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Three key genes expression profiling in Egyptian rheumatoid arthritis patients

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Abstract

Rheumatoid arthritis (RA) is a multi-system autoimmune disease with synovial joints involvement. The triad of autoimmunity, genetics, and environment is the key player in RA pathogenesis. We intended to investigate gene expression of *C-C Chemokine Ligand 2 (CCL2), protein tyrosine phosphatase non-receptor type 22* (*PTPN22), and Cytotoxic T-lymphocyte associated protein 4* (*CTLA-4)* in RA patients versus controls, and its correlation with the activity of the disease. The relative expression of *PTPN22*, *CTLA-4*, and *CCL2* in the peripheral blood of 59 RA patients and 50 controls was determined using RT-PCR. There was a significantly higher median (inter-quartile range) expression of *CTLA-4* and *CCL2* in RA patients in comparison to controls ($P<0.05$). However, in RA patients, *PTPN22* expression was significantly lower than in controls ($P=0.0001$). A weak significant correlation was detected between *PTPN22* and either *CTLA-4* or *CCL2*. Also, on comparing RA patients with moderate to severe disease activity versus those who have a mild disease activity, *CCL2* was significantly over-expressed ($P < 0.05$). Thus, in Egyptian RA patients, there was a significant *PTPN22* down-expression and greater expression of *CTLA-4* and *CCL2*. Moreover, over-expression of *CCL2* in RA patients with moderate-to-severe disease activity was significant. We conclude that these three key genes could become useful diagnostic markers for RA and *CCL2* expression as a good prognostic tool for RA disease activity.

Keywords: Rheumatoid Arthritis, *PTPN22, CTLA-4, CCL2*

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Introduction

Rheumatoid arthritis (RA) is a multi-system autoimmune disorder with synovial joints affection.[1] Synovial swelling, joint stiffness, and the loss of cartilage are the main RA features.[2] Many factors contribute to RA pathogenesis, but the triad of autoimmune, genetics, and environment is the most crucial.[3]

In RA, abnormal immune system activation causes an increase in pro-inflammatory cytokines and chemokines, which leads to angiogenesis and leukocyte invasion in the synovium. Because of the secretion of proteinases and the promotion of osteoclast differentiation, the synovium frames a hyperplastic pannus in addition to the invasion of fibroblast-like and macrophage-like synoviocytes that attack joints.[4]

In addition to the discovered *HLA* genes that are closely related to rheumatoid arthritis pathogenesis, a variety of *non-HLA* genes are strongly linked to it. One of them is *protein tyrosine phosphatase non-receptor type 22* (*PTPN22*), the gene encodes the intracellular phosphatase,[5] which is identified as one of the main authoritative *non-MHC* gene inducing autoimmune diseases through various mechanisms.[6]

The *PTPN22* gene has 24 exons and encoding the lymphoid tyrosine phosphatase (LYP) protein.[7] It is predominantly found in lymphoid tissues and regulates the activation of immune receptors negatively by inhibiting T-cell activation.[8] Moreover, it is counted to be the strongest associated gene with RA coming in the first order according to the MalaCards database.[9]

Moreover, autoimmune diseases were reported to be associated with impaired T-cell responses. *CTLA-4* gene encodes the cytotoxic T-lymphocyte associated protein that sends inhibitory signals to T-cells.[10,11] Among RA patients, serum soluble CTLA-4 (sCTLA-4) was significantly over-expressed than controls, with a positive correlation to disease activity.[12] However, early rheumatoid arthritis patients had lower levels than those who were not treated.[13] On the other hand, a CTLA-4 Ig (Abatacept) was demonstrated to rescue immune function in RA patients not responding to the first anti-TNF-α agent[14] via inhibiting activation of T-cells by blocking CD80/CD86 on B-cells and is therefore linked to RA disease activity.[15]

In inflammation, the immune cells trafficking, and infiltration are known to be coordinated by two chemokines (CXC and C-C). The C-C chemokine ligand 2 (CCL2), sometimes is known as monocyte chemoattractant protein 1 (MCP-1), directs monocytes/macrophages relocation and infiltration.[16] In induction of inflammation, CCL2 drives myeloid and lymphoid cells chemotaxis and organizes their movement in many autoimmune diseases.[17] It also controls remodeling of bone due to stimulation via hormones or mechanical boosts.[16]

