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Effect of Bacterial Infections of The Respiratory Tract System on The Activity of Rheumatoid Arthritis

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ABSTRACT

Background: Several infectious lung diseases often develop in patients with Rheumatoid arthritis (RA), especially during immunosuppressive medication, including disease-modifying anti-rheumatic drugs (DMARDs). The present study aimed to determine the role of respiratory tract bacterial infection in RA activity.

Methods: Blood and sputum samples were collected from 31 patients with RA and 12 healthy subjects as control. The bacterial isolates were isolated and identified in collected sputum by biochemical tests and Vitec 2 system.

Results: In the present study, thirty-one patients with RA were compared with 12 healthy subjects. Eight patients with RA were not infected with pathogenic bacteria (RA-NIPB) (25.8%). Twenty-three RA patients were infected with pathogenic bacteria (RA-IPB) (74.19%). From 23 RA-IPB, 10 RA patients were infected with *Staphylococcus aureus*, 6 RA patients were infected with *Klebsiella pneumonia*, 4 RA patients were infected with *Streptococcus pneumoniae*, and 3 RA patients were infected with *Pseudomonas aeruginosa*. No pathogenic bacteria were isolated from the sputum of a healthy subjects. There was no significant difference between RA-NIPB and RA-IPB in terms of the level of disease activity score DAS28.

Conclusion: The current study showed that patients with RA have a higher incidence of being infected with pathogenic bacteria than the healthy control group. No significant effect of bacterial species infection on the level of DAS28. The present study is the pioneer study that showed no effect of bacterial respiratory infections and different RA treatments on RA disease activity.

Keywords: Rheumatoid Arthritis, DAS28, MTX, ETC, Pathogenic Bacteria.

INTRODUCTION

Serious infections in RA patients were frequently caused by bacterial infections [1,2]. The lung, urinary tract, and skin are the sites that are most often affected [3–6]. Compared to the general population, there were no major differences in the types of isolated pathogens, may be with the exception of intracellular bacteria in patients using anti-tumor necrosis factor-alpha (anti TNF-alpha)[7]. Pulmonary diseases are most popular causes of extra-articular morbidity and mortality in a percentage of 60-80 % of patients with rheumatoid arthritis (RA) and the second cause of death by infection [8]. Although pulmonary infection and therapy toxicity were frequent complications of lung disease associated with RA[9–11]. When treating RA patients with respiratory comorbidities, Physicians frequently need to decide how best to treat them and establish plans for their long-term care.

Although there was no conclusive evidence or general agreement and there was conflicting data, conventional and biological disease modifying antirheumatic medications (DMARD) have been linked to the progress of treatment related pulmonary damage[12,13]. Moreover, due to there is an increased incidence of smoking in RA patient [14], RA is linked with an increased prevalence of lung cancer compared to the healthy subject [15].

The paucity of controlled studies on the effect of treatment on bacterial infections in the lung are scanty in literature unclear the role of pathogenic bacteria in RA patients. The exposure to bacterial lipopolysaccharide causing an imbalance between pro-inflammatory and anti-inflammatory cytokines followed by systematic inflammations [16,17]. Alternatively, bacteria through the activation of the innate immune response such as the toll-like receptors may induce the release of inflammatory cytokines as Interleukin (IL-8) and TNF-α that could stimulate neutrophilic inflammation [18]. Taken together the above, the study aimed to highlight the role of RA in the incidence of bacterial respiratory tract infection and role of this and type of treatment on the RA disease activity in terms of score DAS28.

PATIENTS AND METHODS

The samples were collected from 31 patients with RA and 12 healthy subjects as control group. The average of age of patients with RA was 46.4 ± 12.03 and the control subjects was 41.06 ± 7.8 years. The disease duration was more or equal to one year, and the subjects were successively recruited between November 2021 and March 2022 during routine visits to the rheumatology outpatient clinic of Baghdad Teaching Hospital. The 2010 ACR/EULAR criteria [19] were used in diagnosis of the patients. All patients underwent a baseline of disease activity score (DAS28) to assess RA activity [19] and tested for erythrocyte sedimentation rate (ESR); the C-reactive protein (CRP); Rheumatoid factor (RF); Anticitrullinated protein antibody (ACPA). Based on DAS28, subjects were divided into four groups: remission, low, moderate, and severe.

The sputum samples were taken into sterile screw cap containers (aseptically) in the early morning.

