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Peripheral Blood mRNA and Protein Levels of CCN1 and CCN3 in Psoriatic Arthritis: A Case-Control Study

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ABSTRACT

Background: Patients with psoriasis may develop psoriatic arthritis (PsA), an inflammatory joint disorder. Measuring cellular communication network (CCN) expression levels can help in disease diagnosis and prognosis. The purpose of this study was to assess the diagnostic value of CCN1 and CCN3 expressions for PsA.

Patients and methods: Thirty-seven PsA patients, 37 psoriasis patients, and 37 matched healthy controls participated in a case-control study. ELISA was used to detect protein levels while qRT-PCR was used to evaluate gene expression.

Results: Patients with PsA and psoriasis had greater serum and expression levels of CCN1 compared to controls ($p< 0.0001$). Patients with PsA and psoriasis had greater serum and expression levels of CCN1 compared to controls ($p= 0.003$, $p< 0.0001$). Both CCN3 levels in PsA patients were greater than in controls ($p< 0.0001$), but patients with psoriasis did not significantly differ from controls ($p=0.13$, $p=0.38$). When compared to psoriasis patients, PsA patients had greater serum and expression levels of CCN3 ($p< 0.0001$). In terms of psoriasis duration and severity, CCN1 exhibited a positive relation. It was shown that CCN3 and both the duration and severity of arthritis correlated positively. All markers were positively correlated with inflammatory markers.

Conclusions: The most accurate marker for PsA detection was CCN3 expression, followed by the serum level of CCN1. However, CCN1 expression was the most accurate marker for the differentiation between psoriasis and PsA, followed by CCN3 expression. CCN3 could be a marker for PsA severity. These preliminary data need further studies to confirm their findings.

Keywords: CCN; Psoriasis; Psoriatic Arthritis; Severity.

INTRODUCTION

Psoriatic arthritis (PsA) is an inflammatory condition that damages psoriatic patients' joints and has an immunological basis [(1)]. About 3% of people worldwide are affected by PsA. PsA is found in 30% of psoriatic patients [(2, 3)]. Psoriatic arthritis can strike at any age, but most people get it between the ages of 30 and 50, and both men and women are affected equally [(4)]. PsA causes a variety of joint symptoms and is frequently linked to signs of nail involvement [(5)]. Joint damage caused by PsA not only reduces articular function and increases mortality, but also affects patients' capacity to work and social connections [(6)].

Six matricellular proteins make up the cellular communication network (CCN) family that are all involved in angiogenesis and wound healing [(7)]. CCN genes have been linked to the pathophysiology of chronic inflammatory disorders like rheumatoid arthritis (RA), neuroinflammatory diseases, and inflammatory kidney disease [(8)]. CCN1 is a crucial proinflammatory mediator in RA based on increased levels of CCN1 in synovial fluid from RA patients [(9)]. By stimulating the generation of monocyte chemoattractant protein-1 in osteoblasts, CCN1 promotes monocyte movement [(10)].

In addition, knocking down CCN1 in an animal model reduced joint swelling and cartilage degradation [(11)]. CCN3 is a matrix molecule that may be released from the cytoplasm and participates in angiogenesis and fibrosis, among other functions [(12)]. CCN3 was found in arthritis patients' synovial tissues [(13)]. CCN3 has been linked to osteoarthritis and inflammatory diseases [(14)]. Increased serum CCN3 levels are linked to disease severity in RA [(15)]. However, the role of CCN1 and CCN3 in PsA is uncertain. Psoriasis and PsA are evidently complex genetic illnesses [(16)], with improperly expressed genes. Biological processes are determined by proteins, which are the end products of gene expression. Therefore, measuring gene and protein expression levels can help with disease diagnosis, prognosis, and therapy planning [(17)]. This study hypothesizes that CCN1 and CCN3 gene expressions increased in PsA patients. So, this work aimed to evaluate the potential diagnostic roles of CCN1 and CCN3 genes expressions and proteins levels in PsA patients, as well as the relation between these markers and disease severity.

SUBJECTS AND METHODS

Study Design:

This study, conducted in 2022, used a case-control design. The patients were found from the Departments of Rheumatology and Dermatology. At the Clinical Pathology and Microbiology Departments, the samples were examined.