Also, CCL2 functions as a ligand for the C-C chemokine receptor type 2 (CCR2).[11] For monocytes and basophils, this cytokine has chemotaxis activity, but not for eosinophils or neutrophils. It has been linked to the development of several illnesses, including psoriasis, rheumatoid arthritis, and atherosclerosis, which are characterized by monocyte infiltration.[18,19]

Herein, we selected three key genes; *PTPN22, CTLA4,* and *CCL2* based on integrated bioinformatics analysis and ranking in databases. Then we investigated the relative expression level of these genes in Egyptian RA patients at various disease stages in an attempt to find a good biomarker that could provide a better diagnostic or prognostic role and to monitor disease activity.

Subjects and Methods

Study population and design

Fifty-nine RA Egyptian patients were recruited from the Rheumatology Department at the Faculty of Medicine, Suez Canal University Hospital, Ismailia, in this case-control study. The rheumatoid arthritis classification criteria (ACR/EULAR, 2010) were used to recruit participants in the study.[20] Patients who were less than 16 years of age or had chronic conditions (e.g., heart failure), other

autoimmune diseases, malignancy or complaining of disability, having cognitive deficits, psychiatric symptoms, or handicapped or illiterate patients were excluded.

RA patients were subjected to a thorough medical history and examination. The 28 joint-Disease Activity Score-C Reactive Protein (DAS-28 CRP) was used to assess disease activity. Fifty apparently healthy people were assigned as a control group and were matched the RA patients in terms of age and sex. The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Suez Canal University (approval no. 4309, September 2020). Written informed consents were obtained from each subject before included in the study.

Laboratory assessment

Complete blood count (CBC) was performed using an automated cell counter (Sysmex XT 5 parts, differential cell counter, Germany). Rheumatoid factor (RF), C-reactive protein (CRP), and anti-citrullinated protein antibody (anti-CCp) were assessed using an immunoassay analyzer (COBAS e411 Roche diagnostics, Germany), according to the manufacturer's instructions. The Westergren technique was used to calculate the Erythrocyte Sedimentation Rate (ESR).

The relative expression of PTPN22, CTLA4, and CCL2

Total cellular RNA was extracted from fresh whole blood samples, collected in EDTA coated tubes using the QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany, cat. no. 52304) according to the manufacturer's instructions. The NanoDrop ND-1000 spectrophotometer was used to determine the quantity and quality of RNA and cDNA (NanoDrop Tech., Inc. Wilmington, DE, USA). Reverse transcription was done using a thermocycler (Robocycler Gradient 96, BIOMETRA®, LA, USA) with the High-Capacity cDNA Reverse Transcription Kit (Invitrogen/Life Technologies, USA, cat. no. Archive). The relative expression of *PTPN22, CTLA4,* and *CCL2* was determined using StepOne™ Real-Time PCR System (Applied Biosystem) by utilizing Maxima SYBR Green qPCR master mix (Thermofisher Scientific, USA, cat. no. K0251) and genes' specific primers (willowfort, UK) as described in Table 1, with the thermal cycling protocol shown in Table 2. As an internal reference, primers of *glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* were employed. The results were expressed as ($2^{-\Delta\Delta CT}$).[21] For all PCR reactions, the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines was applied.[22]

In silico data analysis

MalaCards database was used to select our study target genes[9]. The National Center for Biotechnology Information[18] and the Uniprot databases were used to obtain gene and protein data[11]. The STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) version 11.0 was utilized to show predicted protein-protein interactions among our study target genes and other ones, involving functional and physical associations[19]. Furthermore, STRING was used to study the gene ontology concepts of biological processes, molecular functions, and cellular compartment for the gene targets that were chosen. Using online Bioinformatics tools and in silico PCR, we developed and assessed target gene-specific primer tests[23]. Moreover, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway has been accessed to have more clarification of gene product interaction and molecular basis of the disease[4].

