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The Clinical Significance of Nucleotide-Binding Oligomerization Domain-Like Receptor Family Pyrin Domain Containing 3 Inflammasome for the Health of Rheumatoid Arthritis Patients

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Abstract

Background: A recurring autoimmune disease with severe inflammation and joint destruction resulting from an immune-mediated inflammatory reaction is of concern these days, known as Rheumatoid arthritis (RA). It induces intracellular multi-protein signaling hubs, or inflammasomes, linked to pathogen sensing and triggering inflammatory processes in healthy and sick individuals. The Nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) protein forms an inflammasome complex that regulate pro-inflammatory cytokine production, including interleukin-1Beta and 18. Nowadays, numerous experimental agents have been studied for investigating various approaches to improve rheumatoid arthritis treatment, including pyrin domain-containing protein 3 inhibitors.

Methods: The current case-control experimental work involved 82 subjects separated into 3 groups: 22 patients were newly diagnosed with rheumatoid arthritis, 30 rheumatoid arthritis patients taking methotrexate (MTX), and 30 healthy subjects. The samples of saliva were obtained from all specimens, and the salivary inflammasome levels (NLRP3) were detected using ELISA.

Results: The study discovered a significant increase in salivary pyrin domain-containing 3 in patient groups in comparison with the healthy group (control). However, the results discovered that no noteworthy variance (p>0.05) was observed in salivary NLRP3 level between the newly diagnosed rheumatoid arthritis group and the rheumatoid arthritis patients in the methotrexate treatment group.

Conclusion: The study suggested that the elevated level of NLRP3 has a significant impact on disease etiology and could be used as diagnostic biomarkers for rheumatoid arthritis, and could be targeted for the treatment of RA by developing novel and beneficial agents.

Introduction

Rheumatoid arthritis is a chronic, symmetrical inflammation of joints, causing cartilage and bone deterioration, resulting in bone weakness [1]. Initially, some bone joints are afflicted; however, as the condition progresses, more joints are damaged, and extra-articular symptoms are frequently observed [2]. A study conducted in Iraq found that females had greater incidences of RA than males [3].

Rheumatoid arthritis, early and later, poorly managed stages vary clinically. Exhaustion, sore and swollen joints, and morning stiffness are common symptoms of early-stage rheumatoid arthritis (RA), along with high CRP and ESR [4]. RA is linked to hyper-gammaglobulinemia and higher levels of acute-phase proteins. The synovial membrane contains mononuclear cells, which are then stimulated by T lymphocytes to aid B cells in producing rheumatoid factor [5]. However, inadequately treated rheumatoid arthritis demonstrated a complicated feature, including the emergence of systemic manifestations like lung nodules and pleural effusions, vasculitis, lymphomas, keratoconjunctivitis, atherosclerosis, and hematologic abnormalities, rheumatic nodules, cartilage degeneration, bone erosion, decreased range of motion, and joint malalignment are some symptoms. These systemic symptoms in RA patients of the prolonged inflammation pave the way for an increased mortality when they are all considered together [1,2,6].

Still, the most accurate and sensitive methods for diagnosing RA include clinical examination and history. It is crucial to understand that all currently used criteria for rheumatic disorders are classification criteria rather than diagnostic criteria, which creates a significant challenge in the diagnosis of RA [7].

The ACR and EULAR redefined rheumatoid arthritis classification in 2010 to emphasize early illness, autoantibody levels, ESR/CRP, and other immune activation and inflammatory markers [8]. Disease activity score 28 includes sore joints, swollen joints, the ESR, and the patient's overall evaluation score [9-11]. The ACR approved utilizing the DAS28 as an outcome measure for nonbiologic and biologic disease-modifying antirheumatic medication therapy in RA in 2008 [12]. A number of clinical trials using the DAS and DAS28 demonstrated the advantages of strict control in the management of RA [13-15]. The DAS28 assesses 28 sensitive joints (0-28), 28 swollen joints (0-28), a patient's overall rating on a visual analogue scale (0-100), and ESR. Similar to the original DAS [11]. There are three categories that are used to categorize the degrees of disease activity. Some of these categories are low (DAS28 ⩽3.2), moderate (3.2 < DAS28 ⩽5.1), and severe (DAS28 > 5.1) [16].

