# The sensitivity of Rheumatoid Arthritis patients for bacterial infection

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**Abstract**

Rheumatoid arthritis (RA) is a complex autoimmune disease that affects 1-2% of the population worldwide. Multiple genetic risk factors and environmental triggers contribute to the pathogenesis of rheumatoid arthritis. Microbial infection is thought to play an important role in the initiation and persistence of this disease, and one of the most important factors of virulence originating in the Enterobacter family is the specific Curli species A. The current study was conducted in Karbala governorate, (75) blood and urine samples were collected, (55) samples were collected from patients with rheumatoid arthritis RA and (20) samples were from healthy people, and the group with RA patients was divided into two groups, group (A) suffering from urinary tract infection,group(B) uninfected urinary tract infection to determine the effect of bacterial infection on the level of (CD4 + Tcell, CD20 + Bcell, IL-17, IL-10),Use the bacterial sequencing (PCR) technique to determine the presence of CsgA in the bacteria.

Twenty three infections with different types of bacteria that infect the urinary tract were recorded in patients with rheumatoid arthritis. Bacterial isolates were identified using Viteck biochemistry test. The study showed a significant increase in the level of CD4 + T cells in patients with rheumatoid arthritis, whether they suffer from a bacterial infection (with the presence of the CsgA gene) or not, compared to the healthy group ($P \leq 0.001$), as well as a significant increase in the level of these cells among those with rheumatoid arthritis. Rheumatoid arthritis patients who had a bacterial infection compared to those with arthritis who did not suffer from a bacterial infection ($P \leq 0.05$). There is also a significant increase in the level of CD20 + Bcell cells in patients with rheumatoid arthritis ($P \leq 0.001$) without bacterial infection compared to the healthy group. Finally, the results of the current study showed that there was a significant increase in the concentration of the anti-inflammatory cytokine IL-10 ( $P \leq 0.05$) in patients with rheumatoid arthritis bacterial disease compared to patients without bacterial diseaseas well as compared with the healthy group.the pro-inflammatory cytokine IL-17 did not show significant differences with healthy controls and all groups of patients. Conclusion that bacterial infection (UTI) in patients with rheumatoid arthritis, especially bacteria that express CsgA gene, activate the adaptive immune response (B and T cells). The current language of the study is to know the sensitivity of rheumatoid arthritis to entero bacter infection and to identify the effect of the Csg A gene on some immune factors.

Keywords: Rheumatoid Arithritis ,bacterialinfections, T cells, B cells, csgA gene,urinary tract infection.

## 1. Introduction

Autoimmune disease is one of the disorders that are the cause of the immune system.Which targets the tissues of the body itself, genetics, biological factors and infection play a leading role in Causing autoimmune diseases (17).

Rheumatoid arthritis (RA) is one of the most common autoimmune inflammatory diseases. It is characterized by synovial membrane inflammation, systemic inflammation, and the production of autoantibodies (19). RA is mostly affected in the small joints of the hand as well as the foot, knee and ankle, thus targeting the synovial membrane (which is either two or three layers of epithelial cells containing fluid Synovia), as this membrane in the affected joints thicker with a large concentration of inflammatory cells (T lymphocytes and macrophages), which leads to its damage(2)(6).

In case of inflammation, the albumin contains neutrophils that destroy Synovial cartilage by enzymes that are excreted from these cells, and in the advanced stages the bone eats and in fact this erosion leads to the possibility of disability and complete disability (1).

Factor necrosis Tumor - α (TNF - α) is a protein secreted by cells The body's immune system, or protein cell, so that if excreted in a quantity, it can lead to the emergence of rheumatic arthritis. Cytokines, especially TNF-α, activate the inflammatory state by activating lymphocytes CD4+T(T) will stimulate the activity of single inflammatory cells Monocyte, Macrophage, Which Is Called Fibroplast Like Synoviocyte (FLS), and the stimulation of these inflammatory conditions leads to the appearance of cartilage and bone tissue demolition cells in the synovial fluid. In addition, these cells are stimulated. By the expression of cell surface molecule with T cells and then CD4 T cells stimulate B-cell lymphocytes that activate and differentiate to produce immune proteins such as rheumatoid factor (Rheumatoid factor-RF) (14).

Interleukin-10 (10-IL): is a cytokine that has multidirectional effects in the regulation of immunity and inflammation. It reduces the expression of Th1 cytokines and MHC class II antigens as It enhances B-cell survival, proliferation and antibody production (16).