Statistical analysis

The statistical package for the social science (SPSS) version 25 (SPSS Inc., Chicago, IL, USA) software was used to analyze the data. Frequency and percentages were used to present qualitative data. To assess the normality of quantitative data, the Shapiro test was used. Mean and standard deviation were used to represent the normally distributed values, whereas the median and inter-quartile range (IQR) were assessed to express the skewed variables. To determine if a significant difference was found between categorical variables, Fisher exact test was used. To test the significant differences between continuous

variables, Kruskal-Wallis, Mann-Whitney U test, and t-test were utilized. For determining the relationship between the relative expression of our study target genes and several rheumatoid arthritis variables, the Spearman correlation coefficient was utilized. All tests were two-tailed, and significance was defined as a *p* value less than 0.05.

Table 1. The nucleotide sequence of primers used in Real-time PCR.

Genes' symbol	Forward primer (5'->3')	Reverse primer (5'->3')
PTPN22	CTGTACTAGCAACTGCTCCA	TCCAGCTTCCTCAACCACAA
CTLA-4	TGGCTTGCCTTGGATTTCAGC	ACACACAAAGCTGGCGATGC
CCL2	CCGAGAGGCTGAGACTAAC	CTTGCTGCTGGTGATTCTTC
GAPDH	CTCCTCACAGTTGCCATGTA	GTTGAGCACAGGGTACTTTATTG

Table 2. Thermal cycling protocol for StepOne™ Real-Time PCR System.

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	15 S	
Annealing	56°C	30 S	40
Extension	72°C	30 S	

*It was followed by a melting curve analysis to exclude primer dimer

Results

Patients' characteristics

Table 3 summarizes the socio-demographic characteristics, clinical data, laboratory results, and disease activity of RA patients. Mostly, the studied patients were middle-aged females, 54/59 (91.5%) with a median±SD of age was 41.5±11.7 years. RA family history was reported in 4 RA patients (6.8%), and only 4 patients were smokers (6.8%). The median (IQR) of the 28 joint-Disease Activity Score-C Reactive Protein (DAS-28 CRP) of RA patients was 4.3 (2.8), indicating moderate to severe disease activity.

Table 3. Demographic and clinical characteristics of the 59 studied patients.

Variables	Rheumatoid Arthritis
Age, years (median ±SD)	41.5±11.7
Female, n (%)	54 (91.5)
Family history, n (%)	4 (6.8)
Disease duration, years (median ±SD)	2.3 ± 2.1
Smoking, n (%)	4 (6.8)
28-Tender Joint Count, median (IQR)	4.0 (8.0)
28-Swollen Joint Count, median (IQR)	2.0 (3.0)
ESR, mm/hr, median (IQR)	50.0 (48.0)
CRP, mg/L, median (IQR)	6.0 (14.0)
Rheumatoid Factor, n (%)	44 (74.6)
ACPA, n (%)	29 (49.2)
DAS-28 CRP, median (IQR)	4.3 (2.8)
Co-morbidities, n (%)	7.0 (11.8)
Medications	
Methotrexate, n (%)	43 (72.9)
Hydroxychloroquine, n (%)	34 (57.6)
Leflunomide, n (%)	18 (30.5)
Sulfasalazine, n (%)	2 (3.4)
Prednisolone, n (%)	16 (27.1)

IQR, interquartile range; ESR, Erythrocyte Sedimentation Rate; CRP, C-Reactive Protein; ACPA, Anti-Citrullinated Protein Antibodies; DAS-28 CRP, 28 joint-Disease Activity Score-C Reactive Protein.

Expression of PTPN22, CTLA-4 and CCL-2 genes

In RA patients, the median (IQR) of *CTLA-4* gene expression was significantly higher in comparison to controls [1.353 (0.73) vs. 1, respectively, *P*=0.005]. Also, RA patients demonstrated a significantly higher *CCL2* expression than the controls [19.023 vs. 1, respectively, *P*<0.0001]. However, RA patients demonstrated a significantly lower *PTPN22* gene expression compared to its expression in controls [0.161 (0.12) vs 1, *P*=0.0001)], Figure 1.

The correlation matrix of the three genes in rheumatoid arthritis patients revealed a significant weak positive correlation between *PTPN22* and either *CTLA-4* or *CCL2* (r_s=0.269, *P*=0.040 or r_s=0.359, *P*=0.005, respectively). However, there was no correlation between expression of *CTLA-4* and *CCL2* (r_s= 0.087, *P*=0.551), Table 4.

