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Research article

Role of Bacterial Urinary Tract Infection in the Activity of Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is considered one of the most common autoimmune diseases in the world. The nature of this disease, as well as the medications used to treat it, contributes to the occurrence of various bacterial infections. The current study aims to determine the effect of bacterial urinary tract infections (UTI) on the activity of RA in terms of DAS28. In the present study, urine samples were collected from 70 patients suffering from RA and 30 healthy volunteers who did not suffer from RA. The bacterial isolates were isolated and identified using different enrich, selective, and differentiation media. Biochemical tests were also used to identify bacterial isolates. Bacterial diagnosis has been enhanced using VITIC technology. The study proved that the incidence of bacterial UTI patients with RA was higher than in the healthy control group who do not suffer from RA. The highest bacterial infection was found in the case of Staphylococcus aureus followed by *Staphylococcus haemolyticus.* While the lowest bacteria infection was found in the case of *Klebsiella pneumoniae*. There is no significant difference in the DAS28 of the cases (RA) infected with *Escherichia coli, K. pneumoniae, Micrococcus luteus, S. aureus, S. epidermidis, Enterococcus faecium, and Streptococcus spp. The infection (UTI –RA) with S. haemolyticus involved with disease activity (high DAS28) P<0.05 as compared with DAS28 of all RA cases.* It can be concluded from the current study that the rate of UTI in patients with RA was higher than in healthy people who do not suffer from RA. The species of bacteria that causes UTIs does not affect the level of activity of RA (DAS28) except the *S. haemolyticus*.

Keywords: Bacterial infections, DAS 28, Rheumatoid arthritis, Urinary tract infection.

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1. INTRODUCTION

Rheumatoid arthritis is one of these autoimmune disorders, a chronic inflammatory disorder that can lead to severe disabilitie (RA).Over the past 2 decades, RA management has improved. The disease may lead to severe damage to the targeted joints, although some RA patients can experience mild disease and limited joint erupting. RA is systemic and can affect multiple joints in a balanced way. Its main symptoms include muscle pain, weakness, and joint pain or stiffness. Joint impairment occurs in the last stage of disease progression. Extra-articular signs can also be present [1]. This is a joint inflammation that generally occurs in the small joints of the hands and feet, such as wrists, fingers, and toes, and is multi-joint, symmetrical, and disruptive [2]. The cause of RA is still a matter of debate, but both environmental and genetic variables may be involved. In a joint affected by RA, along with persistent discomfort, the synovial tissue that fills the joint capsule becomes inflamed. By activation of T-lymphocytes and B cells, the inflammatory process is triggered and the autoantibodies are synthesized by plasma cells. Anti-citrullinated protein antibodies (ACPAs) and rheumatoid factor (RF) autoantibodies can be detected earlier than in clinical illnesses. In 90 percent of patients, RF is normally present and is a polyclonal immunoglobulin (Ig) IgM antibody.

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Severe RA may represent its higher levels [3]. Urinary tract infection (UTI) is the most frequent bacterial infection that affects millions of people annually. Infection-induced inflammation of the urethra (urethritis), urinary bladder (cystitis), and kidneys (pyelonephritis) are the clinical symptoms of symptomatic UTI, and the presence of elevated levels of bacteria in the urine (bacteriuria) is diagnosed with concomitant urination [4]. The majority of pathogenic bacteria are common in the human urinary system and become harmful when this microbiota becomes unbalanced. After a fecal transplant, the urinary tract's health may be restored. It is necessary to investigate the putative gastrointestinal origin of the human bacterial microbiota. That is why the high percentages of the bacterial pathogens that cause UTI may be the gastrointestinal tract microbiota [5]. A microbial invasion is a urinary tract infection that results in an inflammatory response in the urinary tract epithelium any portion of the urinary system may be affected by UTI: kidneys (pyelonephritis), uterus, bladder (cystitis), and urethra (urethritis). The lower urinary tract involves much of the UTIs (bladder and urethra). Bacteria (Gram-negative and Gram-positive), as well as fungi, can cause the infection Furthermore, 70% of community-acquired UTIs and 50% of hospital-associated UTIs are caused by Uropathogenic *Escherichia coli* (UPEC) and other pathogens are responsible for the lowest rate of infection [6].

Previous studies showed that rheumatoid arthritis is caused by an upper urinary tract infection by Proteus bacteria [7]. The common infections in the gastrointestinal and urogenital tract are associated with a lowered risk of rheumatoid arthritis, which contradicts the hypothesis that infections trigger the onset of RA [8]. There is no previous studies showed the role of bacterial UTI in the activity of RA (DAS28). That is why, the present study showed the role of bacterial infection of UTI in the activity of RA.

