

Bacteriological and Immunological Study of Rheumatoid Arthritis

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Abstract: The current study included the collection of 175 samples of blood (Urea - blood) of patients with rheumatoid arthritis from Al -Al-Kindy Teaching Hospital, Baghdad Teaching Hospital and Al-Imamian Al - Kadhimiain Medical City in Baghdad from both sexes with different ages at the period between 1/10/2016 -1/2/2017. Bacterial growth results showed that 80% of urea for bacterial transplantation were positive results, while the number of samples showing no bacterial growth was 20%. The bacterial isolation evaluate for morphological testes and biochemical microscopy, as well as identification by Api system. The highest frequency of inflectional bacteria was *E. coli* (41.97%), followed by *E. cloacae* (21.25%), *P. aeruginosa* (12.5%), *Salmonella* (10%), *K. pneumoniae* (7.5%) and *S. marcescens* (6.25%). The concentration of some cytokines IL-6, TNF was measured by using ELISA technical. The results showed significant decrease at $P \leq 0.05$ in level of IL-6 for patient serum (23.76 ± 14.01 pg/ml) compared with healthy serum (209.11 ± 76.99) pg /ml. The result of TNF showed an increase significantly at $P \leq 0.05$ in level of serum for rheumatoid arthritis patient (437.68 ± 16.80 pg /ml) compared with healthy serum (370.31 ± 26.87 pg /ml). The study found that reverse relation between IL-6 and TNF.

Keywords: Rheumatoid arthritis, IL-6, TNF- α

1. Introduction

Rheumatoid arthritis is defined as a chronic and systemic autoimmune disease that affects various joints in the body. Rheumatoid arthritis, which affects about 1% of the adult population, leads to a doubling of the mortality rate, is a global prevalence infect of female more than male [1]. The serious condition of the disease with the infection of the synovium of the large joints, which contains a lot of synovial fluid, results in infection of synovial fluid containing large numbers of macrophages and fibroblast [2]. The synovium membrane characteristic by leak of mononuclear cells, lymphocytes and plasma cells as well as dendritic cells [3]. Many environmental factors overlap (such as bacterial infections) in the development of rheumatoid arthritis and induce an innate immune response and humoral [2]. The inflammatory process of the joint begins by activating of antigen-presenting cells, either by exogenous epitopes such as the bacterial DNA and the lipopolysaccharide layer [4] or by antigens autologous [5], then introduction of antigens autologous which associated with the human tissue compatibility complex of the second type to the T-helper cells. The activation of T-helper cells stimulates the secretion of interleukin-2 (IL-2) and interferon gamma (INF- γ), which activates B-lymphocyte. The activation of B-lymphocytes leads to the production of antibody-producing cells such as IgM, IgG antibodies in the synovial fluid of patients with rheumatoid arthritis. These antibodies form immune complexes [6] which stimulates the complement system and the production of pro-inflammatory cytokine [7], such as TNF α and IL-6, which is secreted by active phagocytes in response to the components of the bacterial cell wall [8].

2. Materials and Methods

A total of 175 blood and urea of rheumatoid arthritis patients were collected for clinic consultation at the following hospitals: Baghdad Teaching Hospital (Educational

Laboratory), Al-Kindy Teaching Hospital, Al-Imamian Al-Kadhimiain Medical City in Baghdad, from both sexes patient between the ages of 20-60 years at the period 1/10/2016 to 1/2/2017. The current study included 75 blood samples and 100 samples of the urea.

2.1. Collect blood samples

Blood samples were obtained from the vein as 5 ml were withdrawn from each patient. The blood was placed in a test tube free of any preservative material for the purpose of separating the blood and getting the serum. To prepare the serum after the blood clotting put in the centrifuge for 3 minutes at 3000 RPM, then withdrew serum and neglected the deposit and kept at a temperature of -20 °C for the purpose of conducting immunological tests.

2.2. Collecting the urea samples

Serum samples were collected from 100 patients in the current study and from both sexes at different ages by taking a sample from midstream urine sample in sterile bottles with wide-bore and a tight lid. A sterile loop of the donor sample was transferred and cultured by a streak method on the MacConkey medium and blood medium. The plates were reversed and incubated at 37 °C for 24 hours.

2.3 Isolation and identification of bacteria

Bacterial isolates were identified based on cultured and microscopic traits and biochemical tests approved by MacFaddin [9]. Diagnosis was also done using the API20E system and according to the manufacturer's instructions.