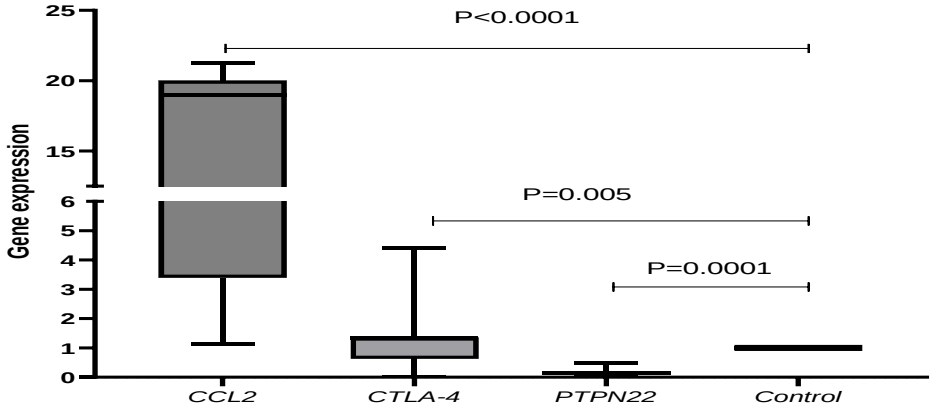

Figure 1. *CCL2, CTLA-4 and PTPN22* genes relative expression in the study participants.

The difference in the relative expression of the three genes in RA patients is significant in comparison to the relative expression level in healthy controls (Kruskale-Wallis, *P*<0.0001). There was a significantly higher level of expression of *CCL2 and CTLA-4* in RA patients compared to controls (*P*<0.0001 and *P*=0.005, respectively). However, there was a significantly lower level of expression of *PTPN22* in RA patients (*P*=0.0001) compared to controls.

Table 4. Correlation matrix of *PTPN22, CTLA4* and *CCL2* relative expression in RA patients:

	PTPN22 rho (**P* value)	*CTLA4* rho (**P* value)	*CCL2* rho (**P* value)
PTPN22	1.00	0.269 (0.040)	0.359 (0.005)
CTLA-4	0.269 (0.040)	1.00	0.087 (NS)
CCL2	0.359 (0.005)	0.087 (NS)	1.00

* *P* value >0.05 is not significant (NS).

Association between expression level of target genes and patients' characteristics

The association between the relative expression of the three genes and selected patients' characteristics is presented in Table 5. Male RA patients showed a significantly higher median (IQR) relative expression of *PTPN22* compared to female patients [0.378 (0.26), vs 0.161 (0.08); *P*=0.030]. In RA patients, there were no differences in *PTPN22, CTLA-4,* and *CCL2* genes expression and family history, smoking, rheumatoid factor, or anti-CCP status.

However, patients with moderate-severe disease activity revealed significantly higher *CCL2* levels (DAS-28 CRP>3.2) [20.0 (13.63), *P*=0.013]. Although patients in remission or low disease activity showed a low-expression of *PTPN22* (DAS-28 CRP ≤3.2), however this did not reach statistical significance (*P*=0.107) as shown in Figure 2.

There was a significantly higher relative expression level of *CCL2* in RA patients with moderate-severe disease activity (DAS-CRP>3.2, *P*=0.013). In contrast, there was a non-significant difference between either *CTLA-4* or *PTPN22* relative expression level with disease activity in RA patients.

Table 5. Association of the relative expression of *PTPN22, CTLA4, and CCL-2* in RA patients with the demographic, clinical and laboratory characteristics.

