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Detection of Nosocomial Respiratory Infections among Hospitalized Patients in Intensive Care Unit: a Survey in Northern Iran

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ABSTRACT

Introduction: Nosocomial respiratory infections are a significant cause of mortality in hospitalized patients in Middle East countries. This study assesses the prevalence of nosocomial respiratory infection and associated factors as a tool for early diagnosis among intensive care unit (ICU) patients at risk for mortality. Methods: From January to November 2021, 357 patients with more than 72 h hospitalization in ICU were monitored. Respiratory samples were examined for the presence of microbial isolates using clinical microbiology procedures based upon microscopic morphology, cultural and PCR methods. Demographic data were collected, including age, gender, date of hospitalization, underlying diseases, date of death, and laboratory data. Results: Out of fiftythree positive cultures, 18 samples (34%) were positive for fungal isolates, and the rest were positive for bacterial isolates. The most common bacterial and fungal isolates were Streptococcus pyogenes (17.9%) and Candida albicans (22.5%). Of the infected patients, 67.9% were male, 39.62 % had kidney diseases, and 15.09% died due to nosocomial infections. The results also showed that the tumor necrosis factor a and complement component 3 levels were significantly associated with the incidence of respiratory fungal or bacterial infections (P < 0.05). Conclusions: The rate of respiratory nosocomial infection in ICU patients was high. It is essential to implement control measures such as managing the length of hospital stay and examining the patient's immune factors to reduce the risk of these infections in ICU patients. Also, ICU patients should be prescribed appropriate antibiotics to prevent respiratory infections.

INTRODUCTION

Nosocomial infections (NI), especially respiratory diseases caused by fungi, bacteria, and viruses in hospitalized patients, complicate clinical manifestations, affect treatment outcomes, and may lead to mortality. Patients over 70 years of age, infants, children, people underlying diseases, patients with severe thrombocytopenia, patients with respiratory diseases, and those with inadequate access to health care services are at higher risk of mortality than other patients [1]. Patients with severe infections, especially those in the intensive care unit (ICU) on ventilators, are at greater risk of developing infections. Such infections occur in approximately 10-30% of critically-ill hospitalized patients, especially those admitted to ICU. In these patients, bacterial and fungal infections are ten times higher than viral infections [2-4]. The most common nosocomial infection is pneumonia, especially ventilatorassociated pneumonia caused by nosocomial bacterial and fungal pathogens. In patients with prolonged ICU stay or intubated patients, these infections are usually caused by

multidrug-resistant Gram-negative bacilli, such as Acinetobacter, and members of the Enterobacteriaceae family. Studies have shown that over 65% of confirmed influenza cases are exacerbated by superinfection with bacterial agents. According to the World Health Organization (WHO), the highest rates of nosocomial infections are reported in the Eastern Mediterranean and Southeast Asia, while the lowest rates are in the Western Pacific and Europe [5-7]. Nosocomial respiratory infections usually involve the lower respiratory tract, and Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, and Staphylococcus aureus are the most commonly isolated pathogens [8, 9].

weakened immune system, broad-spectrum antibiotics, and underlying diseases can lead to pulmonary infection by certain microorganisms, including bacteria and fungi. Over half of the specimens taken from ventilators of ICU patients hospitalized for more than a week were culture-positive for bacteria and fungi such as Aspergillus fumigatus [10, 11]. Severe fungal infections

Saeedi et al.

in people with viral illnesses, such as influenza-associated invasive pulmonary aspergillosis, are commonly reported in critically-ill ICU patients [12]. Hospitalized patients, especially in intensive care units (ICUs), are susceptible to nosocomial infections, leading to high mortality rates and hospitalization costs. Hence, this study assesses the prevalence of nosocomial respiratory infection and associated factors as a tool for early diagnosis among ICU patients at risk for mortality.

MATERIAL AND METHODS

Study population. In this descriptive cross-sectional study, the population included 357 patients (age range: 31-87 years, the average hospital stay: 30.25 days) with underlying conditions admitted to ICUs of nine hospitals in Gorgan, Sari, and QaemShahr in Northern Iran during January to November 2021. The sample size was determined based on the desired accuracy with a confidence level of 95% source (P = 50%).