Within roughly six months, the objective of therapy is to achieve full reduction or at least a significant decline in the illness, likewise to put an end to the degeneration of joints, disability, and systemic indications of rheumatoid arthritis [6,17]. With statistics standing at '40% of patients with work disability after 10 years from onset and 80% of improperly treated patients with joint misalignment,' it's no wonder that early and intensive rheumatoid arthritis therapy is a priority [6,18,19]. RA treatments have improved over the last 30 years because of new drugs. DMARDs, immunosuppressive glucocorticoids, and non-steroidal anti-inflammatory medications are available [20].

Aspirin, diclofenac, and ibuprofen are NSAIDs that are effective in reducing pain and swelling and enhancing joint function. However, as they cannot prevent further joint damage, they are not disease-modifying medications [1]. Prednisolone and other glucocorticoids are extremely effective anti-inflammatory medications that slow the radiologic progression by suppressing the expression of inflammatory genes in the early stages of disease [2,21].

Finally, DMARDs are medications employed to reduce rheumatoid inflammation and prevent subsequent destruction of the joints. Furthermore, these medications mitigate the manifestations of rheumatoid arthritis, improve physical functionality, and inhibit the progression of structural joint degradation [1,6]. There are three categories of DMARDs: conventional synthetic (methotrexate, hydroxychloroquine, sulfasalazine), targeted synthetic (pan-JAK and JAK1/2 inhibitors), and biologic (TNF-α inhibitors, TNF-receptor inhibitors, IL-6 inhibitors, IL-6 receptor inhibitors, co-stimulatory molecule inhibitors, B cell-depleting antibodies). Animal models are used to test numerous rheumatoid arthritis treatments. This includes mesenchymal stem cells, LRR, NOD, and NLRP3 inhibitors, and GM-CSF receptor, GM-CSF, or Toll-like receptor targeting [20].

The NOD-like receptor family pyrin domain-containing 3 (NLRP3) protein can lead to inflammasome complexes that produce IL-1β in response to signals of danger [22]. NLRP3 inflammasome Components have been expressed in synovia of RA patients [23]. It was discovered that "inflammasome activation may be implicated in secretion of pro-inflammatory cytokines in patients with rheumatoid arthritis and that inflammasome inhibition may be a useful therapeutic strategy in future RA therapy". RA patients' synovia and an in vivo collagen-induced arthritis animal model show high NLRP3 inflammasome activity, according to Guo *et al.,* [24]. Administration with MCC950, a "specific NLRP3

inhibitor," resulted in a remarkable reduction in in vivo joint inflammation, bone erosion, and IL-1β production [25].

Inflammasomes were first defined as multiprotein platforms by Martinon *et al.,* [26]. Their development is facilitated by organisms in response to different physiological and pathogenic conditions. These oligomeric protein complexes have distinct activation and regulation processes and can respond to a range of ligands. Inflammation is an immunological reaction to external pathogens and self-damage. Pattern recognition receptors (PRRs) identify PAMPs and DAMPs well [27]. NOD-like receptors are a class of NLRs that belong to the PRRs [28]. The most extensively researched receptor is NLRP3, which belongs to the family of NOD-like receptors. According to Mangan et al., [29], NLRP3 is created and activated when it identifies invading pathogens and self-danger signals [22]. NLRP3 is a PRR critical to innate immunity and inflammation. Inflammasome NLRP3 detects bacteria, viruses, and tissue injury. Activating caspase-1 by NLRP3 inflammasome causes pyroptosis and secretion of pro-inflammatory cytokines IL-1β and IL-18, thereby augmenting inflammation [30]. The inflammatory reactions triggered by the NLRP3 inflammasome's mild activation can rapidly and efficiently eradicate microbial infections and restore damaged tissue. However, excessive NLRP3 inflammasome activation causes unwanted host damage, excessive inflammation, and a pathological condition in the body [31]. Initiating adaptive immunological responses is another crucial function of innate immunity, which enables the host to establish long-lasting, efficient defenses. As a result, adaptive immunity is a consequent expansion of innate immunity [32]. Additionally, adaptive immunity needs the NLRP3 inflammasome. NLRP3 activation is essential for IL-1β and IL-18 production. IL-18 modulates inflammatory and immune responses and is a sensitive inflammation biomarker [33]. Naive T cells can be stimulated to differentiate between memory T cells and effector T cells by the cytokines produced by these two pro-inflammatory cytokines, which will activate adaptive immunity [34]. This study aims to explore NLRP3's involvement in rheumatoid arthritis pathogenesis.

