing risk and diagnosing CVD in patients with RA is critical (Jamthikar et al., 2020). CXCL2 and autophagy genes may serve as markers for the development of CVD in patients with RA.

The goal of this study was to look at CXCL2 expression levels as well as autophagy genes like LC3 and beclin1 and how they relate to clinical findings and carotid intimal–medial thickness (CIMT) in patients with RA.

2. Patients and methods

2.1. Study design and settings

This cross-sectional study included 79 Egyptian adults (age ≥18 years) fulfilling ACR/EULAR 2010 RA classification criteria (Kay and Upchurch, 2012) and subsequently recruited from the 'Rheumatology and Immunology unit (both inpatient and outpatient), Mansoura University hospital, Egypt.' From the outset, patients with other autoimmune or rheumatic diseases or those who underwent cardiovascular surgery were not included. Individuals with hereditary or genetic condition were excluded, as well as patients with family history of genetic or hereditary diseases.

As a control group, 79 healthy individuals of matching age and sex were included.

2.2. Sociodemographic characteristics and clinical evaluation

For patients with RA, sociodemographic characteristics were recorded, including age, sex, weight and height, duration, and age at onset of RA. Thorough medical and clinical examination was conducted to evaluate the disease status, including the number of swollen and tender joints. A visual analog scale of 100 mm was used to assess the patient's overall health, with 0 being the best and 100 representing the worst.

Associated comorbidities such as diabetes mellitus, CVD, and hypertension were also inquired. Therapeutic data were also recruited, including NSAIDs, corticosteroids, conventional disease modifying antirheumatic drugs, and biological agents.

Acute-phase reactants, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR),

serological markers such as rheumatoid factor (RF) and anti-CCP, in addition to complete blood count and serum creatinine were measured at the same day of clinical evaluation. The disease activity score 28 (DAS28-ESR) was computed to assess current disease activity in patients with RA (Wells et al., 2009).

2.3. Isolation of peripheral blood mononuclear cells

A total of 5 ml of whole blood was collected from each participant in EDTA collection tubes, and isolation of peripheral mononuclear blood cells, T cells, B cells, and monocytes was done using Histopaque-1077 (Sigma–Aldrich, Dorset, UK) Ficoll density-gradient centrifugation as per the manufacturer's instructions.

2.4. RNA extraction and cDNA synthesis

Total RNA including lncRNA was extracted by the TRIzol reagent from peripheral mononuclear blood cells (Zymo Research, Irvine, California, USA). NanoDrop2000 (Thermo. Fischer Scientific, Waltham, Massachusetts, USA) was used to quantitate RNA. Before use, the total RNA samples were kept at −80 °C. Using SensiFAST cDNA Synthesis Kit (Bioline, Memphis, Tennessee, USA), reverse transcription was performed on the RNA in a final volume of 20-ml reactions.

2.5. Real-time PCR

The CXCL2, LC3, and beclin1 expression levels were evaluated using GAPDH as an internal control using the Hera plus SYBR Green qPCR kit (Willow fort, Birmingham, UK) according to the manufacturer's protocol. Fold change was calculated using the comparative threshold cycle ($2^{-\Delta\Delta Ct}$) for relative quantification normalized to an endogenous control (Schmittgen and Livak, 2008).

Primers used in this study were as follows:

Cxcl2: forward was 5′-GGCAGAAAGCTTGTCT CAACCC-3′ and reverse was 5′-CTCCTTCAGGAA-CAGCCACCAA-3’.

beclin1: forward was 5′-CTGGACACTCAGCT CAACGTCA-3′ and reverse was 5′-CTCTAGTGC-CAGCTCCTTTAGC-3’.

LC3: forward was 5′-GCTACAAGGGTGAGAAG CAGCT-3′ and reverse was 5′-CTGGTTCACCAG-CAGGAAGAAG-3’.

2.6. Assessment of carotid intima–media thickness

A single examiner with 7 years of ultrasonography experience performed the CIMT measurements. To summarize, the common carotid arteries were assessed using a duplex ultrasound system (Sonoscape, 10-MHz linear array transducer). R-synchronized and recorded longitudinal high-resolution B-mode ultrasound scans were performed over both the right and left common carotid arteries. The off-line measurements were taken in the far wall, 1 cm proximal to the carotid bulb. The CIMT was calculated as the average of 10 observations taken from the first and second echogenic lines from the lumen. The CIMT readings were presented in millimeters.