Ethical approval:

The study protocol was approved by the Faculty of Medicine's Institutional Review Board (Zagazig University) [IRB#9887]. All participants signed written informed consent forms. The study has been carried out in accordance with The Code of Ethics of

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the World Medical Association (Declaration of Helsinki) for studies involving humans.

Subjects:

Calculating the sample size was done using mean and standard deviation of the differences between cases and controls that obtained from a previous study [18]. In this study, there were 37 healthy individuals, 37 patients with psoriasis, and 37 patients with PsA. The controls did not have a history of joint disease or psoriasis in their families. Patients who were pregnant or had other immunological or hepatic illnesses were excluded from the research. The study flowchart is illustrated in Figure 1. All patients had negative rheumatoid factor tests.

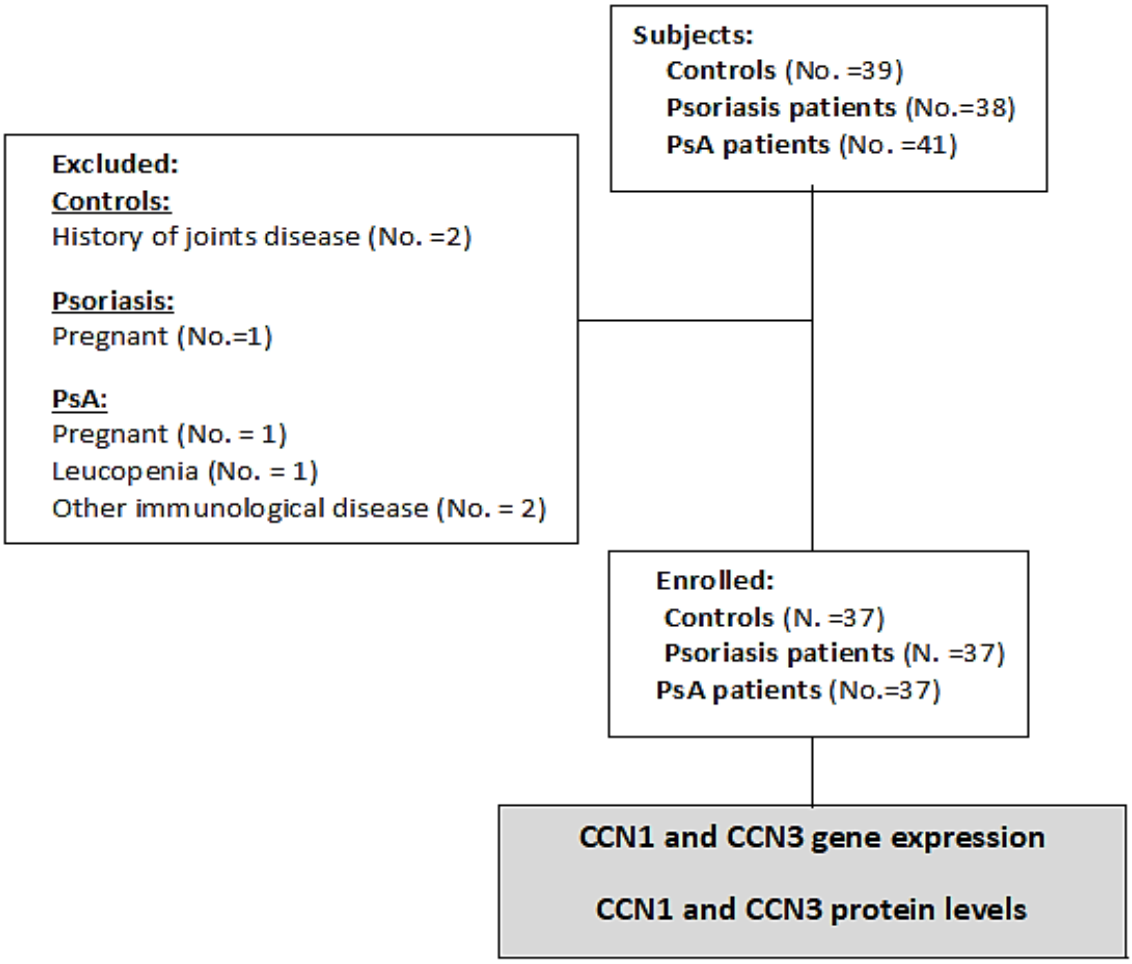


Figure 1: Diagram represents this study workflow

Psoriasis was clinically recognized by its identifiable lesions. The surface area and severity of psoriasis lesions in all patients were evaluated for this study using the psoriatic area and severity index (PASI) [19]. There are two degrees of psoriasis severity (mild and moderate to severe). Patients were categorized as having mild psoriasis if their PASI values were 10 or below, and moderate to severe psoriasis if their PASI values were above 10. [20]. To diagnose PsA patients, the Classification Criteria for Psoriatic Arthritis were applied [21]. To evaluate joint involvement, Moll and Wright's criteria were applied [22]. To clarify the disease activity states, the Disease Activity for Psoriatic Arthritis (DAPSA) was used [23]. The Composite Psoriatic Disease Activity Index (CPDAI) was another tool for assessing disease activity [24].

Samples: Venipuncture was used to obtain whole blood samples from the study participants. An ESR tube, plus plastic serum tube, and EDTA tube were used to collect whole blood from each patient. However, only EDTA and plus plastic serum tubes were used to collect samples from the control subjects.For serum separation, samples were allowed to clot for 30 min at room temperature before being centrifuged for 10 min at 1200 g. Serum was utilized to determine CRP levels. Another aliquot of 1 mL serum was kept at -80°C until the CCN1 and CCN3 ELISA tests were performed. EDTA tubes were processed within 1 hour for RNA extraction.

Methods: The automatic erythrocyte sedimentation rate (ESR) analyzer Vision B (Shenzhen. YHLO Biotech Co., Ltd., Shenzhen, China) was used to determine the ESR. On the Roche Cobas 8000/c702 analyzer, serum was utilized to determine C-reactive protein (CRP) levels (Roche Diagnostic, Mannheim, Germany).

