Archives of Razi Institute, Vol. 77, No. 5 (2022) 1813-1819

DOI: 10.22092/ARI.2022.357980.2126

Original Article

Immunological Evaluation of Individuals Infected with *Acinetobacter baumannii*

Manfi Ahmed, S[1 *], Hashim Yaseen, K[2], Mohammed Mahmood, M[2]

1. Department of Dentist, Al-Rafidain University College, Baghdad, Iraq
2. Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq

Received 28 February 2022; Accepted 21 May 2022
Corresponding Author: aharmanfi75@gmail.com

Abstract

Acinetobacter baumannii (A. baumannii) is a spherical rod-shaped Gram-negative non-lactose fermenting (Coccobacilli, Aerobic bacteria) bacteria. It is a member of *the Moraxellacea* family. *A. baumannii* is a pathogenic, opportunistic organism that infects humans in society and hospitals. In particular, patients with immune system defects are at risk, especially those with burn infections and those hospitalized in intensive care (ICU). It plays a vital role in many illnesses, including septicemia, pneumonia, meningitis, soft tissues, skin infection, endocarditis, and urinary tract infection (UTI). The current study included immunological evaluation of infection with *A. baumannii*. In the current study, 150 blood samples were obtained as follows: 100 blood samples were collected from infected individuals with *A. baumannii* admitted to hospitals in Baghdad. Fifty blood samples were obtained from healthy individuals and considered as the control. 10 ml of blood samples were collected from the venous blood of the participants. *A. baumannii* was collected and isolated from infected patients and diagnosed by traditional methods, using different culture media (MacConkey agar, blood agar, and Chromogenetic agar) and by biochemical assays, then the bacteria diagnosis was confirmed using the VITEK 2 ID-GN cards. Microscopic examination and culture diagnosis of bacteria were conducted, and the diagnosis was confirmed by complete biochemical examinations using VITEK2 Compact System. Assessments included the serum level of IL-17A and TNF-α for hospitalized patients infected with *A. baumannii*. The study recorded a significant increase in the serum level of IL-17A for patients infected with *A. baumannii* (479.83±26.21 pg/ml) compared to control subjects (69.32±4.53 pg/ml). The recorded data showed a significant increase in the serum level of TNF-α for patients infected with *A. baumannii* (98.05±28.89 pg/ml) compared to control (1.40±25.12 pg/ml).

Keywords: IL-17A, TNF-α, *Acinetobacter baumannii*

1. Introduction

Acinetobacter baumannii (A. baumannii) is Gram-negative bacteria that are non-lactose fermenting, characterized by a spherical rod shape (Coccobacilli, Aerobic bacteria), belonging to the family of *Moraxellacea* (1). *A. baumannii* is a non-motile bacterium that does not produce the enzyme cytochrome oxidase, urease citrate, and indole. Many environmental bacterial isolates grow at a temperature ranging from 20C° to 30C°, while *A. baumannii* bacteria grow at a temperature of 44 C° (2, 3). They are short, typically 1.0–1.5 μm by 1.5–2.5 μm in size as measured during the rapid phase of their growth, but often develop into more coccoid in the stationary phase, usually present in pairs or long chains of different lengths (4).

A. baumannii is a pathogenic, opportunistic organism that infects humans in society and hospitals. In particular, patients with immune system defects are at risk, especially those with burn infections and those hospitalized in intensive care (ICU). It plays a vital role

in many illnesses, including septicemia, pneumonia, meningitis, soft tissues, skin infection, endocarditis, and urinary tract infection (UTI) (5).

A. baumannii possesses several virulence factors that increase its pathogenicity, including biofilm formation that increases the ability of bacteria to adhere to both living and non-living surfaces (biotic and abiotic) (6), producing capsular polysaccharides that increase the resistance of bacteria to antibiotics (7), as well as "the production of the enzyme phospholipase," which degrades the host cell wall and increases the virulence of bacteria (7) as well as outer membrane proteins that contribute to the host cell apoptosis process (8).