2.7. Ethical consideration

This study was conducted in compliance with the Helsinki Declaration's (World Medical Association, 2013) principles. The Institutional Research Board of the Faculty of Medicine at Mansoura University approved the study protocol (approval registration number: R.21.10.1500.R1). All participants were provided with detailed information about the study, and their written informed consent was obtained.

2.8. Statistical analysis

IBM-SPSS software (IBM Corp., 2019) was used to enter and evaluate data. IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, New York, USA). Pearson's and Spearman's correlations were used to evaluate the relationship between two quantitative data sets. Pearson's partial correlation was used to estimate the degree and direction of a linear relationship between two continuous variables while excluding one or more of them. To analyze the relationship between a dichotomous variable and a continuous variable, point bi-serial correlation was used. To compare non-normally distributed quantitative data between two groups, the Mann–Whitney *U* test was used. A continuous dependent variable was predicted using multiple regression based on several independent variables. Receiver operating characteristic curve analysis was used to determine a cutoff value for a continuous variable that can distinguish between two circumstances. If the *P* value was less than 0.050 for any of the tests conducted, the results were considered statistically significant. When necessary, appropriate charts were developed to graphically present the results.

3. Results

A total of 79 patients with RA were included in the study, with mean ± SD age of 45.24 ± 10.07 years. Most of them were females (87.3%), and the median duration of RA was 7 years. About one-fourth had a

family history of RA (25.3%). Hypertension and hypercholesterolemia were the most associated comorbidities (12.7 and 11.4%, respectively). The administrated dugs included leflunomide (70.9%), glucocorticoids (70.9%), hydroxychloroquine (68.4%) followed by methotrexate (67.1%) and NSAIDs (43.0%). Other clinical and therapeutic data are illustrated in Table 1.

Approximately 85% had positive RF, whereas 70.9% had positive anti-CCP antibody. The median level of ESR was 31.5 mm/h. Table 2 shows other laboratory data and CXCL2 and autophagy gene expression of the study patients with RA.

The median expression (minimum–maximum) of beclin, LC3, and CXCL2 are 2.55 (0.9–5.1), 2.75 (0.7–6.2), and 2.50 (1.0–5.9), respectively, as shown in Fig. 1.

Table 1. Sociodemographic, clinical, and therapeutic data of the patients with rheumatoid arthritis (N = 79).

Variables	Patients with RA [*n* (%)], (mean ± SD), [median (minimum–maximum)]
Sociodemographic data	
Age (years)	45.24 ± 10.07
Sex	
Male	10 (12.7)
Female	69 (87.3)
Weight (kg)	84.01 ± 20.11
Height (m)	1.60 ± 18.95
Clinical data (years)	
Age at RA onset	36.02 ± 11.58
Duration of RA	7 (0.5–45)
Patient global assessment (0–100)	30 (0–90)
Tender joint count	2 (0–20)
Swollen joint count	1 (0–15)
Morning stiffness (min)	15 (0–300)
DAS28 score	4.07 ± 1.61
HAQ score	1.47 (0–3)
Presence of joint deformities	24 (30.4)
Presence of rheumatoid SC nodules	5 (6.3)
Systolic blood pressure (mmHg)	122.61 ± 14.54
Diastolic blood pressure (mmHg)	80.32 ± 8.48
Family history of RA	20 (25.3)
Associated comorbidities	
Diabetes mellitus	7 (8.9)
Atrial fibrillation	4 (5.1)
Cardiovascular disease	7 (8.9)
Hypertension	10 (12.7)
Hypercholesterolemia	9 (11.4)
Chronic kidney disease	4 (5.1)
Therapeutic data	
NSAIDs	34 (43.0)
COXIB	11 (13.9)
Methotrexate	53 (67.1)
Hydroxychloroquine	54 (68.4)
Leflunomide	56 (70.9)
Sulfasalazine	6 (7.6)
Biologicals	2 (2.5)
Glucocorticoids	56 (70.9)
Antihypertensive drugs	4 (5.1)

DAS28, disease activity score 28; HAQ, health assessment questionnaire; RA, rheumatoid arthritis; SC, subcutaneous.