Whole blood was used to extract total RNA. The Total RNA Purification kit's protocol for RNA extraction was followed **(Cat. No.: PP-210S, Jena Bioscience, Germany).** The Nanodrop 2000 spectrophotometer was used to analyze the quality and quantity of the isolated RNA **(Thermo Fisher Scientific, Waltham, MA, USA)**. The detection of ribosomal RNA bands in gel electrophoresis was used to determine the integrity of the isolated RNA. The total RNA concentration in each sample was set to 50 ng/µL. According to the manufacturer's procedure, single-stranded cDNA was generated from 10 µl RNA samples using the SCRIPT Reverse Transcriptase kit (Cat. No.: PCR-505S) with Oligo-dT primer **(Jena Bioscience, Germany)**. On a PCR thermocycler, the thermal conditions were 28°C for 10 min and 50°C for 60 min **(Gene Amp, PCR system 9700, Perkin Elmer, Singapore).** The cDNA was frozen at -80°C until utilized.

Metabion International AG was the supplier for the primers **(Planegg, Munich, Germany)**. The primers were designed in the following sequence: CCN1 gene [Forward: 5′-TCAAAGACCTGTGGAACTGGTATC-3′, Reverse: 5′-CACAAATCC-GGGTTTCTTTCA-3′]; CCN3 gene [Forward: 5′-CCGCTGTCAGCTGGATGT-3′, Revers: 5′-CTCCAGGCACCTCAACTTTTCT-3′]; and GAPDH gene [Forward: 5′-CATGTTCCAGTATGACTCCACTC-3′, Reverse: 5′-GGCCTCACCCCATTTGATGT-3′]. Real-time qRT-PCR was carried out using the qPCR GreenMaster [Cat. No.: PCR-313S] **(Jena Bioscience, Germany)**. With 5 µL of cDNA, we produced 20 µL PCR reaction mix in accordance with the manufacturer's recommendations. Forty cycles of 95ºC for 15 sec and 60ºC for 1 min were performed after the first denature, which took place at 95ºC for 10 minutes. The specificity of the PCR amplification was ascertained via an analysis of the melting curve. The Stratagene Mx3005P qPCR System was used to perform the PCR reaction **(Agilent Technologies, Germany)**. As a reference gene, the GAPDH gene was selected. The $2^{-\Delta\Delta CT}$ approach was used to report the relative expression of the genes as fold change. Serum CNN1 and CNN3 were quantified by ELISA. Human CYR61 /CCN1 ELISA Kit [Cat. No.: MBS177255] (MyBiosource, Southern California, San Diego, USA) and Human NOV/CCN3 ELISA Kit PicoKine [Cat. No.: EK0833] (Boster Biological Technology, Pleasanton, CA, USA) were utilized. The ELISA procedure was carried out as directed by the manufacturer. The levels of CCN1 and CNN3 were measured in pg/mL. Intra-assay coefficients of variation in percentage for these kits are <3.4 % and <6.2 %, respectively. The percentage of inter-assay coefficients were <6.2 % and <7.7%, respectively.