In the current research, out of 357 patients, 211 (59.10%) were women, and 146 (40.89%) were men. Inclusion criteria were first referral to these medical centers and hospitalization in the ICU for more than 72h with respiratory symptoms including chronic mucus, cough, shortness of breath, noisily breathing, and breath sounds and confirmed radiological examination. Length of stay <48 hours (n =46) and pulmonary shadow due to COVID-19 were considered exclusion Demographic data, characteristics, and clinical features were collected, including age, gender, date of hospitalization, underlying diseases, and date discharge/death. The study was carried out following the Declaration of Helsinki and approved by the Ethics Committee of Islamic Azad University, Chalus Branch, Mazandaran, Iran (ethical code: IR.IAU.CHALUS.REC.1400.069).

Isolation of bacterial strains. Throat swabs, sputum, and fiberoptic bronchoscopy samples were collected three times a week after observing chest x-ray and computed tomography images from 212 patients with respiratory symptoms. Bronchoalveolar lavage (BAL) fluid was sampled by injection of 100 ml sterile normal saline using the bronchoscope and recollection of the fluid by a specialist. The bronchoscope was disinfected with deconex® solution for 15 min, followed by contamination examination before, during, and after the sampling procedure. The sputum and BAL samples were transferred to a Microbiology Laboratory under cold conditions. Then, they were homogenized and centrifuged at 3000 rpm for 5 min, and the precipitate was cultured on blood agar with 5% defibrinated sheep blood using standard procedures. After a 24-h incubation at 37 °C and 5% CO₂, colonies were identified using microbiological and biochemical tests [13] and PCR detection kits with specific primers (Iranian Gene Fanavar Institute, Iran,

Table 1). The results were read based on the institute's protocol, and the microorganisms were confirmed.

Isolation of viral strains. For diagnosis of viral strains, a respiratory viral panel including influenza, parainfluenza, adenovirus, respiratory syncytial virus, and respiratory syncytial virus (RSV) rapid test of BAL specimen was evaluated.

Isolation of fungal strains. The samples were stained with 20% potassium hydroxide mount and calcofluor white dye and then directly examined under a fluorescence microscope to identify fungal agents. The samples were cultured in Sabouraud dextrose agar containing chloramphenicol and brain heart infusion agar and then incubated at 27 °C for at least seven days. During the incubation period, the cultures were examined daily for fungal growth. The culture was identified as positive if fungal growth had occurred at the inoculation point. Finally, the type of fungi grown was determined based on the macroscopic and microscopic characteristics. The grown yeast colonies were more accurately identified by subculture on corn meal agar with Tween 80 and CHROM agar at 35 °C for 48 h, as well as germ tube test using human serum [14].

Estimation of laboratory data. Blood was collected from patients with positive culture (case group=53) and respiratory symptoms but without positive culture (control group=53). Hematologic indices, immunoglobulin G (IgG), immunoglobulin A (IgA), complement C3(C3), C-reactive protein (CRP), aspartate transaminase (AST), lactate dehydrogenase (LDH), interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), white blood cell count (WBC) and blood pH were evaluated.

Statistical analysis. Collected data were presented using descriptive statistics, including mean, standard deviation, and frequency. Analysis was carried out with SPSS Statistics 23.0 using the chi-square test, Fisher's exact test, and one-way analysis of variance (ANOVA) at a significance of 0.05.

RESULTS

Patients characteristics. Of 211 respiratory samples, 96 (45.49%) were related to throat swabs, and 115 (54.50%) were related to sputum/bronchial secretions. Of these samples, 53 (25.11%) were positive for respiratory fungal and bacterial infections, and the rest of the throat swab cultures were negative. The rate of NI in the ICU was 25.11%. Among the clinical symptoms reported in the patients, cough (72.98%) and noisily breathing (46.44%) were common signs. Among the confirmed positive samples, 36 cases (67.9%) were isolated from men. The majority of patients (71.6%) were aged above 50 years.

Table 1. The sequences of the primers used for the detection of microorganisms in this study

Microorganism	Sequence (5`>3`)	Product size (bp)	
S. pneumoniae	F-ATTTCTGTAACAGCTACCAACGA R-GAATTCCCTGTCTTTTCAAAGTC	348	
S. pyogenes	F- AAAGACCGCCTTAACCACCT R- TGCCAAGGTAAACTTCTAAAGCA	407	
H. influenzae	F-GATCCGAATTCCTTAAAAGGAAT R-ATTAAATATTGGATCCAGTAAAAACTGAAC	1100	
M. pneumoniae	F-CAAGCCAAACACGAGCTCCGGCC R-CAGTGTCAGCTGTTTGTCCTTCCCC	543	
S. aureus	F-AAAAACACTTGTCGATATGG R-GTTTCAATACATCAACTGC	950	
C. albicans	F-GCCAGCGAGTATTAACCTTG R-ATTTCAGCCAATGAGGATGG	620	
A. flavus	F-GTAGGGTTCCTAGCGAGCC R-GGAAAAAGATTGATTTGCGTTC	497	