Table 2. Laboratory data, CXCL2, and autophagy gene expression of the study patients with rheumatoid arthritis (N = 79).

Variables	Study patients with RA [*n* (%)], (mean ± SD), [median (minimum–maximum)]
Laboratory data	
Positive rheumatoid factor	67 (84.8)
Positive anti-CCP	56 (70.9)
Hemoglobin (g/dl)	11.11 ± 1.18
Mean corpuscular volume (fl)	79.05 ± 6.52
White blood cells (× 10^9/l)	5.2 (2.40–13)
Platelets (× 10^9/l)	239.45 ± 99.02
Erythrocyte sedimentation rate (mm/h)	31.5 (8–100)
C-reactive protein (mg/l)	2 (10–96)
Serum creatinine	0.87 ± 0.22
Gene expression, median (Q1–Q3)	
CXCL2	2.5 (1.7–3.6)
LC3	2.8 (1.8–4.05)
Beclin	2.55 (1.8–3.58)
Carotid intima–media thickness (mm)	0.74 ± 0.14

LC, light chain 3; RA, rheumatoid arthritis.

Wilcoxon signed-rank tests were run to compare these three gene expressions in RA cases against a hypothesized value (1.0) for control. The median three gene expressions in RA cases were statistically significantly higher than 1.0.

Correlations between CXCL2 gene and autophagy genes with the sociodemographic and clinical are illustrated in Table 3. There was a statistically significant positive correlation of medium strength between CXCL2 and LC3 ($P = 0.026$). Moreover, CXCL2 showed a significant positive correlation with presence of subcutaneous rheumatoid nodules, whereas there was a positive correlation of low strength between CXCL2 and systolic blood pressure, diastolic blood pressure, and health assessment questionnaire score. For LC3 gene expression, it was significantly correlated positively with presence of joint deformities and negatively with hypercholesterolemia. There was a positive correlation of low strength between beclin gene and systolic blood pressure, although it did not reach statistical significance.

As shown in Table 4, CXCL2 was negatively correlated with positive RF ($r = -0.354$, $P = 0.002$), whereas LC3 was strongly correlated with the serum levels of CRP ($r = 0.255$, $P = 0.030$).

Levels of LC3 gene expression were found to be significantly negatively correlated with hypercholesterolemia. Therefore, receiver operating characteristic curve was performed to obtain a cutoff level for discrimination of dyslipidemia as shown in

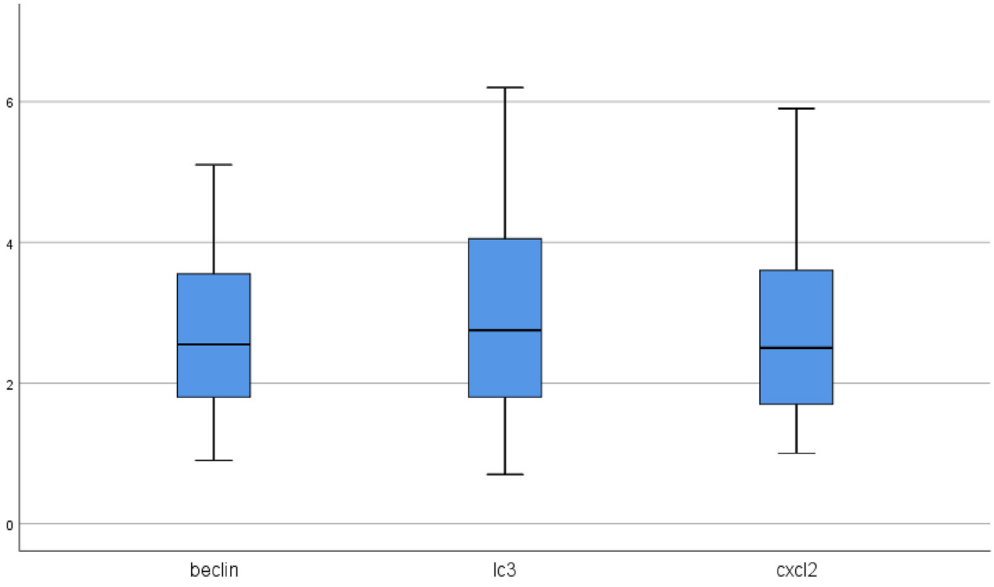


Fig. 1. The median expression of beclin, LC3, and CXCL2. LC3, light chain 3.