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Expectorated sputum was taken to make the patient cough deeply before collecting the sputum samples.

The samples were transported to the laboratory within two hours and processed immediately by culturing on various culture media i.e. nutrient agar, blood agar and MacConkey agar (for primary bacterial identification). The Vitek 2 Compact system was used for further identification of bacterial isolates.

Statistical analysis

The data's mean ± standard deviation (SD) was determined. The correlation between groups was examined using the Pearson r correlation coefficient test. Both the Student's t-test and the Chi-square test were used to assess the differences. The threshold for statistical significance was P 0.05. All statistical analysis done by SPSS (ver. 25).

Ethical approval: The study was conducted following approval from the Human Ethics Committee of the Biology department, Sciences collage, Baghdad university (Reference number: CSEC/1021/0062; Date: 15/10/2021) and the Training and Human Development Center, Committee of Ethics, The Baghdad Karkh Health Department of the Ministry of Health (Reference number: 161; Date: 27/12/2021). The Helsinki Declaration was followed during the Ethical Committee's proceedings. Before enrolling in the study, all the subjects gave their informed written consent.

RESULTS

The clinical manifestations of 31 RA patients and 12 healthy control were shown in table 1. Significant difference was seen among the RA patients and control in terms of clinical and laboratory tests.

Table 1: The clinical manifestations of patients with RA and control.

Parameters	RA patients (n = 31)	Control (n = 12)	P-value
Age (years)	45.0±11.082	40.08 ± 5.53	0.152
Gender (no. [%])			0.02
Male	26	6	
Female	5	6	
BMI	28.2 ± 4.5	29.88 ± 4.98	0.29
Smoking state (no. [%])			
Smoker	3	6	0.004
Non-smoker	28	6	
Disease duration (years)	6.23 ± 3.7	-	-
DAS28	4.158 ± 1.18	-	-
MTX	6	-	-
ETC	11	-	-
MTXETC	16	-	-
ESR (mm/h)	29.3 ± 24.0	11.6 ± 8.5	0.01
RF:			
Positive	28	-	0.0001
Negative	3	12	
CRP (µg/ml)	11.31 ± 12.51	3.2 ± 0.25	0.031
ACPA (U/ml)	336.9 ± 191.5	71.1 ± 19.4	0.0001

The screening of presence of pathogenic bacteria were examine in sputum samples that were collected from patients with RA and healthy control. Table 2 shows the clinical features of thirty-one patients provided sputum samples that divided into two groups that either infected with pathogenic bacteria (RA-IPB) or non–infected with pathogenic bacteria (RA-NIPB). The results showed clearly there is no significant different in terms laboratories, clinical parameters and RA disease activity score (DAS 28) between the two groups (P>0.05).

Table 2: Clinical features of two groups of RA patients. First group, RA patients suffering from pathogenic bacterial infection in respiratory tract (RA-IPB). Second group, RA patients showing clear sputum from pathogenic bacteria (RA-NIPB).

Parameters	RA-IPB (n = 23)	RA-NIPB (n = 8)	P-value
Age (years)	46.65 ± 11.65	40.25 ± 8.04	0.163
Gender (no. [%]) **Male** **Female**	 4 19	 1 7	0.746
BMI	28.87 ± 4.69	26.25 ± 3.43	0.158
Smoking state (no. [%]) **Smoker** **Non-smoker**	 13 87	 0 8	0.282
Disease duration (years)	7.09 ± 3.81	3.75 ± 1.19	0.025
DAS28	4.18 ± 4.18	4.07 ± 0.65	0.823
MTX	17.4	12.5	0.639
ETC	30.4	37.5	0.319
MTXETC	52.2	50.0	0.916
ESR (mm/h)	32.39 ± 26.79	20.63 ± 9.9	0.239
RF: **Positive** **Negative**	 87 13	 0.00 100.0	0.282
CRP (µg/ml)	10.75 ± 10.75	12.92 ± 14.15	0.679
ACPA (U/ml)	339.81 ± 177.17	328.55 ± 241.86	0.889

Figure (1a) showed significant difference between patients with RA and suffering from pathogenic bacterial respiratory tract (RA-IPB) and patients with RA but their respiratory tract was not infected with pathogenic bacteria (RA-NIPB). Significant differences were seen between two groups in terms of percentages of infected with pathogenic bacteria ($P<0.05$). That proved the RA patients are highly incident to be infected with pathogenic bacteria. Figure 1b shows the percentages of different bacterial isolates (pathogenic bacteria) that isolated from RA patients suffering with respiratory tract infection. The results showed that the infection with *S. aureus* represents the highest percentages of bacterial infection (43.5%) followed by *K. pneumonia* (26.1%), *Streptococcus pneumonia* (17.4%) while, the infection with *P. aeruginosa* (13.0%) represented the lowest percentages of bacterial infection.