Table 2. Demographic characteristics of ICU patients with bacterial/fungal infection

		Fungal In	fection	Bacterial 1	nfection	_	
Variables		Number	%	Number	%	<i>P</i> -value	
Gender –	Male	12	22.60	24	45.30	0.36	
	Female	6	11.30	11	20.80		
Age —	31-49	5	9.40	10	19	- 0.2	
	50-61	6	11.30	12	22.60		
	62-87	7	13.20	13	24.50	-	
Specimen -	Sputum	4	7.60	23	43.40	- 0.01*	
	Pulmonary alveoli	14	26.40	12	22.60		

^{*}Significant difference between the study groups based on the chi-square test

Table 3. The frequency distribution of underlying diseases in ICU patients with a respiratory infection

Disease	Number (%)	Number of positive cases (%)	Number of deceased patients (%)	
Diabetes	103 (28.85)	9 (16.98)	1 (1.88)	
Gastrointestinal diseases	29 (8.12)	3 (5.66)	0 (0)	
Kidney diseases	82 (22.96)	21 (39.62)	3 (5.66)	
Stroke	13 (3.64)	2 (3.77)	0 (0)	
Cancer	27 (7.56)	8 (15.09)	4 (7.54)	
Postoperative care	94 (26.33)	10 (18.86)	0 (0)	
Drug or chemical poisoning	9 (2.52)	0 (0)	0 (0)	

Microbial cultures from patients with nosocomial respiratory infections. Eighteen cases (34%) and 35 (66%) had fungal and bacterial infections, respectively. The majority (73.6%) of samples positive for fungal or bacterial infections were related to sputum specimens (Table 2).

Respiratory infections were more common in patients with kidney disease; those under postoperative care and 8 cases (15.09%) died due to nosocomial infections (Table 3), but there was no significant difference between underlying diseases and the frequency of deceased patients (P=0.23).

The most commonly isolated bacteria and fungi were *S. pyogenes* (17, 9%) and *C. albicans* (22.5%). A total of 53 organisms were isolated from patients with these respiratory infections; Gram-positive bacteria frequency was two times more than Gram-negative bacteria. In addition, no bacterial/fungal coinfection and no viral strains were detected (Table 4).

Hematologic Indices. Examination of clinical and laboratory characteristics of patients showed that patients with respiratory infection showed significantly high levels of C3 (41, 77.35%) and TNF α (38, 71.69%) (P< 0.05, Table 5).

Table 4. Results of the microbial culture of samples taken from the ICU patients with respiratory nosocomial infection

Microorganism	Samp	ples	7 (2()
	Sputum	BAL*	Frequency (%)
S. pneumoniae	4	3	7 (13%)
S. pyogenes	7	2	9 (17%)
H. influenzae	3	1	4 (7.5%)
M. pneumoniae	5	2	7 (13%)
S. aureus	5	3	8 (15%)
C. albicans	11	1	12 (22.5%)
A.flavus	3	2	5 (9.5%)
Trichoderma	1	0	1 (2.5%)
Overall	39	14	53 (100%)

^{*} Bronchoalveolar lavage

Table 5. Clinical and laboratory characteristics of patients with respiratory infection and control group

Variables	Unit	Respiratory Infection	No Respiratory Infection	P-value
Hospitalization duration	Days	$37\pm11/00$	33±10/07	0/07
Gender	-	3.01 ± 0.04	3.00 ± 0.03	0.25
Age	Years	64.01±9.07	55.06±8.01	0.07
Immunoglobulin G (IgG)	mg/ml	14/02±5.05	11/07±3.09	0.37
Immunoglobulin A (IgA)	mg/ml	$3/02\pm1.05$	$3/00\pm0.75$	0.31
Complement component 3	mg/ml	$1/15 \pm 1/03$	$2/66 \pm 2/19$	0.02*
CRP	mg/l	42.09±32.02	38.06±29.02	0.62
Aspartate transaminase (AST)	U/L	50.07±42.02	41.00±28.01	0.37
Lactate dehydrogenase (LDH)	U/L	312.11±239.02	268.09±116.22	0.41
Interleukin 6 (IL ₆)	μg/L	41.02±79.00	28.77±53.66	0.38
$TNF\alpha$	μg/L	0.19±0.17	0.50 ± 2.38	0.03*
White blood cell	$\times 10^9/L$	9.98±1.25	10.01±1.00	0.47
Blood pH	-	7.00±0.02	7.05±0.02	0.33