2.4. Antibiotic susceptibility test

The resistance and sensitivity of the bacterial isolates to 10 antibiotics were examined using the tablet method for the following antibiotics (Amikacin, Impenem, Cefotaxim,

Tobramycin, Cefixime, Tetracycline, Gentamycin, Amoxicillin, Ceftazidime, Trimethoprim). The measurement of the inhibition diameter in millimeters around the antibiotic discs and compared with global measurements according to CLSI, 2013.

3. Results and Discussion

3.1 Isolation and Identification:

A total of 80 positive samples of bacterial culture were obtained from 100 samples (80%) of the total number of patients with rheumatoid arthritis as in Table 1.

Table 1: Number and percentages of bacterial isolates from rheumatoid arthritis patients

Bacterial species	No. of isolation	Percentage (%)
<i>Escherichia coli</i>	34	41.97
<i>Enterobacter cloacae</i>	17	21.25
<i>Pseudomonas aeruginosa</i>	10	12.5
<i>Salmonella SPP</i>	8	10
<i>Klebsiella pneumoniae</i>	6	7.5
<i>Serratiamarcescens</i>	5	6.25

The genetic factors, age, sex and environmental factor as infection interacted with begin of rheumatoid arthritis [10]. The results of the present study show that *Escherichia coli* is the most frequent type of bacteria, isolated from 34 patients infection with percentage 41.97%, this result was agreed with a local study by Mohsin [11], followed by *Enterobacter cloacae* 17 samples with percentage 21.25%, this result was differed to Hashim [12]. *Pseudomonas aeruginosa* (12.34%), which represents 10 infections in the third site, this result was agreed with Zine El-Abidine and Ahmed [13]. *Salmonella* spp. infection was ranked fourth by 10%, which represents 8 cases, this result was a violation with Ahmed and Aziz (14). While *Serratiamarcescens* show (5 isolates) with percentage 6.25%, this result of the current study did not agree with the results of Hameed [15].

3.2. Antibiotic susceptibility test

All isolates of *E. coli*, as shown in Table 2, showed efficacy against the antibiotics Tobramycin, Amoxicillin and Gentamycin. All these isolates were sensitive to (52.94%) while Cefixime resistance was 52.94% these results were differed with Hameed (15). While there was a decrease in the resistance of bacteria to antibiotic Ceftazidime (38.2%) and the results of the current study approached with Al-Hamadani [16] that appointed a resistance rate (31.6%). For the antibiotic Cefotaxime percentage of resistance (44.11%) as the results were disagreed with Hashim [11] which recorded a resistance percentage (28%). While carbapenems antibiotic group which include Impenem antibiotic the resistance (52.94%), this result was differed with Faidah