Variable	*PTPN22* Level median (IQR)	*CTLA4* Level median (IQR)	*CCL-2* Level median (IQR)
Family History			
Positive	0.190 (0.26)	1.266 (0.42)	1.676 (14.8)
Negative	0.163 (0.11)	1.35 (0.90)	19.7 (20.0)
P value	NS	NS	NS
Gender			
Male	0.378 (0.26)	1.353 (1.10)	20.0 (10.1)
Female	0.161 (0.08)	1.353 (1.14)	16.0 (16.8)
P value	0.030	NS	NS
Smoking			
Smoker	0.286 (0.29)	1.353 (0.69)	20.4 (14.8)
Non-Smoker	0.169 (0.15)	1.354 (1.11)	17.5 (16.8)
P value	NS	NS	NS
Rheumatoid Factor			
Positive	0.153 (0.11)	1.353 (0.14)	17.9 (16.5)
Negative	0.180 (0.24)	1.122 (1.6)	19.7 (17.9)
P value	NS	NS	NS
ACPA			
Positive	0.180 (0.17)	1.353 (0.80)	20.0 (16.6)
Negative	0.177 (0.49)	1.353 (1.10)	7.45 (18.0)
P value	NS	NS	NS
Methotrexate			
Yes	0.177 (0.09)	1.353 (0.73)	20 (16.6)
No	0.151 (0.20)	1.353 (0.85)	16.3 (17.5)
P value	NS	NS	NS
Leflunomide			
Yes	0.151 (0.24)	1.353 (0.95)	16.3 (15.2)
No	0.177 (0.10)	1.353 (0.47)	20 (16.9)
P value	NS	NS	NS
Hydroxychloroquine			
Yes	0.178 (0.11)	1.353 (0.70)	20 (16.85)
No	0.159 (0.17)	1.353 (0.74)	15.6 (16.3)
P value	NS	NS	NS
Glucocorticoids			
Yes	0.147 (0.13)	1.353 (0.75)	16.03 (15.8)
No	0.180 (0.17)	1.353 (0.80)	19.9 (16.9)
P value	NS	NS	NS

* *P* value >0.05 is not significant (NS). Abbreviations: IQR, inter-quartile range; ACPA, Anti-Citrullinated Protein Antibodies; DAS-28 CRP, 28 joint-Disease Activity Score-C-Reactive Protein.

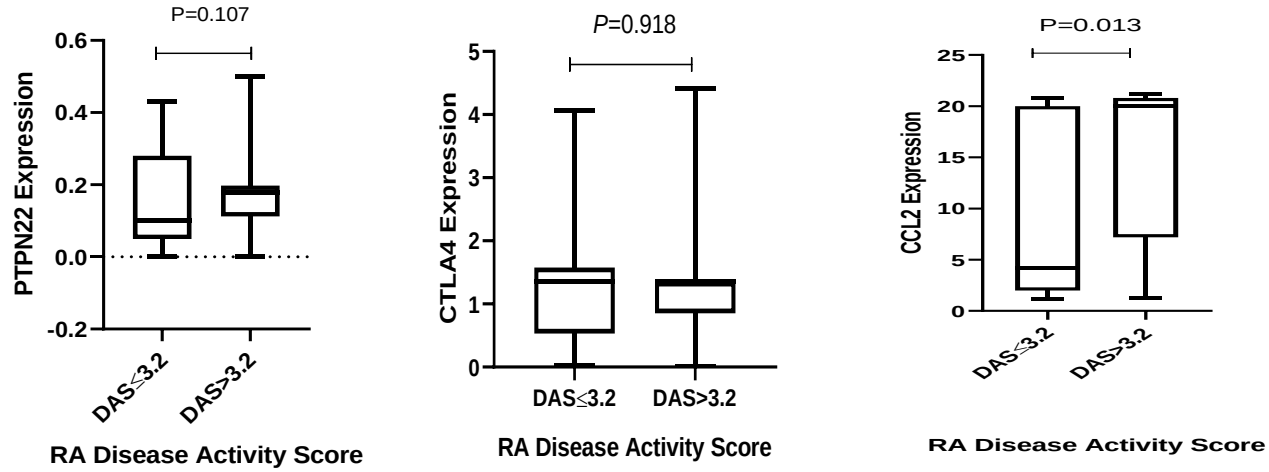

Figure 2. Association of *PTPN22, CTLA-4*, and *CCL2* relative expression in RA patients with disease activity.

Correlation of PTPN22, CTLA4, and CCL-2expression in rheumatoid arthritis patients and some disease activity parameters

PTPN22 expression and 28-swollen joint count demonstrated a significant weak correlation (rs=0.285, *P*=0.030). Also, a significant, albeit weak correlation was noted between *CCL2* gene expression and 28-swollen joint count and ESR (r_s=0.280, *P*=0.033, and r_s=*0.*290, *P*=0.027, respectively), Table 6.