A. baumannii causes many diseases, especially in people with immunodeficiency (immunosuppression) and children who have Tracheostomy, an opening in the trachea that leads to bronchiolitis and tracheobronchitis (9). In this situation, an infection occurs because of the bacteria *A. baumannii*. The incidence of *A. baumannii* increases in smokers, people who drink alcohol, diabetics, and patients with chronic obstructive pulmonary disease (COPD) (10).

In response to the infection, the explosion and production of cytokines and chemokines generally occurred to amplify the immune response against the pathogen agent. The interleukin-17 (IL-17) pathway is essential for neutrophil recruitment in response to various pathogens. The control of *A. baumannii* infection depends on neutrophils (11). This shows that IL-17 may be significant in *A. baumannii* infection; nevertheless, there is still a lot to learn about the protective immune responses triggered by these bacteria and their potential therapeutic implications (12).

Tumor necrosis factor-α (TNF-α), also called TNF ligand superfamily member 2 (TNFSF2), is a proinflammatory cytokine that is mainly produced by activated monocytes and macrophages in response to infection, injury, and tumor burden (13). TNF-α production has also been found in many other inflammatory cell types, including T cells, NK cells, and neutrophils, as well as non-immune cells, including keratinocytes and astrocytes (14). It is also recognized that TNF-α is involved in tissue inflammation and injury and appears to be a prominent ligand for the activation of programmed cell death through apoptosis. This latter function occurs not only during normal growth and development but may also result from pathologic conditions in which local and systemic production of TNF-α is increased (15).

Therefore, the current study aimed to investigate the immunological function of the immune system in the case of *A. baumannii* infection.

2. Materials and Methods

2.1. Sampling

In the current study, 150 blood samples were obtained as follows: 100 blood samples were collected from infected individuals with *A. baumannii* admitted to hospitals in Baghdad. Fifty blood samples were obtained from healthy individuals and considered as the control—10 ml of blood samples were collected from the venous blood of the participants.

A. baumannii was collected and isolated from infected patients and diagnosed by traditional methods, using different culture media (MacConkey agar, blood agar, and Chromogenetic agar) and biochemical assays; then, the bacteria diagnosis was confirmed using the VITEK 2 ID-GN cards.

2.2. Identification of *Acinetobacter baumannii* via VITEK2 Compact System

The bacterial isolate was identified using the VITEK2 technology. On MacConkey agar dishes, the bacterial isolates were subcultured. Bacterial suspensions in 0.45 percent sterile NaCl solution were employed, similar to MacFarland 0.5x 10^8 standards. A densitometer was used to regulate the turbidity of the bacterial suspension. The VITEK 2 compact system was manually loaded with VITEK 2 ID-GN cards, AST-No. 12 cards, and bacterial suspension. A bacterial suspension was physically put into each test card, sealed, and incubated for 6 hours. During this time, the cards were recited every 15 minutes using kinetic fluorescence measurement. The VITEK 2 compact system software examined the data first, then mechanically reported the results (16).

2.3. Assessments of Serum Levels of IL-17A and TNF-alpha

Serum levels of IL-17A and TNF-alpha were detected using commercial kits, which were products of KOMA ELISA (Labiskoma, Korea).

The methods were based on the principles of sandwich enzyme-linked immunosorbent assay (ELISA). The microplate wells were pre-coated with a specific anti-marker antibody (Capture antibody: anti-human IL-17A or TNF-alpha). A specific antibody reacts after adding standards or serum samples to the appropriate wells. This step is followed by adding horseradish peroxidase (HRP)-conjugated with the specific antibody. After incubation, followed by a washing step, the TMB substrate solution (3,3′,5,5′-tetramethylbenzidine) is added to each well. At this point, the blue color is developed in the wells, and after adding the stop solution, the color turns yellow. The density of the color is proportional to the level of IL-17A or TNF-alpha. At a wavelength of 450 nm, optical density (OD) is measured spectrophotometrically. After that, an EXCEL sheet is used to create a standard curve, and a curve-fitting equation is used to determine the level of the unknown serum sample (Figure 1 and 2).