Fig. 1. LC3 at a cutoff value less than or equal to two-fold change statistically significantly discriminates dyslipidemia from normal lipid profile. A simple binary logistic regression was run to ascertain the effect of low LC3 expression on the likelihood that participants will exhibit dyslipidemia (Fig. 2). Participants with LC3 expression less than or equal to two-fold change have 9.7 times higher odds to exhibit dyslipidemia ($P = 0.007$, 95% confidence interval = 1.8–51.2).

According to median duration of the disease, patients with RA were divided into two groups: those who had the disease less than 7 years and those who had it more than 7 years, and the gene expression levels were correlated with the DAS28 and CIMT, as shown in Table 5. There was a statistically significant positive correlation of medium strength between LC3 and CIMT in those with disease duration more than or equal to 7 years after controlling (adjusting) for patient's age.

Table 3. Correlation between CXCL2 and autophagy gene expression and sociodemographic, and clinical data.

Variables	CXCL2		LC3		Beclin	
	r_s	*P* value	r_s	*P* value	r_s	*P* value
Sociodemographic data						
Age (years)	0.041	0.725	−0.145	0.208	0.121	0.229
Sex	0.046	0.693	0.028	0.812	0.064	0.585
Weight (kg)	0.134	0.246	−0.157	0.174	0.076	0.512
Height (m)	−0.007	0.950	−0.021	0.853	0.034	0.771
Smoking	−0.018	0.876	−0.078	0.499	−0.125	0.280
Clinical data						
Duration of RA	0.036	0.759	−0.081	0.484	0.136	0.243
DAS28 score	0.098	0.405	0.090	0.448	−0.009	0.938
HAQ score	0.196	0.088	0.056	0.626	0.005	0.964
Presence of joint deformities	0.105	0.363	**0.252**	0.027[a]	0.133	0.253
Presence of rheumatoid nodules	**0.273**	0.016[a]	−0.032	0.779	0.196	0.089
Systolic blood pressure (mmHg)	0.137	0.237	0.022	0.849	0.162	0.166
Diastolic blood pressure (mmHg)	0.173	0.134	−0.020	0.862	0.008	0.945
Family history of RA	−0.083	0.475	0.167	0.147	0.081	0.488
Associated comorbidities						
Diabetes mellitus	−0.004	0.971	−0.163	0.156	0.194	0.093
Atrial fibrillation	−0.058	0.616	0.074	0.525	−0.019	0.868
Cardiovascular disease	−0.040	0.728	−0.026	0.823	0.060	0.606
Hypertension	0.096	0.405	−0.098	0.394	0.133	0.252
Hypercholesterolemia	−0.054	0.639	**−0.263**	0.021[a]	−0.115	0.324
Chronic kidney disease	−0.139	0.229	−0.016	0.893	0.144	0.216

DAS28, disease activity score 28; HAQ, health assessment questionnaire; RA, rheumatoid arthritis. * and bold value means that the P value is significant.

[a] *P* value less than 0.05.

Table 4. Correlation between CXCL2 and laboratory data.

Laboratory data	CXCL2		LC3		Beclin	
	r_s	*P* value	r_s	*P* value	r_s	*P* value
Positive rheumatoid factor	**−0.354**	0.002[a]	−0.176	0.126	0.068	0.557
Positive anti-CCP	−0.149	0.196	−0.060	0.606	0.078	0.505
Hemoglobin (g/dl)	−0.097	0.406	−0.131	0.258	−0.033	0.766
Mean corpuscular volume (fl)	−0.087	0.455	−0.213	0.064	−0.010	0.929
White blood cells (× 10^9/l)	−0.013	0.913	−0.018	0.878	0.068	0.561
Platelets (× 10^9/l)	0.179	0.124	−0.023	0.847	−0.119	0.313
Lymphocytes (× 10^9/l)	−0.013	0.912	−0.117	0.319	−0.128	0.278
Erythrocyte sedimentation rate	−0.017	0.886	0.078	0.508	−0.021	0.863
C-reactive protein (mg/l)	0.050	0.677	**0.255**	0.030[a]	−0.211	0.075
Serum creatinine	0.100	0.473	−0.097	0.477	−0.076	0.560
Gene expression						
CXCL2	–	–	**0.254**	0.026[a]	−0.050	0.669
LC3	**0.254**	0.026[a]	–	–	−0.139	0.231
Beclin	−0.050	0.669	−0.139	0.231	–	–
CIMT	−0.092	0.434	−0.084	0.471	0.180	0.125

CIMT, carotid intima–media thickness. * and bold value means that the P value is significant.
[a] *P* value less than 0.05.