Statistical Analysis: Quantitative variables were presented as minimum, median, and maximum. Categorical data were displayed as number and percent. For quantitative factors that weren't related, the Mann-Whitney test was used. The chi-squared test was employed to compare categorical ones. The receiver operating characteristic (ROC) curve analysis was used to evaluate the markers' prediction capabilities. To assess the association of variables, the Spearman correlation test was used. SPSS-19 software (SPSS Inc., IL, USA) was used for the statistical analysis. The assessed test was considered significant if the p-value was less than 0.05.

RESULTS

The control group had a median age of 45 [32-59 years]. Eighteen of them were males and 19 were females. Age did not significantly differ between the control and patient groups (p= 0.98 and 0.68 when controls were compared with psoriasis patients and PsA patients, respectively). Additionally, psoriasis patients and PsA patients were matched based on sex with controls (p= 0.64 and 0.81, respectively). Table 1 displays the clinical and laboratory parameters of the patients. Regarding psoriasis activity categorization based on the PASI score, PsA patients exhibited a mild psoriasis prevalence of 62.2%, and a moderate to severe psoriasis prevalence of 37.8%. Nearly 40% of psoriasis patients fell into the moderate to severe category. In terms of psoriasis severity, PsA patients and psoriasis-only patients were comparable.

Table 1: PsA and psoriasis patients' demographics, clinical and laboratory data

Parameters	PsA (No.: 37)	Psoriasis (No.: 37)	p
Age	47 [32-65]	46 [33-57]	0.72
Sex (Male/Female)	17/20 (45.9/54.1)	16/21 (43.2/56.8)	0.82
Psoriasis duration	7 [3-17]	8 [3-15]	0.48
Psoriasis severity			
• PASI	5 [1-20]	6 [1-13]	0.33
• Mild cases	23 (62.2)	22 (59.5)	0.81
• Moderate to severe	14 (37.8)	15 (40.5)	
Nail involvement	20 (54.1)	2 (5.4)	<0.0001*
Arthritis duration	3.5 [1-9]		
Moll and Wright subgroups:			
• DIP joint	6 (16.2)		
• Asymmetrical oligoarthritis	9 (24.3)		
• Polyarthritis	8 (21.7)		
• Spondylitis	12 (32.4)		
• Arthritis mutilans	2 (5.4)		
Arthritis severity			
• DAPSA	30 [12-66]		
• CPDAI	10 [6-11]		
Laboratory findings			
• ESR, mm/h	36 [9-90]	19 [11-40]	0.047*
• CRP, mg/L	30 [6-87]	17 [8-45]	<0.0001*

Data are expressed as median [Range] or number (%)
No: Number; PsA: Psoriatic Arthritis; PASI: Psoriasis Area and Severity Index; DIP: distal interphalangeal DAPSA: Disease Activity for Psoriatic Arthritis; CPDAI: Composite Psoriatic Disease Activity Index; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein.
**: Significant*

The serum level of CCN1 was significantly higher in PsA patients (206 [98-227] pg/mL) and psoriasis patients (184 [143-219] pg/mL) in comparison with controls (55 [36-195] pg/mL). When compared to patients with psoriasis, those with PsA had significantly higher levels of CCN1 (Figure 2a).

The serum level of CCN3 was significantly higher in PsA patients (3960 [1590-8450] pg/mL) in comparison with controls (1852 [1005-4260] pg/mL). There was no significant difference between psoriasis patients (1895 [1102-3258] pg/mL) and controls. When compared to psoriasis patients, the amount of CCN3 in PsA patients was significantly higher (Figure 2b).