Methods

Subjects:

This case-control research had 82 participants categorized into three groups: 22 individuals newly diagnosed with rheumatoid arthritis, 30 patients with rheumatoid arthritis on methotrexate treatment, and 30 ostensibly healthy individuals. Each candidate in the patient group received a DAS 28 disease activity score, the Rheumatologist's disease activity assessment. The College of Dentistry/University of Baghdad Ethical Review Board approved this study project (Ref. No. 465, January 2022). Participants in this study had to meet the 2010 ACR/EULAR inclusion criteria, be older than 20 years and not have any infectious diseases like hepatitis, malignancies, cardiovascular complications, or other autoimmune or inflammatory diseases. Saliva from each subject was collected, and an enzyme-linked immunosorbent assay was used to measure salivary concentrations of inflammasome NLRP3.

Sample size calculation:

The G power 3.1.9.7 analysis tool was used to calculate the size of the sample with a power of research of 95% and an alpha error of 0.05 on a two-sided scale [35].

Estimation of DAS28:

According to DAS 28, each candidate in the patient group received a score for disease activity. This index includes three measurements: an ESR measure (as a marker of inflammation), a TJC (tender joint count) range of 0-28, and an SJC (swollen joint count) range of 0-28. The following equation was used for calculating DAS28:

$$DAS28 = 0.56 \times \sqrt{(TJC28)} + 0.28 \times \sqrt{(SJC28)} + 0.70 \times ln(ESR) + 0.014 \times (Patient\ Global\ Assessment) + 0.96$$

Saliva Samples Collection:

All subjects fasted for an hour as per instructions, sat comfortably, and saliva samples visibly contaminated with blood were discarded. The participants were also instructed to sit comfortably. The saliva was obtained between 9 and 12 that morning. After cleaning their mouth with sterile water for one to two minutes, polyethylene tubes were used to collect up to 5 ml of unstimulated saliva. Saliva was centrifuged for 10 minutes at 3000 rpm and stored in Eppendorf tubes at -20 °C.

Measurement of Salivary Biomarkers:

The ELISA kit (Shanghai/China) was used to measure the concentration of the NLRP3 protein.

Statistical analysis:

The SPSS version 24 statistical software suite was used. Differences between groups were assessed using an Analysis of Variance (ANOVA) with a Tukey HSD post hoc test. The Pearson correlation coefficient test determined factor associations. Significant P-values are 0.05 or below.

Results

ESR-based disease activity score 28 (DAS 28) estimation

This study found a non-significant difference (p>0.05) in mean DAS-28 scores between two RA patient groups upon diagnosis. The mean DAS-28 score for the RA patients receiving MTX was (5.04±0.71), while the newly diagnosed RA group had a mean score of (4.83 ± 1.07), as illustrated in Table 1 and Figure 1.

DAS28	Study groups		T-test (p-value)
	New RA patients' group No.=22	RA Patients on MTX group No.=30	
Range	(2.90-6.30)	(2.86-6.20)	0.406^{NS}
Mean± SD	4.83 ± 1.07	5.04 ± 0.71	

Table 1: The variances in mean values of DAS-28 for two groups of patients (new patients and patients on MTX).
NS: non-significant; SD: Standard Deviation; No.=number

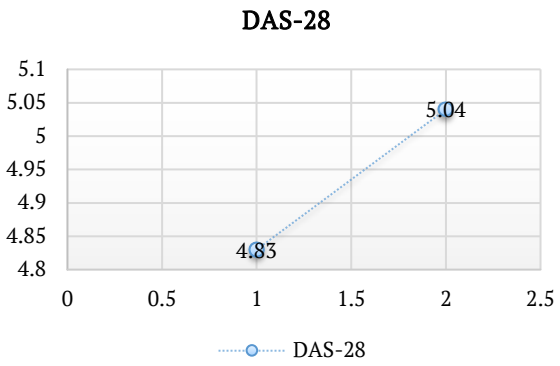

Figure 1: Mean values of DAS-28 for two groups of patients.

Salivary NLRP3 Level

The current study noticed a highly significant increase in salivary NLRP3 (P<0.05) among patient groups in comparison with the control group, as clearly shown in Table 2 and Figure 2. Compared to the control, NLRP3 levels were significantly increased in both the MTX therapy and newly diagnosed RA groups. Salivary NLRP3 levels were not, however, significantly different (p>0.05) between these two groups of patients, as in Table 3.