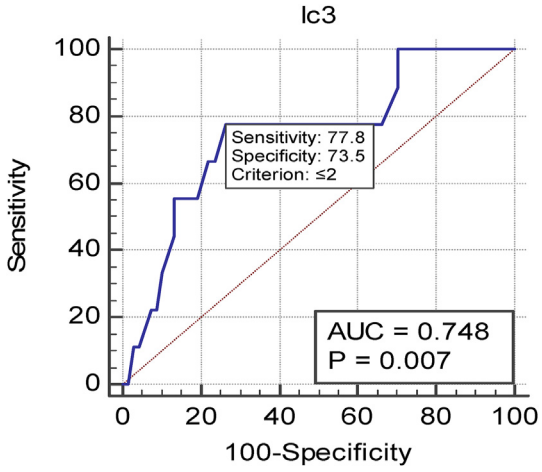


Fig. 2. ROC curve for LC3 in discriminating dyslipidemia. LC3, light chain 3; ROC, receiver operating characteristic.

4. Discussion

Atherosclerotic CVD is the major cause of death in individuals with RA. Chronic inflammation raises these patients' cardiovascular risk (CVR) (Wah-Suarez et al., 2018). CVR in these patients has been observed to be increased by standard CVR factors as well as nontraditional risk factors such as inflammation and RA-specific characteristics (disease activity, disease duration, and RA medication) (Wah-Suarez et al., 2018).

In this work, we evaluated the levels of CXCL2 and autophagy genes such as LC3 and beclin1 in relation to clinical findings and CIMT in patients with RA. CXCL2 level was significantly correlated with the existence of subcutaneous rheumatoid nodules, systolic blood pressure, diastolic blood pressure, and health assessment questionnaire score. LC3 gene expression was strongly positively linked with the occurrence of joint deformities and a high CRP level. There was a low-strength positive correlation between the beclin gene and systolic blood pressure. There was a correlation among the three gene expression levels and the DAS28 and CIMT.

In this study, CXCL2 expression serum levels were higher in patients with RA than in the control group. This is consistent with the results of the study by Ha et al. (2010), in which RA synovial fluid and

Table 5. Correlation of the CXCL2 and autophagy gene expression with disease activity score 28 and carotid intima–media thickness mean based on median disease duration (<7 years and ≥7 years).

	DAS28				CIMT mean			
	<7 years (N = 39)		≥7 years (N = 40)		<7 years (N = 39)		≥7 years (N = 40)	
Biomarker	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
CXCL2	0.091	0.561	0.074	0.692	**−0.330**	0.031[a]	0.079	0.668
Controlled for age	0.056	0.730	0.091	0.637	−0.296	0.060	−0.018	0.926
LC3	0.060	0.702	0.128	0.492	**−0.309**	0.044[a]	0.266	0.141
Controlled for age	0.037	0.820	0.146	0.451	−0.285	0.071	**0.390**	0.037[a]
Beclin1	−0.202	0.194	0.226	0.229	0.288	0.061	0.046	0.804
Controlled for age	−0.201	0.208	0.200	0.299	0.274	0.083	−0.040	0.836

CIMT, carotid intima–media thickness; DAS28, disease activity score 28; *r*, correlation coefficient. Test of significance is Pearson's correlation and partial correlation to control for age. * and bold value means that the *P* value is significant.
[a] *P* value less than 0.05.

serum contained considerably higher amounts of CXCL2 than that in osteoarthritis, implying that enhanced CXCL2 is implicated in bone destruction throughout the pathogenesis of RA. Furthermore, CXCL2 expression levels in ACPA + patients with RA are higher than in ACPA− patients with RA and healthy controls (Wang et al., 2021). As a result, targeting CXCL2 may have therapeutic potential in the treatment of RA.

