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Research Article

Impact of CD39 and CD73 Expression in Rheumatoid Arthritis Patients Mohammed

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Abstract
Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by persistent synovial inflammation, progressive joint damage and systemic manifestations. The condition arises from a complex interplay of genetic predisposition, environmental triggers and immune dysregulation. A hallmark of RA is the disruption of cytokine signaling, which promotes continuous inflammation and tissue destruction. Recent focus has shifted toward understanding the role of purinergic signaling, particularly through ectonucleotidases like CD39, which regulate extracellular ATP and immune cell responses. This study aimed to assess the expression levels of CD39 in peripheral blood mononuclear cells (PBMCs) of RA patients and evaluate its relationship with interleukin-6 (IL-6) levels. The goal was to explore the potential involvement of CD39 in the immunopathogenesis of RA. A case-control study was conducted involving 100 participants, divided equally into RA patients and healthy controls. CD39 gene expression in PBMCs was quantified using qRT-PCR, while serum IL-6 levels were measured using ELISA. Demographic and clinical data were collected, and molecular analyses were carried out on extracted RNA samples. The findings showed a significant downregulation of CD39 expression in RA patients compared to healthy individuals ($p<0.0019$), suggesting impaired ATP hydrolysis and a possible role in sustaining inflammation. Concurrently, IL-6 levels were markedly elevated in RA patients, with a significant correlation between IL-6 and disease markers such as RF and ESR. The data also revealed a negative correlation between CD39 and CD73 expression levels in patients. The results support the hypothesis that disrupted purinergic signaling, marked by reduced CD39 expression, contributes to RA pathogenesis. Elevated IL-6 further emphasizes the inflammatory state. CD39 and IL-6 could serve as valuable biomarkers for understanding disease mechanisms and tailoring personalized therapies.

INTRODUCTION

Rheumatoid arthritis (RA) is a common systemic auto-immune disease principally affecting synovial joints, RA is characterized by immune cell infiltration in the joint around 0.5-1% of the world population is affected Hasan[1]. Ectonucleotidases, particularly CD73, play a crucial role in regulating immune responses through the modulation of extracellular adenosine concentrations, as their coordinated enzymatic activity maintains the balance between pro-inflammatory and anti-inflammatory signals, a mechanism that is especially relevant in the context of autoimmune disorders such as rheumatoid arthritis (RA), which is then converted by CD73 into adenosine, a potent immunosuppressive molecule Jiang[2]. Interleukin-10 (IL-10) is an anti-inflammatory cytokine that plays a protective role in RA, It modulates immune responses by suppressing the production of pro-inflammatory cytokines, inhibiting antigen-presenting cell activity and enhancing regulatory T cell function, which is essential for maintaining immune tolerance. Despite these protective effects, the levels of IL-10 in the synovial fluid of RA patients are often insufficient to counterbalance the pro-inflammatory environment dominated by IL-6 and other cytokines Chang[3]. This study aimed to investigate the relationship between the expression of ectonucleotides CD73 and the risk of developing RA. By determine the expression levels of CD73 in peripheral blood mononuclear cells (PBMCs) using quantitative PCR. Measure the levels of anti-inflammatory cytokines (IL-10) in the serum of RA patients using ELISA kits.

MATERIALS AND METHODS

Patient: A case-control study was conducted from October 2024 to the end of January 2025. A total of 100 adult participants were enrolled from multiple healthcare institutions in Al-Diwaniyah province, including Al-Diwaniyah Teaching Hospital, Al-Hamidiyah General Hospita and Al-Sadr Medical Center. The participants were divided into two main groups:

Group 1 (Rheumatoid Arthritis Patients): This group included 50 patients who were clinically diagnosed with Rheumatoid Arthritis (RA) based on the classification criteria of the American College of Rheumatology (ACR). Among them, 35 were females and 15 were males, with an age range of 20-70 years.