Table 6. Spearman's correlation for *PTPN22, CTLA4, and CCL-2*relative expression in RA patients and some disease activity parameters.

Variable	*PTPN22* Level rho (*P value)	*CTLA4* Level rho (*P value)	*CCL-2* Level rho (*P value)
28-Swollen Joint Count	0.285 (0.030)	0.193 (NS)	0.280 (0.033)
ESR, mm/hr	0.177 (NS)	0.057 (NS)	0.290 (0.027)
CRP, mg/L	-0.173 (NS)	-1.26 (NS)	0.224 0(NS)

* *P* value >0.05 is not significant (NS). Abbreviations: CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate; CRP, C-Reactive protein.

Discussion

Several non-HLA genes are associated with RA. Here, we reported the relative expression of *PTPN22, CTLA-4,* and *CCL-2* in a cohort of Egyptian RA patients. The current results demonstrated that RA patients showed a significant reduction of *PTPN22* expression level compared to controls and a significantly higher expression of *CTLA-4* and *CCL2* compared to controls. Moreover, rheumatoid arthritis patients with moderate-severe disease activity demonstrated a significant over-expression of *CCL2* in comparison to low disease activity.

In the current study, the results revealed a significant reduction of *PTPN22* expression level in RA patients compared to controls which can be explained based on the inhibitory effect of the down regulated *PTPN22* on the immune receptors signaling pathways in RA patients. *PTPN22* gene is widely expressed in myeloid lineages, including B cells, natural killer cells, and neutrophils, acting as the safeguard for both B cell receptors and T cell receptors signaling pathways.[24]

In our study, there was a significant higher *CTLA-4* relative expression in RA patients versus controls which was consistent with findings of Aihaiti *et al.,* 2020 that demonstrated a

significant increase in *CTLA-4* expression in RA synovial tissue,[25] but other studies showed minor to a non-detectable level of CTLA-4 membrane expression on the helper or cytotoxic T cells.[15, 26] The increased expression of CTLA-4 was reported only at the level of the soluble molecule (sCTLA-4)[12,13,15] or on synovial membranes of RA joints.[26]

We showed higher expression of *CCL-2* in peripheral blood and its association with disease activity. This finding agrees with several previous studies as follows, Szekanecz et al., 2003 explained the role of CCL2 as a chemotactic factor for and T-lymphocyte, B-cell, and monocyte/macrophage.[27] Furthermore, Kawano and Nakamachi, 2011 revealed the role of CCL2 as a pro-inflammatory agent in RA synovial inflammation.[28]

A study conducted on RA patients by Sucur et al., 2017 found increased CCL2, CCL3, CCL4, and CXCL10 levels in both plasma and synovial fluid.[29] Also, Stankovic et al., 2009, reported high concentrations of CCL2, a CCR2 ligand, in the synovial liquid of rheumatoid arthritis patients.[30] Such findings were explained by Diaz-Rubio et al., 2019 who reported that the stimulation and movement of monocytes to the inflamed area during the RA inflammatory process were performed by a chemokine system [CCR2/CCL2, Chemerin Chemokine-Like Receptor 1/resolving E1 (CMKLR1/RvE1)]. Also, more expression of these chemokine receptors and communication with their ligands potentiated the chronic inflammatory phase of RA.[31]

Moreover, Kumar et al., 2018 found that RA peripheral blood monocytes showed more production of pro-inflammatory cytokines and higher expression of cellular surface antigens and chemokine receptors. This illustrates the migration, accumulation of peripheral blood monocyte to inflamed synovium, and occurrence of local inflammation.[32]

In addition, Shahrara et al., 2010 demonstrated that IL-1β and TNF-α cytokines induced the production of CCL2, C-X3-C motif chemokine Ligand 1 (CX3CL1), and chemoattractant CCL20 (referred to as macrophage inflammatory protein-3 alpha; MIP-3α) in Synovial fibroblasts in RA patients.[33]

The current study results showed that patients with rheumatoid arthritis ranging from mild to severe activity had significantly higher CCL2 levels. Recently, Gschwandtner and his colleagues in 2019, explained the occurrence of exaggerated cell recruitment and inflammatory process due to increased CCL2 expression. Also, they declared many roles of CCL2 as chemotaxis for myeloid and lymphoid cells, stimulating the production of inflammatory cytokines by activated monocytes, modulating integrin expression and localization which controls cell adhesion, as well as arachidonic acid release.[17]