2.4 Statistical Analysis

The mean±SE of the mean was calculated using the IBM SPSS version 26.0 (Armonk, 2019). The probability was also examined using the student T-test and at the probability level≥ (0.05, 0.01).

The WinPepi application version 11.65 was used to calculate the probability for non-parametric data using Pearson's chi-square test (17).

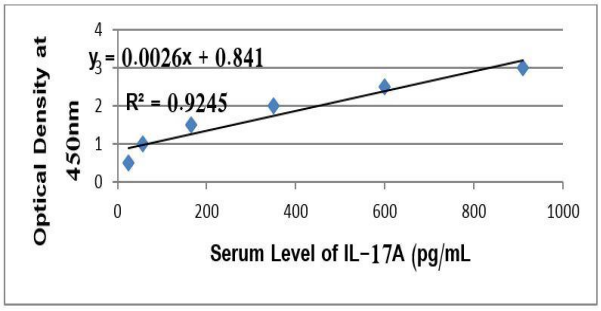

Figure 1. Standard Curve of IL-17A

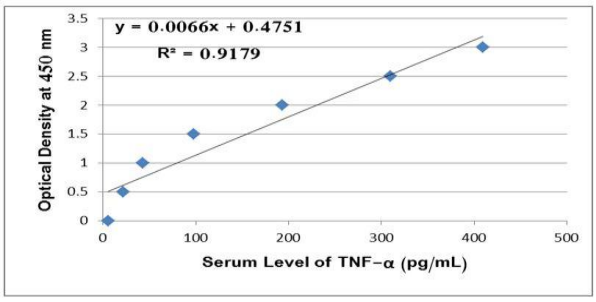

Figure 2. Standard Curve of TNF-alpha

3. Results

The results of bacterial diagnostic tests are tabulated in table 1.

Table 1. Results of Biochemical Identification tests of *Acinetobacter baumannii* isolates

No.	Biochemical tests	Result
1	Catalaseproduction	(+)
2	Citrate Utilization	(+)
3	Hemolysin production	(-)
4	H_2S production	(-)
5	Lactose fermentation	(-)
6	Motility	(-)
7	Oxidase production	(-)
8	Sucrose and glucosefermentation	(-)

(+)= Positive (-) = Negative

Most samples were isolated as *A. baumannii* using the VITEK 2 system with its identification card for Gram-negative strains (ID-GNB). This system has been employed in several previous research and has produced positive findings regarding biochemical test identification and confirmation.

A significant (P≤0.001) increase in IL-17A levels was observed in sera from *A. baumannii*-infected individuals. In this group, the mean level of this interleukin was 479.83 26.21 pg/ml, compared to 69.32 4.53% in controls (Figure 3 and Table 2). These findings were in line with those of Yan, Yang (11), who found elevated levels of IL-17 in the serum of patients.

A significantly (P≤0.01) increased level of TNF-α was observed in sera of patients infected with *A. baumannii* infection with a mean of (98.05±28.89) pg/ ml compared to controls (25.12±1.40) pg/ml (Table 3 and Figure 4).

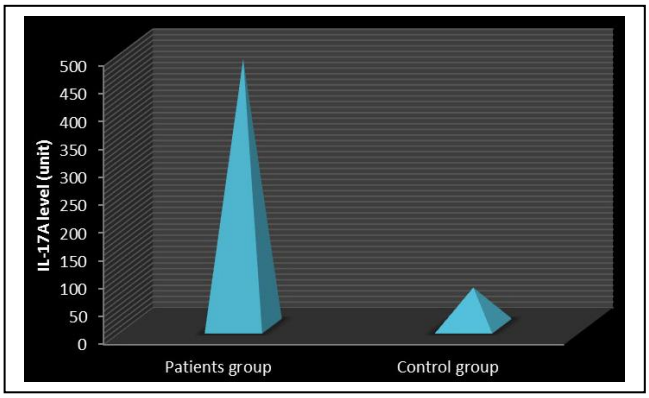

Figure 3. Serum level of Interleukin-17A in total *Acinetobacter baumannii* patients and controls