2. MATERIALS AND METHODS

2.1. Patients' samples

The present study was subjected to 100 cohorts composed of 70 patients with RA and 30 healthy control subjects. The patients were indoor patients who were taking care and medications in Baghdad Teaching Hospital, Baghdad, Iraq, and 30 healthy cohorts obtained from normal persons who visit the hospital for routine checking and from normal persons working in the University of Baghdad, Baghdad, Iraq. 2010 RA classification criteria were followed by rheumatologists in specialist hospitals [9]. They had never received disease-modifying drugs or corticosteroids for one month prior time point of the present study. Among RA patients there were 11 males and 59 females, 40 % tested positive for IgM rheumatoid factor, and 60% tested positive for anti-CCP antibodies; their age was 48.2 ± 8.2 years (mean + SD), and disease activity score 28 (DAS28) [10] was 3.95 ± 1.12 (mean ± SD). The standard methods of Prevoo et al. (1995) and Carpenter et al. (2018) were followed to measure the values of DAS28 [10, 11].

2.2. Urine samples and Bacterial identification

The urine samples were collected from 100 subjects and the samples were taken by sterile screw cups and immediately transported to the laboratory to be inoculated onto appropriate media. The urine samples were inoculated onto MacConkey agar and then incubated at 37°C for 18 h to different organisms according to their ability to ferment lactose and onto blood agar for detecting the type of hemolysis. The collected samples were cultured on MacConkey agar, and Blood agar under aerobic conditions and sterile conditions. To diagnose the isolated bacteria the select colonies were re-cultured on mannitol salt agar. The morphological identification of the isolates as bacilli were confirmed microscopically by performing Gram staining, for which a single colony of each isolate was picked up and stained as per the standard protocol and viewed under oil immersion for similar types of cells. Bacterial identification was done by using biochemical tests which included Gram stain, growth characteristics, and other test methods. The Gram stain slides were prepared using the Gram stain kit for all isolates according to the common Gram stain procedure. This stain makes the determination of cell morphology and arrangement in addition to the Gram reaction and is usually the first differential test run on a specimen for identification [12]. The VITEK 2 DensiCheck instrument, fluorescence system (bioMe´rieux) (ID-GNB card) includes 43 nonenterobacterial gram-negative taxa was used. Testing was performed according to the instructions of the manufacturer [13].

2.3. Preservation of bacterial isolates

For short-term storage, on the surface of the nutrient agar plates, pure isolates of bacteria were maintained for a short time (maximum of two weeks). The plates were wrapped tightly with parafilm and kept at 4°C. While for medium-term storage, pure isolates of bacteria were maintained in a slant culture for a period of one month. In screw-capped tubes containing 5 ml of nutrient agar medium as slant, this culture was prepared, tightly wrapped with parafilm, and stored at 4˚C. For long-term storage, the pure colony was inoculated into nutrient broth containing 15% of glycerol with pure colonies, incubated at 37 °C for 24 h, and then kept at -20 °C; bacteria can be stored for months or years in low temperatures without significant loss of viability [14].

2.4. Statistical analysis

All data represented in mean and standard deviation the ANOVA test was used to identify the significant difference between patients and control groups, a p-value less than 0.05 was considered a significant difference.

3. RESULTS

3.1. Bacteriological studies

The results of the current study showed that out of the seventy patients suffering from RA 38 cases were suffering from UTI (54 % of total cases) comparing the 11 (36 of total healthy control) healthy control suffering from UTI (Table 1). The results of present study showed clearly that patients who suffer from RA are more likely to develop UTI as compared with healthy people.

Table 1. The percentages of bacterial growth that isolated from urine sample.

	Number of cases	Positive		Negative	
		Number	Percent	Number	Percent
RA patients	70	38	54.28	32	45.71
Healthy Control	30	11	36.66	17	56.66

3.2. Species of bacteria isolated from urine samples

The present study showed that the highest incidence of pathogenic bacteria isolates that isolated from urine of patients suffering from RA was found in the *S. aureus* with a percentage of (42.10%), followed by *S. haemolyticus,* while the lowest percentage of incidence of bacteria that isolated from urine of patients suffer from RA was seen in case of *K. pneumonia* with percentage 2.63. The highest incidence of bacterial isolation in urine of group of healthy cohort was seen in case of *S. haemolyticus* with percentage of incidence of isolation 45.45 (5 isolates), followed by *E. coli* with percentage of incidence of isolation 36.36 (4 isolates).

Table 2. Incidence of bacterial isolation in urine of patients suffer from RA and healthy control group.