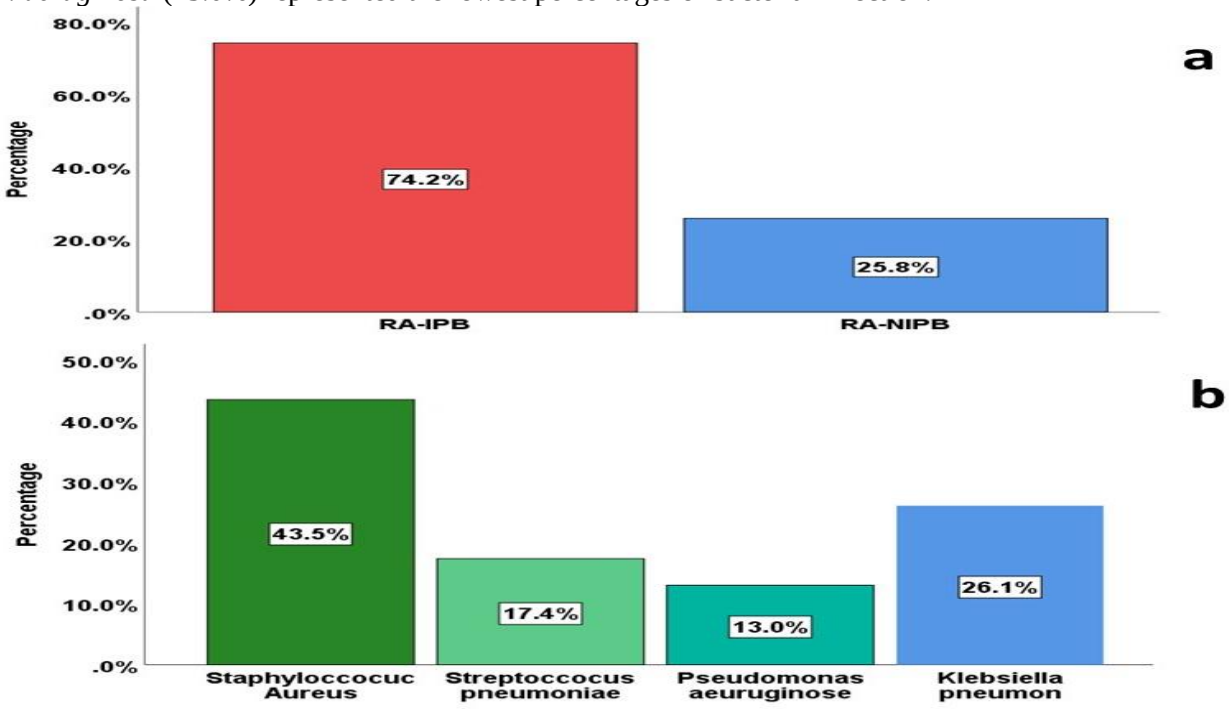
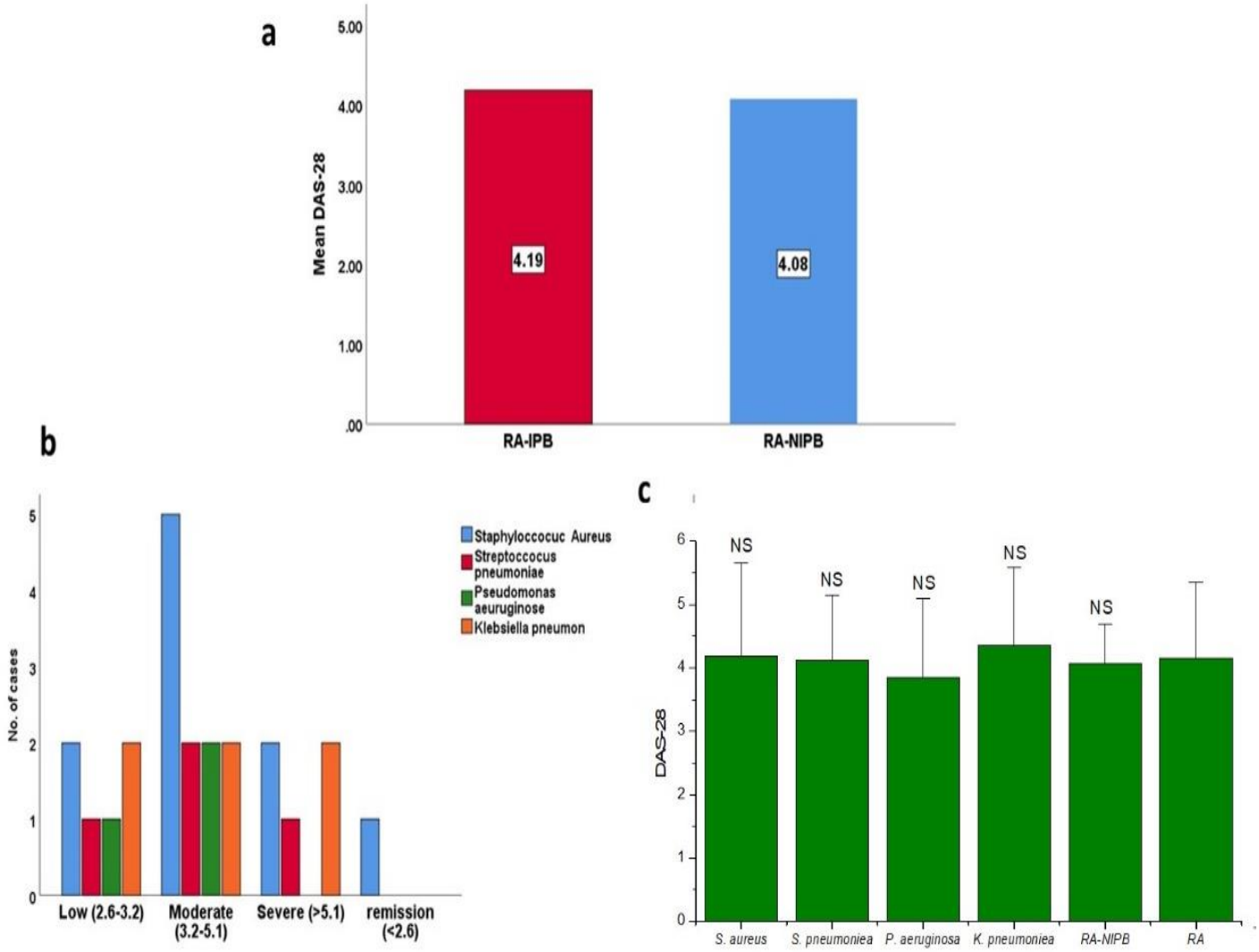