CsgA is the major structural subunit of plexus fibers and

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forms several hundred units of long homopolymers both in vivo and in vitro. The amino acid sequence of CsgA includes three well-described domains: the signaling peptide, the N-terminal 22 (N22) amino acid targeting sequence, and the core domain of the terminal amyloid. Curli are the heterogeneous polymeric non-covalent strands of the CsgA and CsgB subunits, present in the CsgA wild type fibres in vivo in ratios of about 20:1 CsgA:CsgB Curli belongs to an ordered class of CsgA:CsgB. stable proteins known as amyloids (21).

## 2. Materials and Methods

### Sample collection

The study was carried out on 55 RA Iraqi patients and 20 controls for comparisons, the samples were collected in Karbala (Alhussein Educational Hospital) and (Alzahra Hospital) during the period from December 2020 to April 2021. The patients were categories into two groups, patients urinary tract infection (groupA) and patients without bacterial infection (group B). The patient's and control's were with age ranged between 19-81 years.

### Bacterial isolation

Urine samples were inoculated onto blood agar and MacConkey agar. The isolates incubated at 370C for 24 hour. Isolated microbes were identified by colonial characteristic, Gram stain and confirmed by Vitek-2 compact system.

### Assessment of cytokines and CD markers precentage in blood sample

Sera of patients and controls were assessed to test CD4+Tcell and CD20+Tcell interleukine-10 and interleukine-17 levels by ELISA method using commercially available kit (Elabscience, USA).

### DNA extraction

Chromosomal DNA was extracted from fresh overnight culture grown in brain heart broth at 370C using Gen Elutebacterial genomic DNA kit (sigma-USA).

Table 1 : shows oligonucleotide sequence of primers and PCR product size ofcsgA gene.

| Reference | Product size(bp) | Sequence of primer | Name of primer | Gene |
|---|---|---|---|---|
| (Silva etal, 2013). | 178 bp | ATCTGACCCAACGTGGCTTCG | Forward | CsgA |
| | | GATGAGCGGTCGCGTTGT TACC | Reverse | |

### Polymerase chain reaction amplification

Monoplex PCR was used in the current study, 12.5µL master mix (Biolab-England), 3µL of template DNA, 2µL of each primer, 7.5µL of deionized water (total reaction volume 25µL) to investigate csgA gene.

## 3. Statistical Analysis

Statistical analysis was done by using the software statistical package for social science (SPSS; version 22). Results were given as mean±Standard Error (mean± S.E). Statistical analysis for the significance of differences of the quantitative data was done by using independent-sample T test. The probability levels were indicated as follows (one sign $P < 0.05$, two signs $P < 0.001$, three signs $P < 0.001$).

## 4. Results & Discussion

Results of the current study showed 73.33% of SLE patients have UTI and according to age categories the study showed infection distributed among age ranged between (19-39) years, among age ranged between(40-60) years and among age ranged between(61-81) years as shown in Table 2.

| Groups | No. | Age categories (years) | | |
|---|---|---|---|---|
| | | (19-39) | (40-60) | (61-18) |
| Control | 20 | 11 | 7 | 2 |
| Group A | 23 | 6 | 12 | 5 |
| Group B | 32 | 6 | 20 | 6 |
| Total | 75 | 23 | 39 | 13 |

In the current study 73.33% of SLE patients were observed to have UTI, which were distributed as 26.08 % with E. coli, 4.34% with K.pneumoniae, 4.34% with Proteus spp, 21.74% with Pseudomonas putida , 4.34% with Pseudomonas fluorescens ,4.34% with Acinetobacter baumannii ,17.4% with staphylococcus epidermidis and 17.4 % staphylococcus haemolyticus.

Infection and RA are equivalent because they both cause immune-system reactions, but one function protects the body, while the other one allow to do damage to the body (13).