Table 2. Serum level of Interleukin-17A in *Acinetobacter baumannii* infected patients and controls

Groups	Number	Serum Level of IL-17A (pg/ml)			*P*≤
		Mean ± SE.	Minimum	Maximum	
Patients	100	479.83 ± 26.21	107.52	964.03	0.001
Controls	50	69.32 ± 4.53	20.00	215.54	

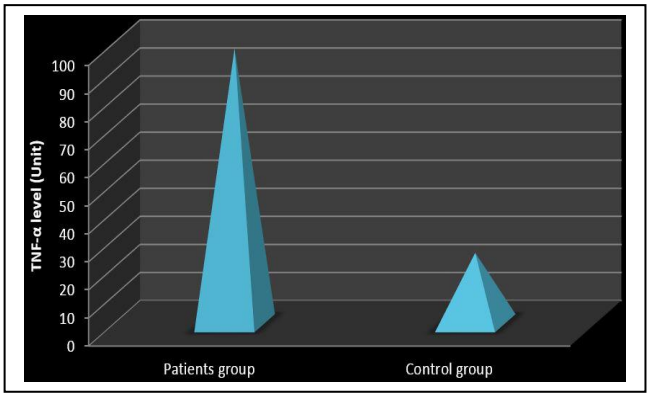

Figure 4. Serum level of Tumor Necrosis Factor-alpha in patients infected with *Acinetobacter baumannii* and in controls

Table 3. Serum level of Tumor Necrosis Factor-alpha in *Acinetobacter baumannii* infected patients and controls

Groups	Number	Serum Level of TNF-α (pg/ml)			*P*≤
		Mean ± SE.	Minimum	Maximum	
Patients	100	98.05 ± 28.89	34.0	116.88	0.001
Controls	50	25.12 ± 1.40	4.17	42.08	

4. Discussion

In this study, the accuracy of the VITEK 2 system was shown to be 99 percent to 100 percent for identifying *A. baumannii,* which agrees with Bagudo, Obande (18), and Ganda and Soehita (19), who showed through their research that the accuracy of this device in diagnosing a gram-negative card BioMérieux can identify *A. baumannii* with up to 99%t but 98.56% for Gram-positive bacteria. Instruments had a 98.56% identification accuracy for Gram-positive bacteria and a 100 % identification accuracy for Gram-negative bacteria (19). BioMérieux's gram-negative card can detect *A. baumannii* with up to 99% accuracy (18).

Emerging evidence shows that the IL-17 pathway is critical in the host's defense against various bacterial pathogens (11); this is because most Gram-negative bacteria secrete vesicles Outer-membrane vesicles (OMVs) of the outer membrane, which consists of lipopolysaccharide and outer membrane proteins. These bacteria use these vesicles to deliver toxins to the host cells and thus work to modify the balance of the host cells and stimulate the effects of cytopathic. As a result, epithelial and myeloid cells recognize pathogen-associated molecular patterns (PAMPs) in the vesicles and release innate immune signals that induce proinflammatory cytokines to be secreted (20). OMVs can also activate T and B cells, resulting in adaptive immunological responses (21). The immune response due to *A. baumannii* leads to the high production of proinflammatory cytokines and chemokines, and such immune response varies according to the nature of the individuals and the nature of the infection *of A. baumannii* (22).