Moreover, CXCL2 expression was positively correlated with DAS28 and CRP. This is in agreement with the finding of the study conducted by Wang et al. (2021), in which CXCL2 was positively correlated with DAS28, ESR, and CRP.

CXCL2 was positively correlated with CIMT. The inflammatory CXCL2 has been shown to be involved in the pathogenesis and progression of CVD. Overexpression of CXCL2 in the myocardium can causes myocardial cell injury. Moreover, CXCL2 is a powerful chemokine for neutrophil recruitment and adherence in inflammation, which leads to the formation of atherosclerotic plaques in atherosclerosis. Furthermore, CXCL2 promotes chronic inflammation in obese individuals, hastening the degenerative process of atherosclerosis which has a negative effect on myocardial infarction. Furthermore, elevated levels of CXCL2 in the pancreatic islets are especially linked to the onset of diabetes (Guo et al., 2020).

LC3 gene expression, one of the autophagy genes, was significantly linked with the occurrence of joint deformities and CRP elevation in our study patients with RA. In accordance with our findings, the expression of LC3 is elevated in patients with RA and is correlated with ESR, DAS28, CRP, and RF (Shao et al., 2019). Autophagy has been demonstrated to promote immune and nonimmune cell survival, peptide citrullination, citrullinated peptide presentation, and immune and nonimmune cell maturation such as osteoclasts, B, and T cells. Despite this, it is thought that the increased autophagy rate in these cells is responsible for apoptosis resistance, proliferation, and the production of inflammatory mediators, which ultimately leads to higher joint and cartilage deterioration in patients with RA. Furthermore, autophagy of osteoclasts is increased in experimental arthritis and patients with RA (Karami et al., 2020). Autophagy is a naturally occurring intracellular catabolic system that degrades intracellular pathogens, damaged organelles, and protein aggregates while also resynthesizing macromolecules, giving extra energy, and boosting cell survival (Buckland, 2013). Recent research suggests that autophagy may play a role in osteoclastogenesis. Hypoxia, an autophagy-activating stimulus, appears to be able to boost osteoclast development (Knowles and Athanasou, 2009). These findings give autophagy a significant protective function against apoptosis. For these reasons, therapy based on autophagy regulation may be helpful in RA (Vomero et al., 2018).

Autophagy is linked to many diseases and conditions, such as infection, cancer, neurodegenerative disease, and heart disease (Levine and Kroemer, 2008; Ravikumar et al., 2010). Moreover, autophagy-related proteins (beclin1, Atg5, and LC3) were found to be overexpressed in the synovial tissue of people with active RA (Zhu et al., 2017). In a murine model of RA, Lin et al. (2013) showed that tumor necrosis factor-activates autophagy. Connor et al. (2012) showed that tumor necrosis factor stimulates autophagy by inducing the endoplasmic reticulum stress response.

Autophagy gene expression levels (LC3 and beclin1) were positively correlated with CIMT in this study. Autophagy has been linked to a variety of diseases, including atherosclerosis (Grootaert et al., 2018). Excessive autophagy activation may result in plaque instability. Furthermore, autophagy may be harmful to plaque construction as endothelial injury or death is a major mechanism for acute clinical events by encouraging thrombosis (Liu et al., 2015).

To the best of our knowledge, this is the first study among Egyptian patients with RA that looks at the relationship between autophagy genes and CIMT. Our study, however, has several drawbacks. First, there is a lack of data on echocardiography, which might provide more detailed information regarding the effect of autophagy genes on heart disease. Second, there is a dearth of long-term follow-up on RA cases with CVD, which could aid in determining the effect on prognosis and treatment response. These constraints should be considered in future research.

5. Conclusions

CXCL2 and autophagy gene expression levels (LC3 and beclin1) are positively correlated with the clinical manifestations and CIMT in patients with RA. These genes may serve as predictors for CVD in patients with RA.

Author contributions

Conceptualization was done by M.E. and D.K.N. Investigation was done by all authors. Data curation and formal analysis were done by M.K.N. and KT.A. Writing the original draft was done by S.T. Writing, reviewing, and editing were done by all authors.