The peripheral blood expression fold change of CCN1 was significantly higher in PsA patients (1.99 [0.9-3.3]) and psoriasis patients (1.47 [1-1.8]) in comparison with controls. When compared to psoriasis patients, the expression level of CCN1 in PsA patients was significantly greater (Figure 2c).

The peripheral blood expression fold change of CCN3 was significantly higher in PsA patients (3.6 [1-5.2]) in comparison with controls. There was no significant difference between psoriasis patients (1 [0.88-1.6]) and controls. In comparison to patients with psoriasis, PsA patients had significantly higher levels of CCN3 expression (Figure 2d).

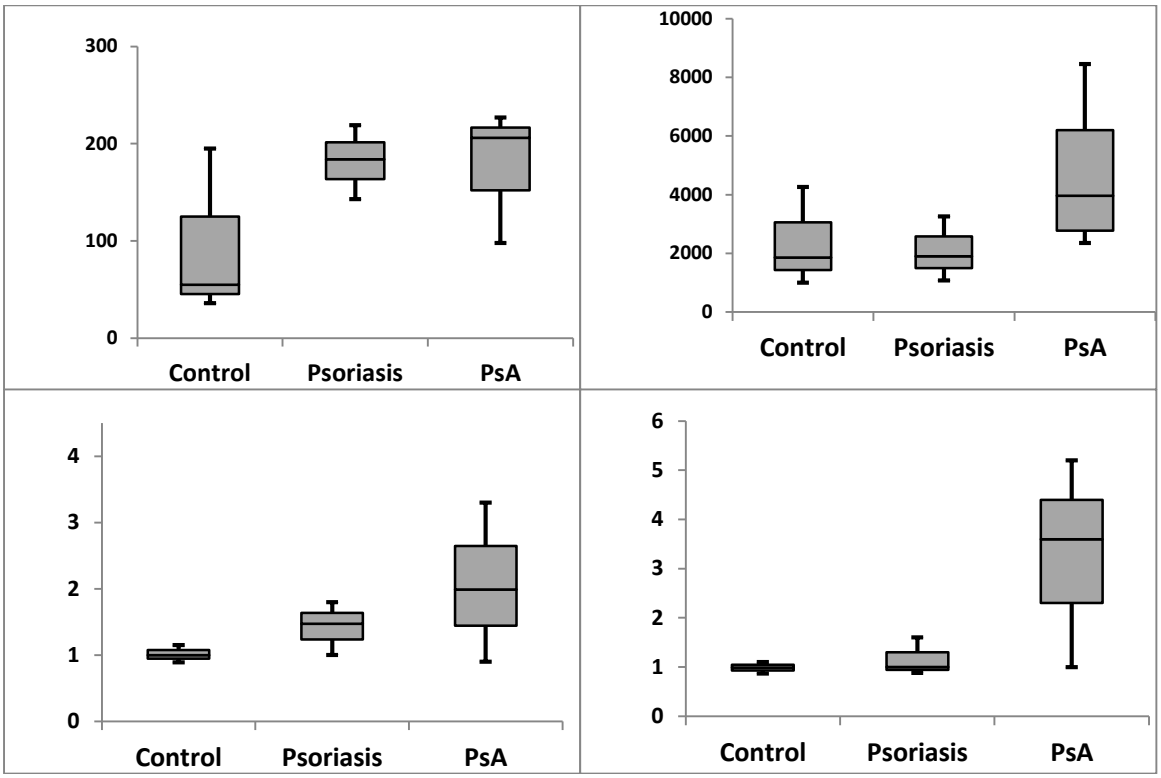
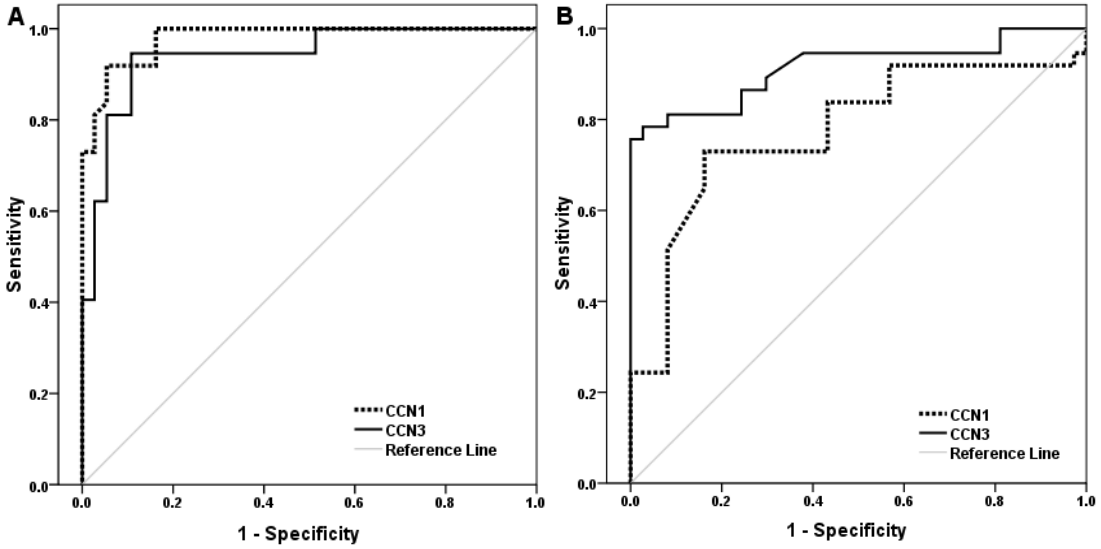


