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Evaluation Of Some Haemato-Immunological Parameters In Patients With Pneumonia

Ali S. Kadhim*, Ahmed K. Atya, Alla NH. AL-Waheeb
Department of Biology, College of Science, University of Thi-Qar, Iraq.
*Corresponding author: alisaad20003@gmail.com

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Abstract
Background: Prior research has shown that pneumonia illness is associated with interleukin-17 (IL-17), CD 4+, and other immunological criteria. However, it is still unclear how IL-17 and CD 4+ relate to ventilator-acquired pneumonia (VAP), community-acquired pneumonia (CAP), and hospital-acquired pneumonia (HAP). Through a prospective study, this research attempts to investigate the relationships between serum levels of IL-17, CD 4+, ESR, CRP, and WBCs with the severity and prognosis in hospitalised RCU patients. Methods: All 57 hospitalised patients in RCU were recruited. Serum IL-17 and CD 4+ were detected by enzyme-linked immunosorbent assay (ELISA). ESR, CRP, and WBCs were recruited also. Results: It was noted that in all 57 patients admitted to respiratory care units in Baghdad Providence, who were included in our current research and diagnosed with pneumonia, the levels of IL-17, ESR, CRP, WBC, and neutrophil were high, while CD 4+ was low level. Conclusions: These tests can be used to diagnose patients with pneumonia because there is a close relationship between them.
Keywords: Klebsiella pneumoniae, Pneumonia, Immunocompromised patients, Respiratory Care Units.

Introduction

Pneumonia is defined as "new lung infiltrates plus clinical evidence that the infiltrate is of an infectious origin, which includes the new onset of fever, purulent sputum, leukocytosis, and decline in oxygenation" [1]. Klebsiella pneumoniae is a gram-negative, aerobic, facultatively anaerobic, non-motile bacilli and is a common cause of many human infections [2]. Hospital-acquired pneumonia (HAP), or nosocomial pneumonia, is a lower respiratory infection that was not incubating at the time of hospital admission and presents clinically two or more days after hospitalization. Ventilator-associated pneumonia (VAP) refers to nosocomial pneumonia that develops among patients connected to mechanical ventilators (MV). VAP is defined as pneumonia that presents more than 48 hours after endotracheal intubation (ETT). The term healthcare-associated pneumonia (HCAP) was defined as pneumonia in nonhospitalized patients who had significant experience with the healthcare system and were believed to be at an increased risk for infection with multidrug-resistant (MDR) organisms

because of such contact [3]. Patients who would have met the criteria for HCAP should not be empirically treated with antibiotics to cover MDR bacteria unless they have valid risk factors for acquiring MDR organisms [5,6]. Inhalation, aspiration, and hematogenous spread are the three main mechanisms bacteria reach the lungs. The primary route organisms enter the lower airways is aspiration of oropharyngeal secretions into the trachea. Primary inhalation pneumonia develops when these organisms bypass normal respiratory defense mechanisms or when the patient inhales aerobic gram-negative organisms that colonize the upper respiratory tract or respiratory support equipment. Aspiration pneumonia results from aspiration of colonized upper respiratory tract secretions. In healthy individuals, roughly 45% of the population is estimated to aspirate during sleep, with critically ill patients likely aspirating more frequently [7,8]. The oesophagus appears to be an important reservoir of gram-negative bacilli that can ascend and colonize the respiratory tract. A prospective observational study found that patients who used acid-suppressive medications were more likely to develop hospital-acquired pneumonia (HAP) than were patients who did not (5% vs 2%). The risk for pneumonia was significantly increased with proton pump inhibitors, but not with histamine 2-blocking agents [9]. The development of hospital-acquired pneumonia (HAP) represents an imbalance between normal host defenses and the ability of microorganisms to colonize and then invade the lower respiratory tract. Because aerobic gram-negative bacilli are the major pathogens associated with HAP, the pathophysiology of nosocomial pneumonia relates to the destructive effect on lung tissue. Aerobic gram-negative pathogens may be divided into two categories. The first category includes organisms that cause necrotizing pneumonia with rapid cavitation, micro abscess formation, blood vessel invasion, and haemorrhage [10]. Alternatively, other non-necrotizing gram-negative bacilli may be responsible for nosocomial pneumonia. A common bacteria involved in hospital-acquired pneumonia (HAP) is Klebsiella pneumoniae [11]. Endotracheal intubation (ETT) is an independent risk factor with multiple associated factors such as Micro-aspiration around the endotracheal tube, endotracheal intubation, Prolonged duration of ventilation, Abnormal swallowing function, and secretions pooled above the endotracheal tube. The endotracheal tube, which acts as a foreign body, the pooling of secretions and the resultant need for suctioning by sucker device can damage the tracheal mucosa. This can further facilitate tracheal colonization. Additionally, pathogenic bacteria can form a glycocalyx biofilm on the tube's surface that protects them from both antibiotics and host defenses. This can become a contributing factor to the recurrence of infection and treatment failure as well. Other risk factors include prior antibiotic use, cross-contamination with other patients in the same unit and malnutrition also (12). Nosocomial pneumonia accounted for 22% of all hospital infections in the United States (13). It is the second most common infection in hospitalized patients and the most common infection in the intensive care unit (ICU) responsible for one-fourth of all ICU infections [14]. The most common causes of infiltrates in ventilated patients with fever and/or leukocytosis include the following conditions: congestive heart failure, pulmonary embolus or infarction, acute respiratory distress syndrome (ARDS), pulmonary drug reactions, alveolar haemorrhage, bronchiolitis pneumonia (BP), chronic obstructive pulmonary disease (COPD), hypersensitivity pneumonitis,