A study conducted by Lin Zhang et al., 2015 reported that the *c-Jun*, *c-Fos*, and *CCL2* mRNA expression in RA patients were more than normal controls on peripheral blood as well as in synovial tissues. Also, they showed raised levels of serum CCL2 in RA patients with widespread CCL2 expression in RA Synovial tissue in histological assessment.[34]

In conclusion, *CTLA-4* and *CCL2* expression in Egyptian RA patients were significantly increased, while a significant *PTPN22* down-expression was observed in the same population with a significant over-expression of *CCL2* in RA patients with moderate-to-severe disease activity. Such findings may suggest that these three key genes could become useful diagnostic markers for RA and *CCL2* expression as a good prognostic tool for RA disease activity.

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Author Contributions

MAE, NA, MH, the concept. DB, AH, RE, MA, design, materials, data collection and/or processing. SE, MH, supervision. All authors, resources. MAE, NA., MH, SE, analysis and/or interpretation. DB, AH, RE, MAE, NA, literature search. All authors writing manuscript. DB, AH, RE, MA, MAE, NA, critical review.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Suez Canal University (approval no. 4309, September 2020).

Informed consent

Written informed consents were obtained from each subject before included in the study.

References

1. Pap, T. and A. Korb-Pap (2015). *C*artilage damage in osteoarthritis and rheumatoid arthritis--two unequal siblings. *Nat Rev Rheumatol*,. 11(10): p. 606-15.

2. Sarzi-Puttini, P., et al., (2014). *P*ain in rheumatoid arthritis: a critical review. *Reumatismo*, 66(1): p. 18-27.

3. Kurkó, J., et al., (2013).Genetics of rheumatoid arthritis - a comprehensive review. *Clinical reviews in allergy & immunology*, 45(2): p. 170-179.

4. Qiu, Y.-Q., (2013). KEGG Pathway Database, in Encyclopedia of Systems Biology, W. Dubitzky, et al., Editors. Springer New York: New York, NY. p. 1068-1069.

5. van der Helm-van Mil, A.H., J.Z. Wesoly, and T.W. Huizinga (2005). Understanding the genetic contribution to rheumatoid arthritis. *Curr Opin Rheumatol*, 17(3): p. 299-304.

6. Mustelin, T., N. Bottini, and S.M. Stanford (2019). The Contribution of PTPN22 to Rheumatic Disease. *Arthritis & rheumatology* (Hoboken, N.J.), 71(4): p. 486-495.

7. Cohen, S., et al., (1999). *C*loning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase, Lyp. *Blood*, 93(6): p. 2013-24.

8. Purvis, H.A., et al., (2020). Phosphatase PTPN22 Regulates Dendritic Cell Homeostasis and cDC2 Dependent T Cell Responses. *Frontiers in immunology*, 11: p. 376-376.

9. Rappaport, N., et al., (2017). MalaCards: an amalgamated human disease compendium with diverse clinical and genetic annotation and structured search. *Nucleic acids research*, 45(D1): p. D877-D887.

10. Linsley, P.S. and S.G. Nadler, (2009). The clinical utility of inhibiting CD28-mediated costimulation. *Immunol Rev*, 229(1): p. 307-21.

11. The UniProt, C., (2018). UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Research*, 47(D1): p. D506-D515.

12. Cao, J., et al., (2012). *I*ncreased production of circulating soluble co-stimulatory molecules CTLA-4, CD28 and CD80 in patients with rheumatoid arthritis. *International Immunopharmacology*, 14(4): p. 585-592.

13. García-Chagollán, M., et al., (2020). Expression patterns of CD28 and CTLA-4 in early, chronic, and untreated rheumatoid arthritis. *Journal of Clinical Laboratory Analysis*, 34(5): p. e23188.

14. Picchianti Diamanti, A., et al., (2014). Abatacept (cytotoxic T lymphocyte antigen 4-immunoglobulin) improves B cell function and regulatory T cell inhibitory capacity in rheumatoid arthritis patients non-responding to anti-tumour necrosis factor-α agents. *Clin Exp Immunol*, 177(3): p. 630-40.