Figure 2: CCN1 and CCN3 protein and gene expression levels in studied subjects
CCN: Cellular Communication Network Factor; PsA: Psoriatic Arthritis
**: Significant*

The predictive roles of the studied markers in PsA detection and in differentiating between psoriasis and PsA were evaluated by ROC curve analysis (Figure 3). Table 2 provides the markers' diagnostic performance criteria. Regarding PsA detection, the most accurate marker was the CCN3 expression, followed by the serum level of CCN1. However, CCN1 expression was the most accurate marker for the differentiation between psoriasis and PsA, followed by CCN3 expression.



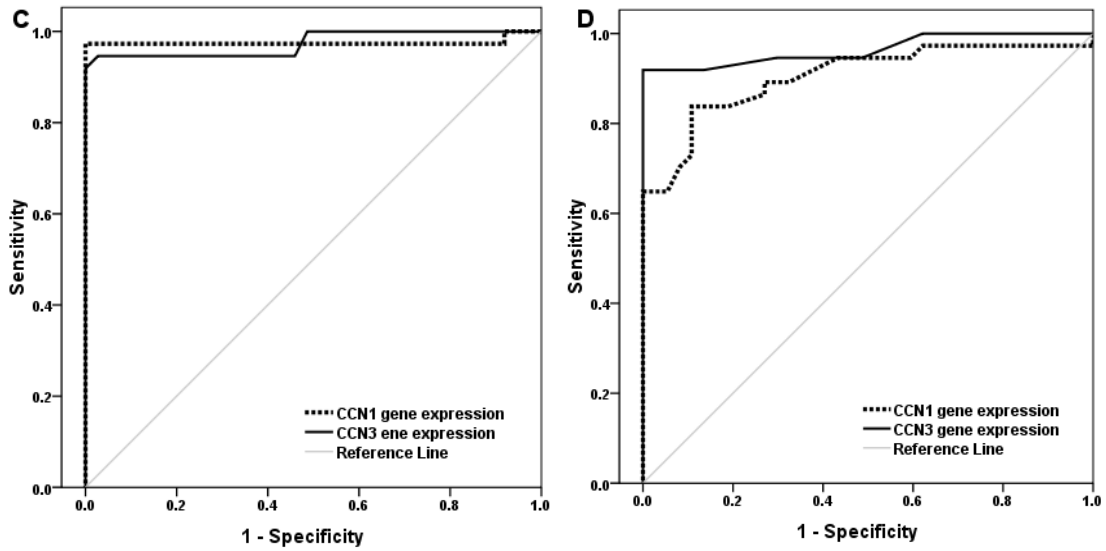


Figure3: (**A**) ROC curve of CCN1 and CNN3 protein for PsA diagnosis (**B**) ROC curve of CCN1 and CNN3 protein for distinguishing psoriasis from PsA (**C**) ROC curve of CCN1 and CNN3 gene expression for PsA detection (**D**) ROC curve of CCN1 and CNN3 gene expression for distinguishing psoriasis from PsA
CCN: Cellular Communication Network Factor; PsA: Psoriatic Arthritis