Group 2 (Healthy Controls): This group included 50 apparently healthy individuals without any known autoimmune or systemic diseases. They were matched in age and gender distribution with the RA group (35 females and 15 males, aged 20-70 years). All participants were directly interviewed using a structured and anonymous questionnaire that collected demographic and clinical data, including age, sex, residence, smoking status, disease duration, family history of RA and treatment history (for RA patients). The study protocol was reviewed and approved by the institutional ethics committees of the participating hospitals and verbal informed consent was obtained from all participants prior to data and sample collection, To explore the potential involvement of CD73 in the pathogenesis of RA, whole blood samples were obtained from all participants. Total RNA was extracted from each sample, and the expression levels of CD73 were quantified using Real-Time Polymerase Chain Reaction (Real-Time PCR).

Primers: The qPCR Primers for CD39 and CD73 as well as GAPDH housekeeping gene were design in this study by using NCBI-Database and Primer3 plus design online. These primers were provided by (Macrogen company, Korea) as following table:

Table 1: The qPCR Primers for CD39 and CD73 as well as GAPDH Housekeeping Gene

Sequence (5'-3')		Primer
CD73 qPCR primer	F	TTTGCACCAAGTGTCGAGTG
	R	ACCATTGGCCAGGAAGTTTG
GAPDH qPCR primer	F	AAAATCAAGTGGGGCGATGC
	R	TTCTCCATGGTGGTGAAGACG

Total RNA Extraction: Total RNA were extracted from Whole blood samples by using (TRIzol® reagent kit). The extracted genomic RNA was checked by using Nanodrop spectrophotometer (THERMO. USA) that check RNA concentration and estimation of RNA purity through reading the absorbance in at (260/280 nm). The extracted RNA were treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme kit) and done according to method described by Promega company, USA. After that, The mixture was incubated at 37C° for 30 minutes. Then, 1µl stop reaction was added and incubated at 65C° for 10 minutes for inactivation of DNase enzyme action. DNase-I treated RNA samples were used in cDNA synthesis step for miRNA by using M-MLV Reverse Transcriptase kit. Than RNA and primer was denatured for 10 min at 65 °C, after that immediately cool on ice. Then the tubes were placed in vortex and briefly spinning down. The RNA converted into cDNA in thermocycler.

RESULTS AND DISCUSSION

Interleukin Profiles in Patient and Healthy Groups: Table 2 demonstrates that the examination of interleukin profiles exhibited substantial and diverse patterns between the patient and control groups. interleukin-10

(IL-10) demonstrated a reverse trend, showing markedly decreased levels in the patient cohort (173.11±11.49 pg/mL) relative to the control group (307.53±16.22 pg/mL), resulting in a statistically significant difference ($p<0.0049$). This indicates an approximate 41% decrease in this anti-inflammatory cytokine in patients compared to healthy individuals.

Table 2: Laboratory Parameters in Patient and Control Groups

Parameter	Patients (n=50) Mean±SE	Controls (n=50) Mean±SE	p-value
IL-10	173.11±11.49	307.53±16.22	<0.0049

Values are presented as mean±standard deviation (standard error of the mean). p-values were calculated using Student's t-test.