[17] result, as well as the resistance of *E. coli* to Trimethoprim antibiotic which record (50%) and this results controvert of the findings Al-Attar [18] with a ratio of resistance (70.4)%. While the resistance of *C. cloacae* to Tobramycin, Gentamycin and Trimethoprim antibiotics were 68.75, 62.5 and 62.5% respectively, as resistance of this bacteria against Impenem was (68.75%) and against Amoxicillin and Tetracycline were (56.25, 68.75)%, these results were differed with Abid and Mahdi [19]. *P. aeruginosa* showed complete resistance to Ceftazidime antibiotic (100%) and that the resistance of the bacteria to aminoglycosides group Amikacin, Tobramycin and Gentamycin (70,70,60%) respectively, while its resistance to Cefotaxime and Cefixime antibiotics (70, 50%) and found to be resistant to Impenem (80%), Tetracycline (60%), Amoxicillin and Trimethoprim (70%). These results were consistent with Al-Salihiet al. [20] for amikacin antibiotic and with Al-Taai [21] for Tetracycline and Gentamycin which found that resistance percentage 60.71%. Our results for Cefotaxime antibiotics were consistent with Mansour et al. [22] (70.4%) by resistance percentage (70.4%) but disagreed with Oreibi [23] for the Cefixime antibiotic who record a resistance percentage 80%. Our results for trimethoprim, Amoxicillin and Impenem were disagreed with Muhsin [24] and Idrees [25] but were agreed with Salem [26] and Ahmed [27] for Ceftazidime, Tobramycin antibiotics. While the resistance of *Salmonella* to Cefixime and Amoxicillin (100%), Impenem, Cefotaxime, Trimethoprim and Gentamycin were (75%), the resistance for Ceftazidime, Tobramycin and Tetracycline (50%) and Amikacin (25%). These results were consistent with the findings of Ismail [28] for Gentamycin, who found that resistance to *Salmonella* isolated from diarrhea was 75% and opposite to Cefotaxime and Tetracycline which resistance was (69% , 75.6)%. Our results differed with Oplustil et al. [29] about Impenem, Amikacin, Ceftazidime, Trimethoprim and Cefixime, which found the antibacterial resistance 0%. The researchers Talal and Yosif [30] found that the resistance of bacteria to Amoxicillin was 100%. The results of the study disagreed with Mohdet et al. [31], which recorded a low resistance for Cefixime (16.92). While *S. marcescens* showed complete resistance to all antibiotics under study. The results were agreed with Faidah [17] for Amikacin, while disagreed with Tetracycline, Ceftazidime, Gentamycin, Impenem, Trimethoprim and Cefotaxime also disagreed with Ahmed and Aziz [14] for Amoxicillin and Cefotaxime. *K. pneumoniae* was show complete resistance against all antibiotic, these results were consistent with Hashim [11] for Amoxicillin and Tetracycline (100%), but disagreed with the following antibiotic: Amikacin, Cefotaxime, Trimethoprim, Tobramycin, and Gentamycin which found no sensitivity to these antibiotic by bacteria (0%). The results showed disagreed with Ahmed [27] which found that the sensitivity of the bacteria to antibiotic Ceftazidime and Ceftazidime was (29.41, 100, 64.70)%.

Table 2: Resistance of isolated bacteria from urea of rheumatoid arthritis patients to antibiotics

Antibiotic Bacteria	No.	AK 30ug	AX 25ug	CN 10ug	TOB 10ug	TMP 10ug	TE 10ug	IPM 10ug	CFM 10ug	CTX 10ug	CAZ 10ug
<i>Escherichia coli</i>	34	17 (50)	16 (47.05)	16 (47.05)	16 (47.05)	17 (50)	17 (50)	18 (52.94)	18 (52.94)	15 (44.11)	13 (38.2)
<i>Enterobacter Cloacae</i>	18	12 (75)	11 (68.75)	10 (62.5)	11 (68.75)	10 (62.5)	9 (56.25)	11 (68.75)	10 (62.5)	10 (62.5)	7 (43.75)
<i>Pseudomonas aeruginosa</i>	10	7 (100)	7 (100)	6 (100)	7 (100)	7 (100)	6 (100)	8 (100)	5 (100)	7 (100)	10 (100)
<i>Salmonella</i>	4	3 (75)	4 (100)	3 (75)	2 (50)	3 (75)	2 (50)	3 (75)	4 (100)	3 (75)	2 (50)
<i>S.marcescens</i>	5	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
<i>Klebsiella pneumoniae</i>	6	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)

3.3. Measuring the level of cytokines in the serum of patients with rheumatoid arthritis

The kit was used by Peprotech (USA) to measure the level of IL-6, TNF α and according to the manufacturer's instructions.

3.3.1 Cytokine IL-6

The results of the statistical analysis of the study shown in Table (3) and Figure (1) a significant decrease of $P \leq 0.05$ in the level of IL-6 in the serum of patient infected with the disease and compared with its level in healthy serum. The serum IL-6 levels were 23.76 ± 14.01 pg/ml while in healthy serum 209.11 ± 76.99 pg/ml therefore the results showed a significance different below the probability level $P \leq 0.05$ in level of cytokine in serum of patients with rheumatoid arthritis.

Table 3: Average concentration of the IL-6 of two groups of patients with rheumatoid arthritis and control estimated at pg /ml.