15. Liu, P.-C., et al., (2020). Cytotoxic T lymphocyte-associated antigen-4-Ig (CTLA-4-Ig) suppresses *Staphylococcus aureus*-induced CD80, CD86, and pro-inflammatory cytokine expression in human B cells. *Arthritis research & therapy*, 22(1): p. 64-64.

16. Siddiqui, J.A. and N.C. Partridge (2017). CCL2/Monocyte Chemoattractant Protein 1 and Parathyroid Hormone Action on Bone. *Frontiers in endocrinology,* 8: p. 49-49.

17. Gschwandtner, M., R. Derler, and K.S. (2019). Midwood, More Than Just Attractive: How CCL2 Influences Myeloid Cell Behavior Beyond Chemotaxis. *Frontiers in immunology,* 10(2759).

18. Coordinators, N.R., (2016). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, 44(D1): p. D7-D19.

19. Szklarczyk, D., et al., (2019). *STRING v11:* protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic acids research*, 47(D1): p. D607-D613.

20. Aletaha, D., et al., (2010). rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against

Rheumatism collaborative initiative. *Arthritis & Rheumatism*, 62(9): p. 2569-2581.

21. Livak, K.J. and T.D. Schmittgen, (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*, 25(4): p. 402-408.

22. Bustin, S.A., et al., (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem*, 55(4): p. 611-22.

23. San Millán, R.M., et al., (2013). Online exercise for the design and simulation of PCR and PCR-RFLP experiments. *BMC Research Notes*, 6(1): p. 513.

24. Diaz-Gallo, L.-M. and J. Martin (2012). *PTPN22* splice forms: a new role in rheumatoid arthritis. *Genome Medicine*, 4(2): p. 13-13.

25. Aihaiti, Y., et al., (2020). Identification of pivotal genes and pathways in the synovial tissue of patients with rheumatoid arthritis and osteoarthritis through integrated bioinformatic analysis. *Mol Med Rep*, 22(4): p. 3513-3524.

26. Liu, M.F., et al., (1999). Increased expression of down-regulatory CTLA-4 molecule on T lymphocytes from rheumatoid synovial compartment. *Scand J Immunol*, 50(1): p. 68-72.

27. Szekanecz, Z., J. Kim, and A.E. Koch, (2003). Chemokines and chemokine receptors in rheumatoid arthritis. *Seminars in Immunology*, 15(1): p. 15-21.

28. Kawano, S. and Y. Nakamachi (2011). *miR-124a a*s a key regulator of proliferation and MCP-1 secretion in synoviocytes from patients with rheumatoid arthritis. *Ann Rheum Dis*, 70 Suppl 1: p. i88-91.

29. Sucur, A., et al., (2017). Chemokine signals are crucial for enhanced homing and differentiation of circulating osteoclast progenitor cells. *Arthritis research & therapy*, 19(1): p. 142-142.

30. Stankovic, A., et al., (2009). Serum and synovial fluid concentrations of CCL2 (MCP-1) chemokine in patients suffering rheumatoid arthritis and osteoarthritis reflect disease activity. *Bratisl Lek Listy*, 110(10): p. 641-6.

31. Diaz-Rubio, G., et al., (2019). *"*Chemokine receptors CCR2, CMKLR1 expression pattern matching to the levels of their respective ligands CCL2, RvE1 in the inflammatory response of insulin resistance in rheumatoid arthritis". C*linica Chimica Acta,* 493: p. S298-S299.

32. Kumar, R.A., et al., (2018). Monocytes in rheumatoid arthritis: Circulating precursors of macrophages and osteoclasts and, their heterogeneity and plasticity role in RA pathogenesis. *International Immunopharmacology*, 65: p. 348-359.

33. Shahrara, S., et al., (2010). IL-17-mediated monocyte migration occurs partially through CC chemokine ligand 2/monocyte chemoattractant protein-1 induction. *Journal of immunology* (Baltimore, Md. (1950), 184(8): p. 4479-4487.

34. Zhang, L., et al., (2015). Chemokine Signaling Pathway Involved in CCL2 Expression in Patients with Rheumatoid Arthritis. *Yonsei medical journal*, 56(4): p. 1134-1142.