NLRP3 (pg/mL)	Study groups			ANOVA (P-value)
	Control group No.=30	New RA patients' group No.=22	RA patients in MTX group No.=30	
Range	(162.6-603.2)	(358.7-1287.0)	(650.2-1130.8)	p < 0.001
Mean± SD	290.7 ± 97.40	868.1 ± 241.67	845.7 ± 127.1	

Table 2: The difference in mean values of NLRP3 (pg/mL) in the studied groups.
SD: Standard Deviation; No. = number; p < 0.05 considered significant.

Grouping	Mean difference	Tukey's HSD (p value)
NLRP3 (pg/mL)		
Control group vs. New RA patient group	577.33	< 0.001 *
Control group vs. RA patients in MTX group	554.94	< 0.001 *
New RA patient group vs. RA patients on MTX group	22.39	0.869 NS

Table 3: Intergroup NLRP3 (pg/mL) mean comparisons between all pairings of groups.
NS: non-significant; *=significant

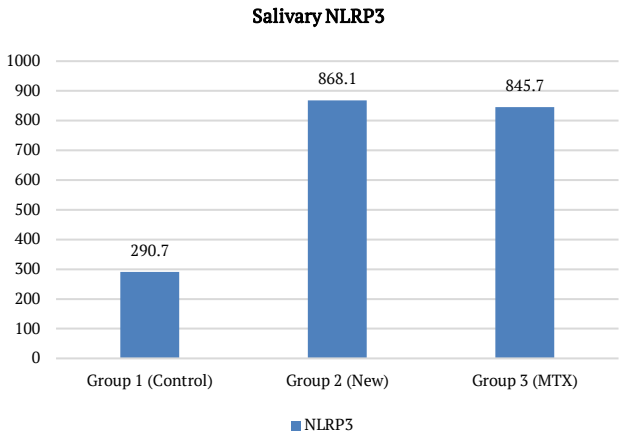

Figure 2: Mean values of NLRP3 in the studied groups.

Discussion

Due to the fact that RA leads to joint inflammation, resulting in joint damage and decreased function, it is vital to evaluate the severity of RA clinically so that the efficacy of current treatment methods can be determined, and whether moving to other medications is necessary [36]. Vis-à-vis disease activity during initial diagnosis of RA, the DAS-28 value for the group recently diagnosed with RA was close to that reported by Smigielska-Czepiel *et al.,* [37] and lower than that reported by Li *et al.,* [38]. The DAS-28 value in the group of RA patients on MTX was comparable to that reported by Filková *et al.,* [39] and Hruskova *et al.,* [40], as well as the mean DAS-28 value in an Iraqi study on RA conducted by Khidhiret *et al.,* [41].

The present study showed an increase in the salivary NLRP3 level in RA patient group compared with control subjects, and it is consistent with several studies that demonstrated that NLRP3 mRNA and NLRP3 inflammasome-related proteins are upregulated in monocytes, macrophages, and DCs of patients with RA [42,43]. The current results align with Choulaki *et al.,* [44], who reported that patients with active RA exhibit heightened expression of NLRP3 and NLRP3-mediated IL-1β secretion in whole blood cells [44]. NLRP3 is raised in the synovial tissue, whole blood, and CD4 T lymphocytes of individuals with active RA [42]. Ramos-Bello *et al.*, [45] in a trial to evaluate the possible effect of MTX and MTX plus Colchicine (CCH) in NLRP3 inflammasome expression and activity in early RA patients, indicated that caspase-1 activity and NLRP3 expression were raised in all patients; patients in MTX had a modification in its expression in month one, but that difference was not subsequently identified in month three [45].

In comparison, patients with MTX + CCH had significantly lower NLRP3 expression and activity at

first and third months. Clinical indicators also improved significantly in the first and third months. CCH treatment reduced NLRP3 inflammasome expression and activity, suggesting that CCH is a powerful NLRP3 inhibitor [45].

These results are in agreement with a study that investigated the correlation between the SNPs in NLRP3 gene and the vulnerability to RA in the Han Chinese population. The study demonstrated that the expression of NLRP3 in neutrophils and Peripheral Blood Mononuclear Cells (PBMCs) was correlated significantly with DAS28, CRP, and ESR [46].

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Author Contributions

Sarah Ibrahim Dhaidan: Conceptualization, study design, data collection, manuscript drafting, and final approval of the manuscript.
Batool Hassan Al-Ghurabi: Reviewing and supervision.
Raja Hadi Al-Jubouri: Manuscript review and supervision.
Osama Saad Madhloom: Technical support, manuscript editing.

Conflict of Interest

The authors state that there is no conflict of interest in the publishing of this work.

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