### Genetic detection using polymerase chain reaction (PCR)

To detect the presence of csgA gene in bacterial sample result of PCR technique showed that all bacterial samples csg A positive with product of 178pb in size as a result in fig.1

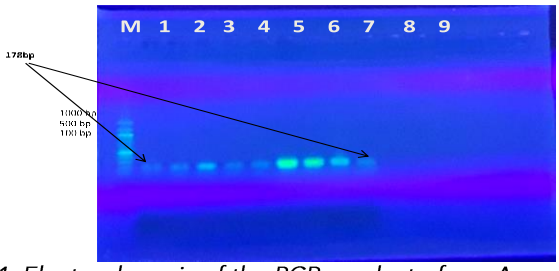


*Fig.1: Electrophoresis of the PCR product of csg A gene (178 base pairs) for all bacterial isolates at 0.8% agarose and a potential difference of 70 volts for one hour, path M represents the volume index (100-1500 base pairs) Paths 1-9 represent bacterial isolates that have the csg gene A path 10 represents the negative control.*

The current study confirmed that the PCR result for all pathological bacterial isolates was positive, noting that the csgA gene has the ability to encode a protein that helps bacteria bypass immune defense and cause inflammation. Interestingly, multiple immune cells such as macrophages and T cells respond by regulating pro-inflammatory cytokines, including IL-6,IL-23,IL-17,IL-22 when convolutional cells cross the epithelial barrier [12].

There are several previous studies that demonstrated that the csgA gene encodes curlin which is the major subunit protein of curli which are

thin coiled surface structures with which pathogenic bacteria participate in surface infection of tissue cells or organs, formation of biofilms, and bacterial association with a variety of extracellular assemblies. Cell and serum proteins [20].

**Immuno-serological tests**

**CD4+T Cell Factor Levels**

**CD4+T Cell Levels by Groups**

Where it was found that there is a significant increase in the level of CD4 in the blood in the two groups with rheumatic disease, with a clear significant difference when comparing between the healthy group and patients infected with pathogenic bacteria, a rate of (0.93 ± 0.12) and between the group of healthy and non-infected patients (0.64 ± 0.078) and there is a significant difference between the group of rheumatic patients infected with pathogenic bacteria and the group of rheumatic patients without bacteria.

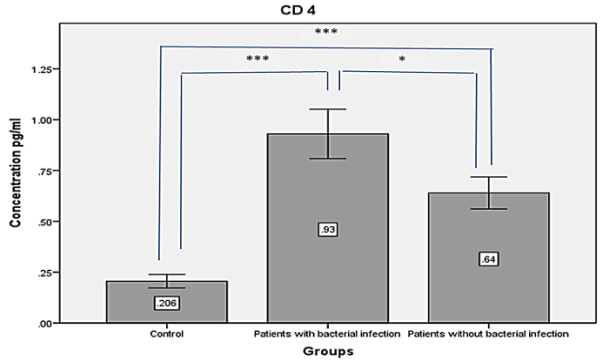


*Fig.2: The concentration of CD4+ T cells in the serum of the healthy group and the two groups of rheumatoid patients. C: the same as the healthy group, A represents rheumatic patients who have a bacterial infection and B: rheumatic patients who do not have a bacterial infection. The significance value was indicated as * between the healthy group and the group of patients. The level of probability was denoted as (* meansP < 0.05, ** means p<0.01, *** means P < 0.001).*

The results of this study agreed with the study of researchers (5)(9) who found that rheumatic disease with bacterial infection leads to stimulating T cells to an immune response, which leads to an increase in CD4 concentration for a certain period, but the body is then adapted to Levels are limited by mechanics (MHC class I and II) and reduce it to lower levels, and perhaps the samples that were currently studied were in high levels of immunity and are not adapted until now because it takes time and varies between individuals according to the patients' immune system and other factors.

## CD20+B Cell Factor Levels by Groups

The study showed that there is a clear and significant increase in the level of CD20 in the blood serum of the two groups affected by rheumatic disease, with or without bacterial infection, compared to the healthy control group at a rate of (1768.29 ± 108.07). Bacterial infections and rheumatic diseases in stimulating B cells to increase the expression of CD20 in patients and perhaps bacterial infections are what stimulate the body to attack some parts of the body, which leads to the emergence of rheumatic disease (23)(22)(4).

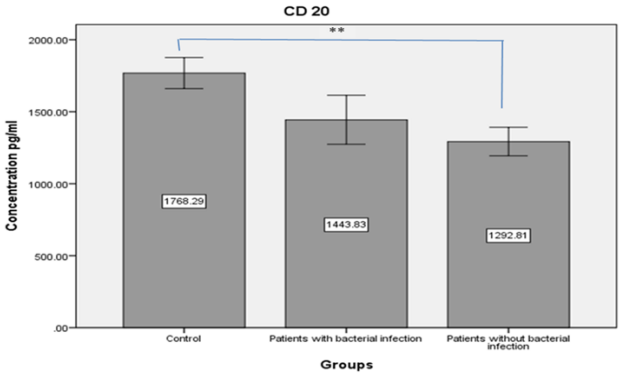


*Fig.3: Bcell CD20+ cell concentration in the serum of three groups of the study. C: Same as the healthy group, A: represents rheumatic patients with bacterial infection and B: rheumatic patients with no bacterial infection Indicated probability level as (* meansP < 0.05, ** means p<0.01 , *** means P < 0.001).*