Figure (1): a, Percentages of subjects who suffering from RA and infected with pathogenic bacteria (respiratory tract infection) (RA-IPB) and patients who suffering from RA and were not infected with pathogenic bacteria (RA-NIPB); **b,** percentages of patients with RA in infected with different species of pathogenic bacteria the isolates were isolated from sputum collected from twenty-three patients with RA (RA-PIB).

Figure (2a) showed the means of DAS28 of RA infected group (RA-IPB, 23 patients with RA) with different bacterial species and RA a non-infected group with pathogenic bacteria (RA-NIPB, 8 patients with RA). The results showed that there is no significant difference among two groups in terms of DAS28 (P>0.05). Figure (2b) showed a divided RA group depending on DAS28 into four groups (remission, low, moderate, severe). The results showed that the infection with *S. aureus* represent the domain infection in all groups (4 groups). Figure 2c the effect of infected with different species of pathogenic bacteria on the level of DAS28, the results were compared with DAS28 of total patients with RA (n: 31). The results showed no significant difference (P>0.05).



Figure(2): a, Level of DAS28 in two groups of patients with RA [patients with RA and infected with pathogenic bacteria in their respiratory tract (RA-IPB) and patients with RA and non-infected with pathogenic bacteria (RA-NIPB); **b,** number of cases that infected with different pathogenic bacteria (RA-IPB) in different group of RA divided to four main categories according to the level of DAS28; **c,** Level of DAS28 in different patients who infected with different bacterial species (respiratory tract infection).