Cytokine	Groups	No.	M \pm SE
IL-6	Control	24	209.1 \pm 76.99
	Patient	75	23.76 \pm 14.01

*Significant differences below the probability level $P \leq 0.05$

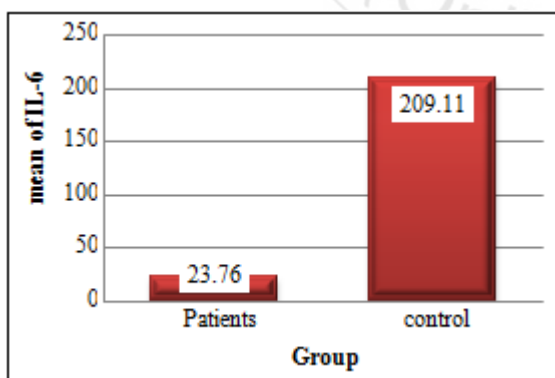


Figure 1: The average concentration of IL-6 for the two groups of patients with rheumatoid arthritis and control estimated at pg / ml.

The results of the present study differed with the findings of Shaban[32], which indicate that there are significant differences in the serum of patients with the disease. These studies show to the role of IL-6 in the disease because ability of cytokine to induce and active B-lymphocytes which are

differentiation into plasma cells and then the production of antibodies, including the rheumatoid factor [33]. It also regulates the proliferation and differentiation of young Th0-lymphocytes to a Th-17 that produces IL-17. This also cytokine causes erosion bone and cartilage by stimulating bone-forming cells [34]. As stimulates IL-6 to dilate blood vessels, increase lymphocytic infiltration, proliferative synovial proliferation and amplify the joint by producing metalloproteinases matrix (MMPs) in synovial cells.

3.3.2. Cytokine TNF- α

The results of the current study showed that in Table (4) and Figure (2) there was a significant increase in $P \leq 0.05$ in cytokine level in patients serum (437.68 ± 16.80 pg/ml) while in health serum (370.31 ± 26.87)pg / ml.

Table 4: Average concentration of TNF- α for the two groups of patients with rheumatoid arthritis and control estimated at pg / ml.

Cytokine	Groups	No.	M \pm SE
TNF- α	Control	24	370.31 \pm 26.87
	Patient	75	437.68 \pm 16.80

*Significant differences below the probability level $P \leq 0.05$

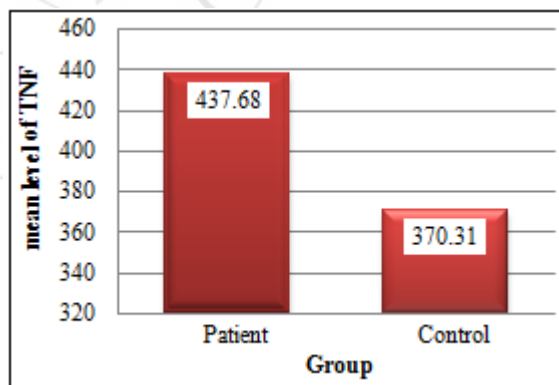


Figure 2: Average concentration of TNF- α for the two groups of patients with rheumatoid arthritis and control is estimated at 1pg/ ml.

The results of the study were consistent with Muhsin [24] and Al-Obeidy and Abdullah [35] and The reason for its rise is due to be being an important part of the inflammatory cytokines involved in the disease by stimulating the leaking cells present in the articular tissue such as fibroblast cells, osteoclast and cartilage cells to secrete the proteolytic enzymes such as Matrix Metalloproteinase MMP when

stimulated by IL-1 and TNF- α . MMP enzymes dissolve connective tissues and consider an important medias in the destruction of the joint [36]. The high levels of cytokine are due to the host's response to antigens that may be bacterial, such as lipopolysaccharide and viral, which activate phagocytes and lymphocytes T, B. The activation of phagocytes results the secretion of a number of inflammatory cytokines such as IL-1 β , TNF- α , IL-6, IL-2 [37]. This cytokine can increase inflammation by stimulating fibroblast to express adhesion molecules by binding them to the receptors on the surface of the white blood cells, thereby facilitating their transmission to the site of inflammation in the synovial membrane.

3.3.3. Correlation Coefficients (r) between cytokines under study

The correlation coefficient between cytokines under studied showed non-significant negative correlation coefficient ($P \leq 0.05$) between (IL-6, TNF- α) and the highest negative correlation between cytokines-0.071. While a positive correlation coefficient between IL-6 and bacterial infection was found with a correlation coefficient of 0.87. The correlation coefficient was significant below the probability level ($P \leq 0.05$).

Table 5: Correlation coefficient between IL-6, TNF- α for rheumatoid arthritis patients

IL-6	correlation coefficient (r)	TNF- α	Bacterial
		-0.071	0.87
	P-Value	0.138	0.005

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