Given its importance in promoting granulopoiesis and triggering the synthesis of cytokines, chemokines, and antimicrobial peptides such as GM-CSF, IL-8 (a neutrophil chemoattractant and homolog human chemokine to KC and MIP-2), and LL-37, IL-17 has emerged as a promising contender (23). Furthermore, other chemokines, such as CXCL1 and CXCL8 (IL-8), promote neutrophil aggregations and activation at sites of infection; additionally, together with IL-22, IL-17 increases the expression of antimicrobial peptides, such as β-defensins, S100A8, and lipocalin 2, that have broad-spectrum antimicrobial activity (24).

A significantly ($P \leq 0.01$) increased level of TNF-α was observed in sera of patients infected with *A. baumannii* infection, and similar results were recorded by Chen (22), who reported that infection with *A. baumannii* leads to an increase in the level of TNF-α in the patient's sera due to such deleterious infection. It represents a mechanism of resistance to multidrug-resistant *A baumannii*. This bacterium interacts with the host innate pattern-recognition receptors, induces a cascade of inflammatory cytokine and chemokine responses, and recruits innate immune effector cells to the site of infection for effective infection control.

TLR4 is a critical receptor for Gram-negative bacteria like *A. baumannii* to recognize their hosts, which interacts with the lipid A fraction of LPS, which is the main component of the cell wall of *A. baumannii*. In turn, the activated TLR4 signaling pathway triggers an innate immune response in macrophages and DCs, which includes NF-B activation and optimum production of interleukin (IL)-6, IL-12, and tumor necrosis factor (TNF)-, as well as *A. baumannii* death (25). It has been shown that mast cells promote bacterial clearance by releasing different mediators, including TNF-α, a chemoattractant for neutrophils (26). *A. baumannii* adheres to mast cells via CD32 expressed in mast cells and stimulates TNF-α, which leads to the development of inflammation and then the release of activated neutrophils (22). However, the interaction of *A. baumannii*LOS with mast cells remains unclear. Although, these studies demonstrated that the interaction of *A.baumannii* cell membrane components with mast cells affects the expression of proinflammatory cytokines and chemokines (27).

In conclusion:

1. The method of diagnosing *Acinetobacter baumannii* using the VITEK device is more accurate

than traditional tests, where the accuracy rate is approximately 99%.

2. Infection with *A. baumannii* is associated with high levels of (IL-17A and TNF-α) in the patients' serum, which reflects the nature of the emerging inflammatory immune response to *Acinetobacter baumannii*.

Authors' Contribution

Study concept and design: K. H. Y.
Acquisition of data: S. M. A.
Analysis and interpretation of data: M. M. M.
Drafting of the manuscript: K. H. Y.
Critical revision of the manuscript for important intellectual content: S. M. A.
Statistical analysis: S. M. A.
Administrative, technical, and material support: S. M. A.