Conflict of interest

There are no conflicts of interest.

References

Buckland, J., 2013. Autophagy: a dual role in the life and death of RASFs. Nat. Rev. Rheumatol. 9, 637, 637.

Chistiakov, D.A., Orekhov, A.N., Bobryshev, Y.V., 2018. Chemokines and relevant microRNAs in the atherogenic process. Mini Rev. Med. Chem. 18, 597–608.

Connor, A.M., Mahomed, N., Gandhi, R., Keystone, E.C., Berger, S.A., 2012. TNFα modulates protein degradation pathways in rheumatoid arthritis synovial fibroblasts. Arthritis Res. Ther. 14, 1–19.

De Filippo, K., Dudeck, A., Hasenberg, M., Nye, E., van Rooijen, N., Hartmann, K., et al., 2013. Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. Blood J. Am. Soc. Hematol. 121, 4930–4937.

Grootaert, M.O.J., Roth, L., Schrijvers, D.M., De Meyer, G.R.Y., Martinet, W., 2018. Defective autophagy in atherosclerosis: to die or to senesce? Oxid. Med. Cell. Longev. 2018, e7687083.

Guo, L.-Y., Yang, F., Peng, L.-J., Li, Y.-B., Wang, A.-P., 2020. CXCL2, a new critical factor and therapeutic target for cardiovascular diseases. Clin. Exp. Hypertens. N Y 42, 428–437.

Ha, J., Choi, H.-S., Lee, Y., Kwon, H.-J., Song, Y.W., Kim, H.-H., 2010. CXC chemokine ligand 2 induced by receptor activator of NF-κB ligand enhances osteoclastogenesis. J. Immunol. 184, 4717–4724.

Jamthikar, A.D., Gupta, D., Puvvula, A., Johri, A.M., Khanna, N.N., Saba, L., et al., 2020. Cardiovascular risk assessment in patients with rheumatoid arthritis using carotid ultrasound B-mode imaging. Rheumatol. Int. 1–19.

Karami, J., Masoumi, M., Khorramdelazad, H., Bashiri, H., Darvishi, P., Sereshki, H.A., et al., 2020. Role of autophagy in the pathogenesis of rheumatoid arthritis: latest evidence and therapeutic approaches. Life Sci. 254, 117734.

Karpouzas, G.A., Malpeso, J., Choi, T.-Y., Li, D., Munoz, S., Budoff, M.J., 2014. Prevalence, extent and composition of coronary plaque in patients with rheumatoid arthritis without symptoms or prior diagnosis of coronary artery disease. Ann. Rheum. Dis. 73, 1797–1804.

Karpouzas, G.A., Bui, V.L., Ronda, N., Hollan, I., Ormseth, S.R., 2021. Biologics and atherosclerotic cardiovascular risk in rheumatoid arthritis: a review of evidence and mechanistic insights. Expet Rev. Clin. Immunol. 17, 355–374.

Kay, J., Upchurch, K.S., 2012. ACR/EULAR 2010 rheumatoid arthritis classification criteria. Rheumatology 51 vi5–vi9.

Knowles, H.J., Athanasou, N.A., 2009. Acute hypoxia and osteoclast activity: a balance between enhanced resorption and increased apoptosis. J. Pathol. Soc. G B Irel. 218, 256–264.

Kraft, L., Kenworthy, A., 2012. Imaging protein complex formation in the autophagy pathway: analysis of the interaction of LC3 and Atg4BC74A in live cells using Förster resonance energy transfer and fluorescence recovery after photobleaching. J. Biomed. Opt. 17, 011008.

Kroemer, G., Jäättelä, M., 2005. Lysosomes and autophagy in cell death control. Nat. Rev. Cancer 5, 886–897.

Levine, B., Kroemer, G., 2008. Autophagy in the pathogenesis of disease. Cell 132, 27–42.

Levine, B., Mizushima, N., Virgin, H.W., 2011. Autophagy in immunity and inflammation. Nature 469, 323–335.

Li, X., Klintman, D., Liu, Q., Sato, T., Jeppsson, B., Thorlacius, H., 2004. Critical role of CXC chemokines in endotoxemic liver injury in mice. J. Leukoc. Biol. 75, 443–452.