Type of bacteria	Patient		Control	
	Number	%	Number	%
Enterococcus faecium	2	5.26	0	0
Escherichia coli	4	10.52	4	36.36
Klebsiella pneumoniae	1	2.63	0	0
Micrococcus luteus	3	7.89	0	0
Staphylococcus aureus	16	42.10	2	18.18
Staphylococcus epidermidis	2	5.26	0	0
Staphylococcus haemolyticus	7	18.42	5	45.45
Streptococcus spp	3	7.89	0	0

3.3. Urinary tract infection and DAS-28

The current study showed that the DAS28 in patients with RA is higher than its levels in healthy people, and this is logical because patients with RA suffer from joint symptoms and problems, which are related to determining the value of DAS28 (Fig. 1). The study also showed that there is no significant difference of DAS28 in patients suffers from RA and UTI in comparing with the value of DAS28 in patients suffer from RA and their not suffer from UTI. Also there is no significant difference in DSA28 was observed between patients of RA and suffering with UTI and the value of DAS28 of all patients group (70 cohorts) of RA, similar finding was observed in value of DAS28 in patients of RA and not suffer from UTI in comparing with the value of DAS28 of total patient group with RA (70 cohorts), (P > 0.05).

When assessing the effect of UTI on the value of DAS28 in healthy people (control group). No significant differences were identified in DAS28 between the group of healthy people with UTI and healthy people without UTI. There is no significant difference in DSA28 was observed between healthy cohorts those suffering from UTI and the value of DAS28 of all healthy group (30 cohorts), similar finding was observed in value of DAS28 in healthy group and not suffer from UTI with the value of DAS28 of total healthy cohorts (30 cohorts), (P >0.05) (Fig. 1, control group). The present study showed clearly that the infection with bacteria in urinary tract system does not effect on the DAS28 in patients with RA and healthy control.

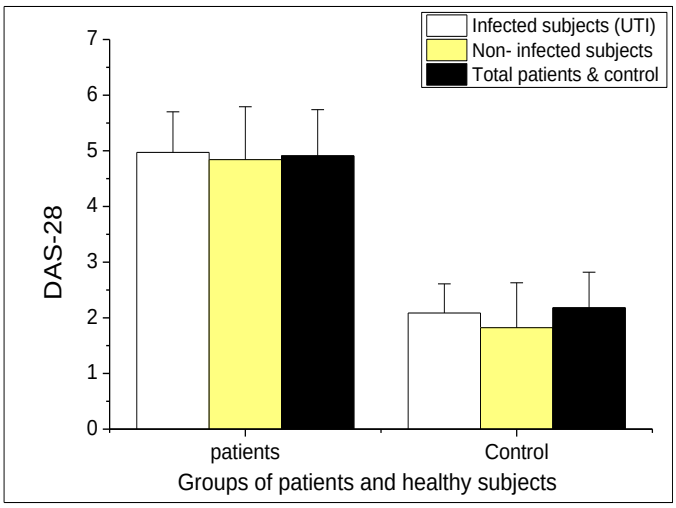

Fig 1. Disease activity score (DAS) 28 score in groups of patients and control. There is no significant effect of infection with bacteria in urinary tract on the value of DAS28 in patients group (RA) and healthy control group, P>0.05).

3.4. Effect of species of bacteria that cause UTI on DAS28

Fig 2 shows the effect of infection of the urinary tract with different species of bacteria on the level of DAS28 of RA. The results showed that the UTI in patients with RA by different species of bacteria led to an effect on the level of DAS-28 when compared with the level of DAS28 for all RA group (70 cohorts). But this change was not a statistically except for patients who infected (urinary tract) with *S. haemolyticus*. The level of DAS28 in patients with RA who infected with *S. haemolyticus* higher significantly (P<0.05) than the mean of DAS28 of total RA patients. In the control group, the infection of urinary tract of healthy people with different bacterial species did not effect on the level of DAS28, concluded the bacterial species did not effect on the level of DAS28 in health control cohorts.

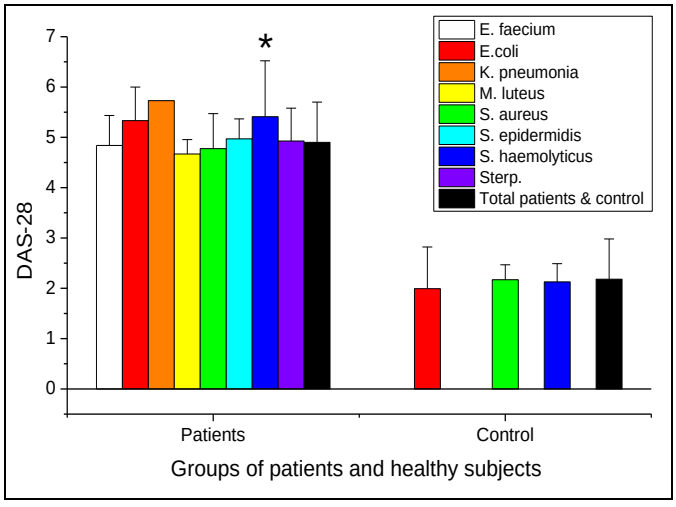

Fig. 2. Disease activity scores (DAS) 28 in groups of patients and controls that suffering from infection (UTI) with different species of bacteria. Asterisk indicates the significant difference from mean of DAS28 score of all patients' subjects (either patients group in case of patients' subjects or control group in case of control subjects).