Ethics

The study was approved by the Human Research Committee at Al-Rafidain University College, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Asif M, Alvi IA, Rehman SU. Insight into Acinetobacter baumannii: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infect Drug Re. 2018;11:1249.
2. Chapartegui-González I, Lázaro-Díez M, Bravo Z, Navas J, Icardo JM, Ramos-Vivas J. Acinetobacter baumannii maintains its virulence after long-time starvation. PloS One. 2018;13(8):0201961.
3. Tille P. Bailey & Scott's diagnostic microbiology-E-Book: Elsevier Health Sciences; 2015.
4. Almasaudi SB. Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. Saudi J Biol Sci. 2018;25(3):586-96.
5. Li L-H, Yang Y-S, Sun J-R, Huang T-W, Huang W-C, Chen F-J, et al. Clinical and molecular characterization of Acinetobacter seifertii in Taiwan. J Antimicrob Chemoth. 2021;76(2):312-21.
6. KhalaweTektook N. Study the virulence factors and patterns of antibiotics resistance in acinetobacter baumannii isolated from hospitalized patients in Baghdad City. Pak J Biotechnol. 2018;15(1):19-23.
7. Gallego L. Acinetobacter baumannii: Factors involved in its high adaptability to adverse environmental conditions. J Microbiol Exp. 2016;3(2):00085.
8. Uppalapati SR, Sett A, Pathania R. The outer membrane proteins OmpA, CarO, and OprD of Acinetobacter baumannii confer a two-pronged defense in facilitating its success as a potent human pathogen. Front Microbiol. 2020:2441.
9. Payam MA, Rasooli I, Owlia P, Talei D, Alipour SD. Correlation of virulence factors and cell sdhesion of clinical isolates of Acinetobacter baumannii. Arch Clin Infect Dis. 2018;13(3):62841.
10. Cillóniz C, Dominedò C, Torres A. Multidrug resistant gram-negative bacteria in community-acquired pneumonia. Annual Update in Intensive Care and Emergency Medicine 2019. 2019:459-75.
11. Yan Z, Yang J, Hu R, Hu X, Chen K. Acinetobacter baumannii infection and IL-17 mediated immunity. Mediators Inflamm. 2016;2016.
12. Morris FC, Dexter C, Kostoulias X, Uddin MI, Peleg AY. The mechanisms of disease caused by Acinetobacter baumannii. Front Microbiol. 2019;10:1601.
13. Sato Y, Unno Y, Miyazaki C, Ubagai T, Ono Y. Multidrug-resistant Acinetobacter baumannii resists reactive oxygen species and survives in macrophages. Sci Rep. 2019;9(1):1-12.
14. Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. Crit Rev Eukaryot Gene Expr. 2010;20(2).
15. Webster JD, Vucic D. The balance of TNF mediated pathways regulates inflammatory cell death signaling in healthy and diseased tissues. Front Cell Dev Biol. 2020;8:365.
16. Bullock NO, Aslanzadeh J. Biochemical profile-based microbial identification systems. Advanced techniques in diagnostic microbiology: Springer; 2013. p. 87-121.
17. Abramson JH. WINPEPI updated: computer programs for epidemiologists, and their teaching potential. Epidemiol Perspect Innov. 2011;8(1):1-9.

18. Bagudo AI, Obande GA, Harun A, Singh KKB. Advances in automated techniques to identify complex. Asian Biomed.14(5):177-86.
19. Ganda RP, Soehita S. Suitability Tests for Bacterial Identification and Antibiotic Sensitivity Tests using Microscan Walkaway on Vitek 2. Indian J Forensic Med Toxicol. 2021;15(2).
20. Eisenreich W, Rudel T, Heesemann J, Goebel W. How viral and intracellular bacterial pathogens reprogram the metabolism of host cells to allow their intracellular replication. Front Cell Infect Microbiol. 2019;9:42.
21. Tiku V, Tan M-W. Host immunity and cellular responses to bacterial outer membrane vesicles. Trends Immunol. 2021;42(11):1024-36.
22. Chen W. Host innate immune responses to Acinetobacter baumannii infection. Front Cell Infect Microbiol. 2020:486.
23. García-Patiño MG, García-Contreras R, Licona-Limón P. The immune response against Acinetobacter baumannii, an emerging pathogen in nosocomial infections. Front Immunol. 2017;8:441.
24. Cheng J, Yan J, Reyna Z, Slarve M, Lu P, Spellberg B, et al. Synergistic rifabutin and colistin reduce emergence of resistance when treating Acinetobacter baumannii. Antimicrob Agents Chemother. 2021;65(4):e02204-20.
25. Kim C, Ndegwa E. Influence of pH and temperature on growth characteristics of leading foodborne pathogens in a laboratory medium and select food beverages. 2018.
26. Metzemaekers M, Gouwy M, Proost P. Neutrophil chemoattractant receptors in health and disease: double-edged swords. Cell Mol Immunol. 2020;17(5):433-50.
27. Kikuchi-Ueda T, Ubagai T, Kamoshida G, Nakano R, Nakano A, Ono Y. Acinetobacter baumannii LOS Regulate the Expression of Inflammatory Cytokine Genes and Proteins in Human Mast Cells. Pathogens. 2021;10(3):290.