## Serum cytokine levels

### Interleukin-10 (IL-10) levels by groups A, B, and C

During this study, the level of IL-10 was determined, as it was significantly increased in the group of people with rheumatism in the presence of bacterial infection at a rate of (41.85 ± 10.03) compared with the healthy control group and the group with rheumatic disease only, while the group with rheumatic disease showed an increase, but this increase It did not reach the moral level when compared with the healthy control group with an average of (18.81±4.04) as shown in the figure.

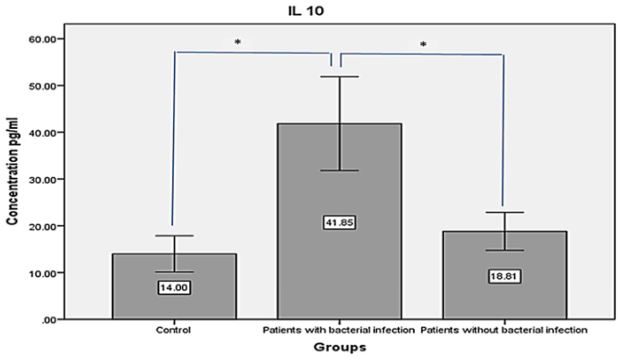


*Fig.4: Serum IL-10 concentration for three study groups. C: Same as the healthy group, A represents rheumatic patients with bacterial infection and B: rheumatic patients with no bacterial infection Indicated probability level (* meansP < 0.05, ** means p<0.01, *** means P < 0.001).*

This result indicates that this interleukin is associated with bacterial infections, where it rises when it is present and also rises with the presence of the disease, but slightly, and this result is consistent with the results of other studies.

(11)(10), which confirmed that rheumatism in the joints with bacterial infections shows a clear rise in the level and concentration of IL-10 and its control reduces the severity of the disease (3) and may have a significant role in doubling the pathogenicity of people with this disease with pathogenic bacterial infections.

## Interleukin-17 (IL-17) levels by groups A, B, and C

This study also dealt with the determination of the level of IL-17, as it was at a clear but insignificant increase between the three groups of the group with rheumatic disease with no bacterial infection (33.13 ± 7.66), the healthy control group and the group with rheumatic disease with an average of (29.58 ± 9.32), Whatever it is, the IL-17 level is high in the group with rheumatic disease with the presence of bacterial infection, less than the group with rheumatic disease without bacterial infection and the healthy control group at a rate of (33.13 ± 7.66) as shown in the figure.

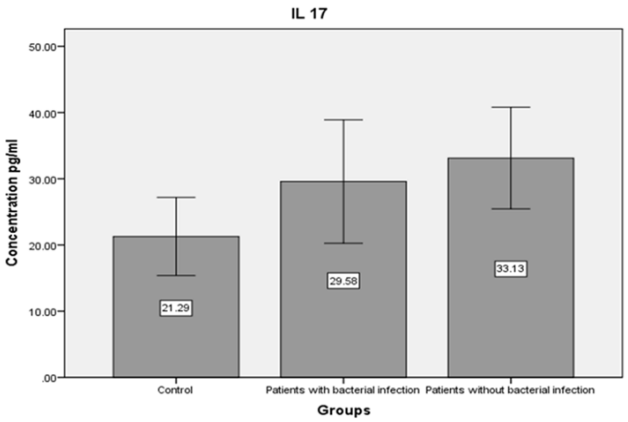


*Fig.5: The concentration of IL-17 in the serum of three groups of the study. C: Same as the healthy group, A: represents rheumatic patients with bacterial infection and B: rheumatic patients with no bacterial infection Indicated probability level (* meansP < 0.05, ** means p<0.01, *** means P < 0.001).*

It is worth noting that IL17 is considered pre-inflammatory and is low after infection because it is affected by the level of anti-inflammatory immunoglobulins (15)(7)(8)(18).this confirms the results of the current study, as IL-17 was high in uninfected patients and slightly lower in the group of infected patients with bacterial infection and healthy control, because it was affected by anti-inflammatory immune markers Cytokines.

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