4. DISCUSSION

Many studies have shown that suffering from RA is usually associated with various bacterial infections, especially UTI [7]. The present study included the collection of urine samples from patients suffering from RA and some of them suffering from bacterial UTIs. The current study confirmed that the rate of UTI in patients with RA was higher than the rate in healthy cohorts. Moreover, the highest rate of infection was caused by *S. aureus* followed by *S. haemolyticus.* Puntis *et al*., (2013) found a high correlation between incidences of UTI and suffering from RA. That happened because this group of patients (patients suffering from RA) use long-term oral steroids as sole therapy was associated with a high incidence of UTI and that was correlated with the duration of taking the anti-rheumatic drugs [15]. Bacteria of the genus *Proteus* of the family Enterobacteriaceae are facultative human pathogens responsible mainly for urinary tract and wound infections, bacteremia, and the development of RA [16]. Sams *et al*. (2015) investigated the risk of and risk factors for S. aureus sepsis in RA [17]. A previous study found that the prevalence of *E. coli* Compared to other related research, among UTI patients was fairly low (10 percent). Another study conducted by Odoki *et al*. (2019) covered patients attending hospitals in the western Uganda district of Bushenyi and found a significantly high prevalence of *E. coli* 41.9 % was registered in patients with RA [18]. A previous study showed that the incidences of infection with specific bacterial species in patients with RA vary, and this depends on the prevalence of the species of bacteria in the area in which people who suffer from chronic arthritis live. We would like to show that the current study is the first to show that *S. aureus* is the most common bacterium present in the urine of patients suffering from RA in Baghdad, Iraq.

In the present study, it was found that the bacterial UTI did not affect the activity of RA (DAS28), but when we studied the effect of the infection with particular bacterial species, we found that only the infection with *S. haemolyticus* effect on the value of DAS28 as compared with the healthy control group.

Most of the previous studies that dealt with the activity of RA disease showed that the severity of the disease is highly correlated with DAS28, as the existing and previously completed studies showed that DAS28 is associated with RA and that there is a significant difference between the value of DAS28 in patients and the DAS28 level in the control group [19], and this is completely agree with the findings of the current study.

Many previous studies have shown the close relationship between RA disease and infection with different microorganisms, and this has been explained by the nature of the treatments that are used by patients that reduce the body's resistance (immune response) to pathogens, which provides a great opportunity for pathogens to cause infection in the body of people suffering from RA [20].

Although very few studies have shown the role of some pathogens antigens and some components secreted by pathogens in increasing the effectiveness of immunity in patients with RA, there is no previous study that shows the relationship between the activity of RA (DAS28) and the UTI infection with bacterial species. That is why; this study is a unique study that attempts to focus on the effect of UTI infection on the activity of RA disease. Although the study did not show that there is a direct and statistical effect of UTI on the activity of RA disease, this does not eliminate the consideration of this study as the first study that sheds light on this topic.

No previous study has been obtained linking the UTI of patients with RA with a specific species of bacteria with the activity of RA disease, so this study is considered the first study that links the type of bacteria causing UTI in patients with RA with the activity of RA disease in terms of DAS28. Therefore, this topic needs extensive independent studies to determine the effect of infection with a certain species of bacteria on specific organs of the human body on the activity (DAS28) and severity of the disease (in terms of clinical and lab findings). This will open a new approach to understanding the reasons for the increase in the severity of RA disease in certain patients and in certain cases without others. We do not say in this study that bacterial infection plays a fundamental role in that, as there are other factors that may contribute to increasing the activity of RA disease in certain patients in special circumstances [21]. However, this study sheds light on a new factor that can play a role, even partially, in the activity of RA disease, so we suggest increasing scientific research in this area.

5. CONCLUSION

It can be concluded from the current study that the rate of UTI in patients with RA was higher than in healthy people who do not suffer from RA. The species of bacteria that causes UTIs does not affect the level of activity of RA (DAS28) except the *S. haemolyticus*.

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Conflict of interest

The authors declare that they have no conflict of interests.

Ethical Approval

This review was approved by the Ethical Committee of the University of Baghdad, Baghdad, Iraq (No 1630, 2021).

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