interstitial lung disease, bronchogenic carcinomas, pulmonary embolism, lung fibrosis, chronic heart disease (CHD), endotracheal aspiration, diabetes mellites (DM) (15). Community-acquired pneumonia (CAP) always causes immune system disorders and local and systemic higher infection and inflammatory responses in patients [16]. Interleukin-17 (IL-17) is primarily secreted by T helper 17 cells (Th17), monocyte and eosinophilia [17,18]. Increasing data have proved that IL-17 can recruit neutrophils, activate T cells, and stimulate macrophages and epitheliums. Finally, IL-17 produces a range of pro-inflammatory cytokines and induces inflammatory reactions [19,20]. in vitro, research has confirmed that IL-17 overexpression or recombinant IL-17 administration elevates chemokines and evokes inflammatory reactions in the lung (21,22). Interleukin-17 is a cytokine belonging to the IL-17 family which contains six members IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F (23).

Material and Methods

Study design and patients: The current study included the investigation of Klebsiella pneumoniae bacteria in (252) samples collected from patients hospitalised in respiratory care units including 143/252 (56.74%) males and 109/252 (43.25%) females aged between (16-95 years) at Baghdad teaching hospital, Al-Yarmouk teaching hospital, Al-Karama teaching hospital, and Al-Imam Al-Kazemin teaching medical city for the period from February to August 2023 in Baghdad providence.

Samples Collection and Culturing Samples

collected via swabs were taken from the endotracheal tube (ETT), tracheostomy tube (TT), and sputum swabs (continuous positive airway pressure CPAP/face mask), then immediately transported by transporter medium to laboratories for culture by using the standard bacteriological technique on MacConkey agar medium for growth under controlled conditions. Petri-dishes plates were incubated upside down aerobically at 37 degrees Celsius for 24 hours.

Identification of Klebsiella pneumoniae isolates: Isolates were identified on the phenotypic characteristics, biochemical tests, Vitek-II system, and 16SrRNA primers.

Serum separation: Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃ before centrifugation for 20 minutes at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Study design and subjects: The study was about patients lying in respiratory care units in Baghdad Governorate who suffer from respiratory problems and have chronic infections in their respiratory system and knowledge of the general status of their immune system, as the antibiotics given to them by specialists' physicians were insufficient to rid them of their diseases and respiratory system infections. Bacterial cultures were performed as 87/252 (34.52%) from endotracheal tube swab, 73/252 (28.96%) from tracheostomy tube swab, and 92/252 (36.50%) from sputum swab; K. pneumoniae was identified 57/252 (22.61%) included 24/87 (27.58%), 12/73 (16.43%), 21/92 (22.82%); from the endotracheal tube (ETT) swab, tracheostomy tube (TT) swab and Sputum swab, respectively. patients who had Klebsiella pneumoniae isolates were targeted, and their number was 57 isolates. Blood samples were taken from them, and the following immunological tests were performed, it was noted that they were suffering from immunocompromised. The research confirmed that the presence of

bacteria is related to decreased immunity in patients. In a later study, plant extracts, namely garlic and red pepper, will be introduced into their nutrition, and with the help of nutritionists specializing in these critical respiratory units, to create a synergistic effect with antibiotics to help their immune system kill bacteria and improve their immune and health status.

Enzyme-linked immunosorbent assay (ELISA) and Assay procedure

1. Determine wells for diluted standard, blank and sample. Add 100 µl each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro-ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decant the liquid from each well, do not wash. Immediately add 100 µl of Biotinylated Detection Ab work in solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.
3. Decant the solution from each well and add 350 µl of wash buffer to each well. Soak for 1 minute and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
4. Add 100 µl of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.
5. Decant the solution from each well and repeat the wash process 5 times as in step 3.
6. Add 90 µl of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual colour change, but not more than 30 minutes. Preheat the Microplate Reader for about 15 min before OD measurement.
7. Add 50 µl of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Statistical Study:

Software Prism Graph Pad 8.0.1 was used for statistical analysis. A T-test was performed to find significance between the average values of patients and healthy controls. All the compared values were shown to be significant. P value was considered $P<0.05$.