Figure (3) showed the percentage of bacterial infections with pathogenic bacteria in three groups of patients under different treatments. Five patients were taken MTX therapy (4 of them was RA-IPB and one of them was RA-NIPB), 10 patients were taken ETC therapy (7 of them was RA-IPB and 3 of them was RA-NIPB), and 16 patients were taken MTXETC therapy (12 of them was RA-PIB and 4 of them was RA-NIPB). The results presented that there was no significant association among treatment type and infection with pathogenic bacteria (P>0.05).

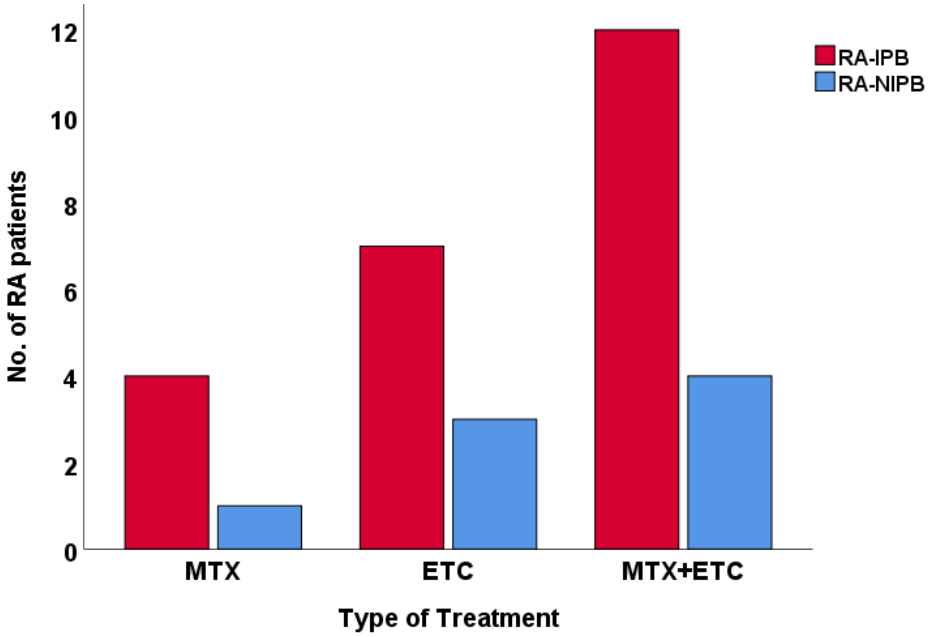


Figure (3): Number of patients with RA either infected with pathogenic bacteria (RA-IPB) or non-infected with pathogenic bacteria (RA-NIPB). The bacteria were isolated from sputum of 31 patients with RA.

DISCUSSION

Lung diseases is one of the extra-articular manifestations of RA that most concerns clinicians, due to the wide range of different types of involvement described and the potential increased risk and severity of respiratory infections. It was found a high prevalence of bacterial colonization by potentially pathogenic microorganism in patients with RA [20–22].

In the current study, it was found that the high incident of respiratory tract infect in patients with RA as compared with healthy control group. It was found that different species of pathogenic bacteria were isolated from sputum of patients with RA i.e. *S. aureus, K. pneumonia, S. pneumonia, and P. aeruginosa*, these data was in line with previously studies [20–22]. The high incidence with pathogenic bacterial infection can be explained by the patients with RA normally taken anti-immune drugs and immune suppressive drugs that will naturally reduce the ability of immune system to defense against external pathogens that is why, logically we got high percentage of infection with pathogenic bacteria in patients with RA as compared with healthy control group. The present study is the pioneer study that showed for the first time there is no effect of infection of respiratory tract of patient with RA on the level of disease activity in terms of DAS28.

While there is a distinct lack of lung microbiome studies in RA or pre-RA and therefore it is not impossible to assume they could be present within the RA/ pre-RA population. Both *S. pneumonia* and *S. pyogenes* are capable of causing respiratory infections [20]. It was interesting to note also the number of respiratory infections has been shown to increase prior to the onset of RA [23]. The previous study found that the possible link between treating RA patients especially DMARDs therapy on respiratory infections [21,24], while other study found no relation between DMARDs therapy and respiratory infections, and this found in the our study no relation between therapy and pathogenic bacteria [25].

It can be concluded that the high incidence of respiratory tract infection in patients with RA. The novelty of this project, there is no effect of respiratory tract infection with pathogenic bacteria on the disease activity.

Conflict of Interest & Financial disclosure

The authors declare that they do not have a known competing financial interest or personal relationship that could have had an impact on the work reported in this paper.

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