Table 2: Markers' diagnostic performance criteria regarding PsA detection and differentiation from psoriasis

Marker	Cutoff	Youden's index	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
PsA detection							
CCN1 (pg/mL)	≥ 167.5	0.87	91.9	94.6	94.4	92.1	93.2
CCN3 (pg/mL)	≥ 2217.5	0.84	94.6	89.2	89.7	94.3	91.9
CCN1 gene expression (Fold change)	≥1.68	0.73	83.8	89.1	88.6	84.6	86.5
CCN3 gene expression (Fold change)	≥1.85	0.92	91.9	100	100	92.5	95.9
PsA differentiation from psoriasis							
CCN1 (pg/mL)	≥196.5	0.57	73	83.8	81.8	75.6	78.4
CCN3 (pg/mL)	≥2290	0.73	81.1	91.1	90.1	82.9	86.5
CCN1 gene expression (Fold change)	≥1.28	0.97	97.3	100	100	97.4	98.6
CCN3 gene expression (Fold change)	≥1.6	0.92	91.9	100	100	92.5	95.9

CCN: Cellular Communication Network Factor; PsA: Psoriatic Arthritis; PPV: Positive predictive value; NPV: Negative predictive *value.*

The association between the markers' levels and PsA features is shown in Table 3. The duration and severity of psoriasis were positively correlated with CCN1 and CCN1 gene expression. There was a significant positive association between the length and severity of arthritis and CCN3 and CCN3 gene expression. All markers were positively correlated with inflammatory markers (CRP and ESR).

Table 3: Correlation of CCN1 and CCN3 protein and gene expression levels with PsA patients' characteristics

Parameters of PsA	CCN1		CCN3		CCN1 gene expression		CCN3 gene expression	
	r	p	r	p	r	p	r	p
Age	0.03	0.87	0.15	0.38	0.21	0.2	-0.07	0.69
Sex (Male/Female)	0.05	0.75	0.26	0.13	0.09	0.61	0.14	0.39
Psoriasis duration	0.36	0.027*	0.1	0.58	0.39	0.016*	0.11	0.54
Arthritis duration	0.21	0.2	0.51	0.001*	-0.03	0.88	0.52	0.001*
Nail involvement	0.11	0.53	0.11	0.52	0.12	0.48	0.05	0.77
Moll and Wright types	0.08	0.65	0.02	0.89	0.06	0.74	0.08	0.62
Psoriasis severity								
• PASI	0.34	0.04*	-0.11	0.52	0.45	0.005*	0.2	0.23
Arthritis severity								
• DAPSA	0.16	0.36	0.4	0.014*	0.04	0.83	0.46	0.004*
• CPDAI	0.09	0.58	0.36	0.029*	0.08	0.65	0.39	0.017*
Laboratory findings:								
• ESR, mm/h	0.38	0.023*	0.37	0.022*	0.32	0.048*	0.34	0.039*
• CRP, mg/L	0.37	0.021*	0.49	0.002*	0.41	0.01*	0.35	0.035*

PsA: Psoriatic Arthritis; PASI: Psoriasis Area and Severity Index; DAPSA: Disease Activity for Psoriatic Arthritis; CPDAI: Composite Psoriatic Disease Activity Index; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein.
**: Significant*

DISCUSSION

Psoriasis and musculoskeletal inflammation co-occur in PsA. Numerous studies are carried out to help predict, diagnose, treat, and prevent PsA [25,26]. Rather than just preserving structural stability, the primary role of CCN members is to promote cell-extracellular matrix contact. By combining with heparan sulphate, and proteoglycan, CCNs play vital roles in a variety of processes in the biological systems [27, 28].