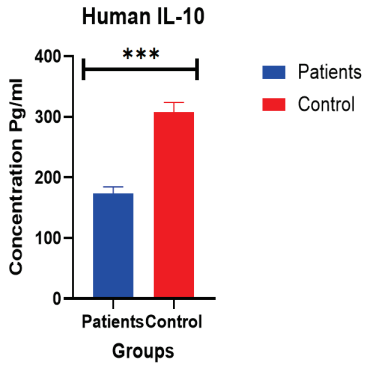

Fig. 1: Human IL-10

The substantial decrease in IL-10 levels in patients (173.11±11.49 pg/mL vs. 307.53±16.22 pg/mL., $p<0.0049$) signifies a dysfunction in counter-regulatory anti-inflammatory systems. This about 41% reduction signifies a significant shortfall in immunomodulatory capacity, potentially leading to uncontrolled inflammation. The positive association between IL-10 and CD73 ($r=0.34$, $p<0.05$) in patients indicates a functional relationship between adenosinergic immunomodulation and IL-10 synthesis. This association aligns with previous mechanistic investigations by Rodriguez-Perea[4], which indicated that adenosine produced via CD73 activity enhances IL-10 production by regulatory T cells and alternatively activated macrophages. Chen[5] revealed this mechanism by identifying the adenosine receptor-mediated augmentation of STAT3 phosphorylation, a crucial step in IL-10 gene transcription. The observed positive correlation between IL-10 and age in healthy controls ($r=0.28$, $p=0.077$), while not statistically significant, is consistent with the inflammaging hypothesis articulated by Franceschi[6], which posits that compensatory anti-inflammatory mechanisms intensify with age to mitigate age-associated inflammatory processes.

CD73 Gene Expression in Patient and Healthy Groups: The examination of ectonucleotidase expression, as shown in Table 3, indicated significant disparities between the sick and healthy groups. CD73 gene expression exhibited a marked decrease in patients (1.94±0.27 relative expression units) compared to control participants (7.30±0.69 relative expression units), resulting in a highly significant difference ($p<0.0023$). This is an approximate 3.8-fold reduction in CD73 expression in the sick population compared to healthy persons.

Table 3: CD39 and CD73 Gene Expression in Patient and Healthy Groups

Parameter	Patients (n=50) Mean ± SE	Controls (n=50) Mean ± SE	p-value
Gene expression			
CD73	1.93 ± 0.27	6.58 ± 0.71	<0.0023

Values are presented as mean±standard deviation (standard error of the mean). p-values were calculated using Student's t-test.

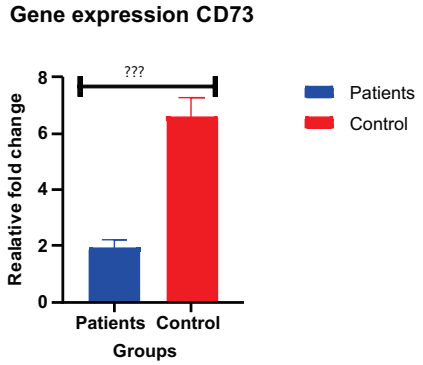

Fig. 2: Gene Expression CD73

The almost 3.8-fold decrease in CD73 expression in patients (1.94±0.27 vs. 7.30±0.69 relative expression units., $p<0.0023$) signifies a significant deficiency in the last phase of adenosine production. This impairment, along with diminished CD39 expression, indicates a thorough breakdown of the adenosinergic immunomodulatory system. The positive correlation between CD73 and IL-10 in both patients and controls ($r=0.34$, $p<0.05$ and $r=0.41$, $p<0.01$, respectively) substantiates a functional relationship between adenosine synthesis and the production of anti-inflammatory cytokines. Recent investigations by Morandi[7] have mechanistically characterised this link, demonstrating that adenosine produced via CD73 activity augments IL-10 production through A2A receptor-mediated cAMP-PKA-CREB signalling in many immune cell types. The enduring association in both groups, despite markedly reduced CD73 levels in patients, indicates that the CD73-IL-10 axis remains functionally intact but functions at a substantially reduced capacity in RA. This discovery corresponds with the "preserved pathway" theory suggested by Zhang[8], which asserts that specific immunoregulatory interactions remain

qualitatively intact while quantitatively compromised in autoimmune disorders.

CONCLUSIONS

The downregulation of CD73 in RA patients may impair the conversion of AMP to adenosine, thus reducing the anti-inflammatory effects of adenosine and contributing to persistent synovial inflammation. Interleukin-10 (IL-10) levels were markedly reduced in RA patients, indicating a weakened regulatory immune response and highlighting an imbalance between pro-and anti-inflammatory cytokines.

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