Result

Out of Fifty-seven blood samples were collected from patients hospitalized in respiratory care units in Teaching Hospital: Al-Yarmouk Teaching Hospital, Al-Karama Teaching Hospital, and Al-Imam Al-Kazemin Teaching Medical City from February to August 2023 in Baghdad Providence. The age was (15 - 95) years old among VAP (ventilator-associated ventilator) patients. 24/57 (42.10%) had an

endotracheal tube, 12/57 (21.05%) had a tracheotomy tube, as they had been lying on a mechanical ventilator for more than two days because they were suffering from several chronic diseases. They have immune problems, as well as 21/57 (36.84%) of sputum, as they were lying down, but they were connected to a CPAP (continuous positive airway pressure) machine. The serum was taken to perform the following analyses: WBC, NEU%, EOS%, BASO%, LYM%, MONO%, ESR, CRP, IL-17, and CD 4+ levels. The demographic characteristics and clinical information in this study of our study show that all of the above patients suffer from pneumonia, as they suffered from high temperatures between (39-39.5), high white blood cell counts from (12.8-17.1), frequent inflammatory pulmonary secretions, and the presence of infections and fibrosis through chest x-rays that were diagnosed by specialist doctors according to CORBX-LAP pneumonia scale (Confusion-low, oxygenation-low, Respiratory rate-low, Blood pressure-low, X-ray of chest-multilobar bilateral, LDH-high, Albumin-low, Platelets-low). The results showed an increase in IL-17, WBC, Neutrophil level, ESR, and CRP level, while a decrease in both (Lymphocyte, CD 4+ level) (Figure 1). This proves that in patients suffering from pneumonia, levels of IL-17 are high and CD4+. These results were compared with twenty-five healthy people without disease and showed a statistically significant value $p>0.05$.

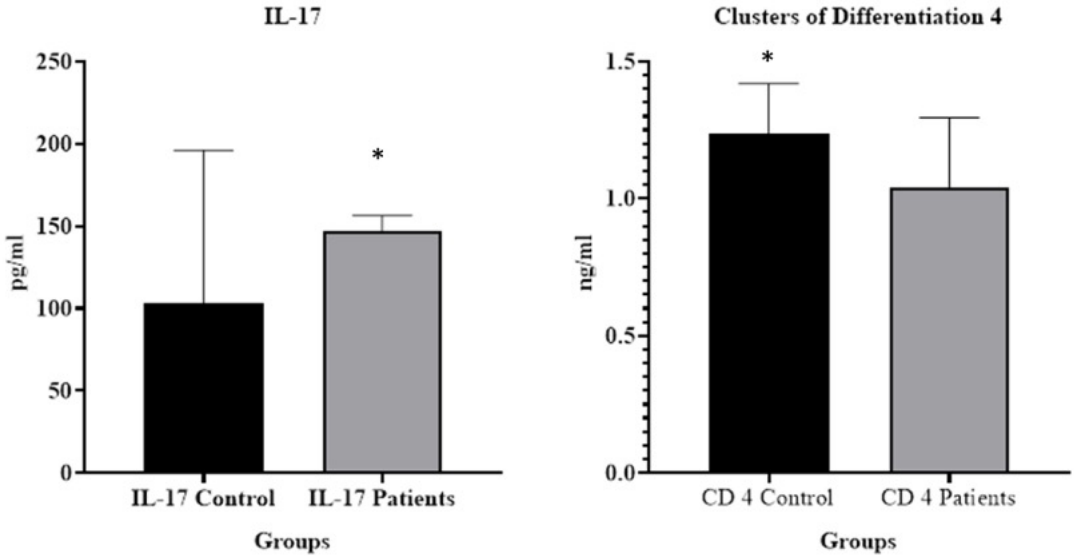

Figure 1. Interleukin-17 level and Cluster of differentiation 4+ level in patients versus control. Data expressed as mean±SD. *indicate significant difference at p value <0.001.

Discussion

This study strengthens the evidence of associations between serum IL-17 with the severity and prognosis among CAP patients. The research mainly found that: (1) Serum IL-17 on admission gradually rose in parallel with CAP severity scores; (2) IL-17 on admission was closely correlated with many clinicopathological features among CAP patients; (3) IL-17 on admission was positively correlated with CAP severity scores in CAP patients; (4) Serum higher IL-17 on admission elevated

the risk of ICU admission, mechanical ventilation, death and longer length of hospitalization among CAP patients during hospitalization. IL-17, one of the robust pro-inflammatory cytokines, which takes part in airway inflammation, is a central factor in the excessive activation of the body's defense system and the excessive inflammatory response (22). Past studies demonstrated that IL-17 plays key roles in the progression of several lung diseases, including lung fibrosis, emphysema, acute lung injury and pulmonary hypertension (23). Serum IL-17 is increased in patients with acute respiratory distress syndrome and participates in the occurrence of pneumonia (24). Nevertheless, the role of IL-17 in CAP has been ill-defined so far. Therefore, we tested the levels of serum IL-17 among CAP patients with different severity scores. In the current research, we discovered that the level of IL-17 was gradually increased in parallel with the CAP severity scores among CAP patients. In addition, logistic and linear regression analysis indicated that IL-17 was positively associated with CAP severity scores among CAP patients. These findings indicated that IL-17 is positively correlated with the severity of CAP patients.

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