CCN1 contributes to the pathophysiology of RA by encouraging synovium hyperplasia and synoviocyte overgrowth. CCN1 increases the activity of neutrophils and T helper-17, which in turn induces inflammation [29-31]. CCN1 enhances pro-inflammatory substances that exacerbates psoriasis skin lesions. [32]. RA, osteoarthritis, and systemic sclerosis are immune-related diseases, and CCN3 has recently been identified as a key mediator in these conditions [33]. To our knowledge, serum levels of CCN1 and CCN3, as well as their gene expression have not been investigated in PsA patients yet. Our study evaluated serum levels of CCN1 and CCN3 in psoriasis and PsA patients as well as peripheral blood expression. The purpose of this study was to assess how well the examined markers could identify PsA and distinguish psoriasis from PsA. The aim of this study was also to evaluate the relationship between the severity of the disease and these markers.

In this study, we found that the serum level of CCN1 was significantly higher in PsA patients and psoriasis patients. **Chen *et al.*** [10] found that when compared to synovial fluid from individuals without RA, RA synovial fluid exhibits significantly greater levels of CCN1 expression. According to the current study, PsA patients had significantly greater peripheral blood levels of CCN1 expression compared to controls and to psoriasis patients, this is consistent with the results of **Sun *et al.*** [32], where they found that in psoriasis patients' lesional skin, CCN1 expression was increased. CCN1 expression is higher in the non-lesional skin of psoriasis patients compared to healthy skin. Furthermore, their data implies that CCN1 may be implicated in the pathophysiology of psoriatic arthritis given that it aids in the formation of synovium hyperplasia in rheumatoid arthritis and epidermal hyperplasia in the pathogenesis of psoriasis [30]. This is also consistent with previous research [34], which revealed that by reducing TGF-signaling and promoting collagen breakage, increased CCN1 in the dermis decreases collagen formation.

Consistent with our results, previous results had documented that numerous autoimmune and inflammatory illnesses, including systemic lupus erythematosus, psoriasis, and Sjogren's syndrome, are characterized by the presence of elevated levels of CCN1. In the pathophysiology of psoriasis, over-expression of CCN1 was essential for inducing keratinocyte hyperplasia because it elevated interleukin-1β expression [35].

We revealed that PsA patients had significantly greater serum levels of CCN3 than controls, while there was no significant variance between psoriasis patients and controls.

Similar findings were made by **Wei *et al.*** ([15]), who discovered that RA patients had significantly elevated serum CCN3 levels than that in controls and that CCN3

was correlated with the RA activity score. These findings raise the possibility that CCN3 may be important for the development of RA. Additionally, when compared to normal controls, the expression of all CCN genes was greater in synovial samples from patients with osteoarthritis (OA) and RA **(13).** In contrast to a review article that found exogenous CCN3 has preventive effects in rats undergoing induced osteoarthritis. OA joints that treated with CCN3 showed reduced cartilage deterioration and had more lubricin expression than untreated joints **(14)**. This may be explained by different pathophysiologic derangements that are noticed in chronic and systemic autoimmune diseases. Additionally, different immune mediators are involved in the inflammatory processes of RA and PsA. Contrarily, articular cartilage loss is the pathological hallmark of OA that is associated with ageing.

In the current study, CCN1 and CCN1 gene expression showed a positive correlation with psoriasis duration and severity. However, there was a positive association between CCN3 and the severity and duration of arthritis. So, CCN3 could be a marker for PsA severity. All markers were positively correlated with inflammatory markers. The production of chemical mediators is controlled by CCN3. When RA patients were compared to healthy controls, their serum levels of CCN3 were significantly higher. CCN3 expression was associated with inflammatory markers and anti-CCP antibodies **(15).**

In summary, our study is an unprecedented step that assesses the roles of CCN markers in identifying PsA and distinguishing it from psoriasis. To recruit more patients in studies that can support our findings, additional research is required. The absence of an evaluation of CCN's role in PsA etiology and an explanation of how medication affects markers are two limitations of this study. Additionally, the levels of CCN in synovial fluid were not evaluated. The idea that PsA's reversal of CCN growth may serve as a potential therapeutic target is also encouraged for further investigation.

CONCLUSIONS

In terms of detecting PsA, CCN3 expression was the most accurate marker, followed by CCN1 serum levels. However, the CCN1 expression was the most accurate marker for differentiating psoriasis from PsA, followed by the CCN3 expression. CCN3 had a significant positive correlation with arthritis duration and severity. So, CCN3 could be a marker for PsA severity. Further research is required to verify the findings of these preliminary data.

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