Lin, N.-Y., Beyer, C., Gießl, A., Kireva, T., Scholtysek, C., Uderhardt, S., et al., 2013. Autophagy regulates TNFα-mediated joint destruction in experimental arthritis. Ann. Rheum. Dis. 72, 761–768.

Liu, H., Cao, Y., Tong, T., Shi, J., Zhang, Y., Yang, Y., et al., 2015. Autophagy in atherosclerosis: a phenomenon found in human carotid atherosclerotic plaques. Chin. Med. J. 128, 69–74.

Lotz, M.K., Carames, B., 2011. Autophagy and cartilage homeostasis mechanisms in joint health, aging and OA. Nat. Rev. Rheumatol. 7, 579–587.

McPherson, R., Davies, R.W., 2012. Inflammation and coronary artery disease: insights from genetic studies. Can. J. Cardiol. 28, 662–666.

Miyabe, Y., Miyabe, C., Iwai, Y., Luster, A.D., 2020. Targeting the chemokine system in rheumatoid arthritis and vasculitis. Jap. Med. Assoc. 3, 182–192.

Mohanty, S.K., Ivantes, C.A., Mourya, R., Pacheco, C., Bezerra, J.A., 2010. Macrophages are targeted by rotavirus in experimental biliary atresia and induce neutrophil chemotaxis by Mip2/Cxcl2. Pediatr. Res. 67, 345–351.

Ormseth, M.J., Solus, J.F., Sheng, Q., Chen, S.-C., Ye, F., Wu, Q., et al., 2021. Plasma miRNAs improve the prediction of coronary atherosclerosis in patients with rheumatoid arthritis. Clin. Rheumatol. 40, 2211–2219.

Ravikumar, B., Sarkar, S., Davies, J.E., Futter, M., Garcia-Arencibia, M., Green-Thompson, Z.W., et al., 2010. Regulation of mammalian autophagy in physiology and pathophysiology. Physiol. Rev. 90, 1383–1435.

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3, 1101–1108.

Shao, X., Yuan, Y., Jiang, X., Jia, A., Liu, J., Liu, F., 2019. Increased expression of LC3 in peripheral blood mononuclear cells is related to disease status in patients with rheumatoid arthritis. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi Chin 35, 1030–1034.

Smolen, J.S., Aletaha, D., Barton, A., Burmester, G.R., Emery, P., Firestein, G.S., et al., 2018. Rheumatoid arthritis. Nat. Rev. Dis. Prim. 4, 18001.

Vomero, M., Barbati, C., Colasanti, T., Perricone, C., Novelli, L., Ceccarelli, F., et al., 2018. Autophagy and rheumatoid arthritis: current knowledges and future perspectives. Front. Immunol. 9, 1577.

Wah-Suarez, M.I., Galarza-Delgado, D.A., Azpiri-Lopez, J.R., Colunga-Pedraza, I.J., Cardenas-de la Garza, J.A., Vera-Pineda, R., et al., 2018. The best cardiovascular risk calculator to predict carotid plaques in rheumatoid arthritis patients. Clin. Rheumatol. 37, 2373–2380.

Wang, X., Sun, L., He, N., An, Z., Yu, R., Li, C., et al., 2021. Increased expression of CXCL2 in ACPA-positive rheumatoid arthritis and its role in osteoclastogenesis. Clin. Exp. Immunol. 203, 194–208.

Wells, G., Becker, J.-C., Teng, J., Dougados, M., Schiff, M., Smolen, J., et al., 2009. Validation of the 28-joint Disease Activity Score (DAS28) and European League against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. Ann. Rheum. Dis. 68, 954–960.

Wolpe, S.D., Cerami, A., 1989. Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. Faseb. J. 3, 2565–2573.

World Medical Association, 2013. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 310, 2191–2194.

Yang, J., Liu, H., Cao, Q., Zhong, W., 2020. Characteristics of CXCL2 expression in coronary atherosclerosis and negative regulation by microRNA-421. J. Int. Med. Res. 48, 0300060519896150.

Zhu, L., Wang, H., Wu, Y., He, Z., Qin, Y., Shen, Q., 2017. The autophagy level is increased in the synovial tissues of patients with active rheumatoid arthritis and is correlated with disease severity. Mediat. Inflamm. 2017, 7623145.