^{*} Statistically significant difference

DISCUSSION

The increased global population has brought some health priorities into greater focus, including the need for more hospitals and the variety of medical services, organ transplant facilitation, increasing the life expectancy of immunocompromised patients, and controlling nosocomial infections. Every year, many people worldwide become infected with nosocomial infections, resulting in many deaths [15].

Currently, the nosocomial infection surveillance program in Iran has focused on five main groups, including urinary tract infection (UTI), respiratory infection (PNEU), bloodstream infection (BSI), surgical site infection (SSI), and device-associated infections, particularly ventilator-associated infection [16]. Critically ill ICU patients are more susceptible to infections due to their exceptional circumstances. Fungal colonization in the respiratory tract can be an important source of infection. Respiratory fungal infections are less common but clinically more severe than bacterial and viral infections. These infections mainly immunocompromised individuals, and their virulence can vary from asymptomatic to fatal [17].

In the present study, the rate of nosocomial respiratory infection was 25.11%, which is different from the study in Mexico (32.2%) [18] and the previous study in Iran (41.6%) [19]. According to a meta-analysis in 2021 and the INISS independent data sources, the estimated incidence of NI in Iran was about 0.32%-9.1% [20]. The current research focused only on respiratory infections, not other infections such as surgical sites and urinary tract infections. In our study, most confirmed nosocomial respiratory infections were bacterial, so fungal infections showed a rate of 34% with a priority of yeast infection (22.5%), and no viral isolate was detected. Inconsistent with our study, a previous study in Iran (2011) reported Candida (64.7%), Aspergillus (19.3%), and Penicillium (7.9%) as the most common fungal isolates [21]. In another study in Iran (2019), 9 of 64 patients (14%) had pulmonary candidiasis. In addition, most of the positive samples (77.8%) were taken from women [22]. In this study, we found no significant relationship between the patient's gender and the incidence of respiratory infections. In another study, the rate of systemic fungal infection was 71.1% in hospitalized patients, which is higher than the rate observed in our study [23]. In the current research, S. pyogenes (17%) and S. aureus (15%) were the predominant bacterial isolates. In Iran, in one study in 2019, *K. pneumoniae* (26%) and *Acinetobacter* sp. (18%) [24], and in another in 2008, *S. aureus* (38.1%) and *P. aeruginosa* (31%) were identified as the most frequently isolated bacterial pathogens from ICU patients [25]. In Serbia (2020), the incidence of NI in ICU was high compared to other European countries. The most common agents identified were *C. difficile*, *Klebsiella* spp., *A. baumanii*, and *Enterococcus* spp. [26].

It is well-demonstrated that microbial infection. especially bacterial and fungal coinfection, can complicate treatment and increase mortality risk. In the present study, we observed no bacterial/fungal coinfection in ICU patients. A study on patients with coronavirus disease 2019 (COVID-19) in the UK reported no case of fungal/bacterial coinfection but confirmed the small impact of secondary bacterial infections on the severity of the disease [27]. In a study in Wuhan, China, in 2020, 3.2% of patients with pneumonia had a concomitant fungal infection [28]. In a study in Egypt, the prevalence of bacterial/fungal coinfection in patients with COVID-19 was reported to be 10.7% [29]. For these reasons, it is necessary to evaluate the signs and biochemical parameters of patients' serum for better treatment and to avoid the overuse of antibiotics. In terms of laboratory tests, findings indicated that the level of C3 and TNFa were significantly associated with the incidence of respiratory infections compared to patients without respiratory infections. Generally, at admission and early stages of diseases, the level of blood and serum factors is usually normal. However, disease progression causes an increase in inflammatory responses and activates cytokines and TNFα, particularly in systemic diseases, decreasing the number of lymphocytes [28]. Based on the results of the present study, age and gender had no association with the incidence of nosocomial respiratory infections. Moreover, other factors such as IgA, IgG, and IL-6 are associated with the severity of the primary disease and do not necessarily increase infections. Aspartate transaminase is usually elevated in liver disease patients receiving antiviral and non-steroidal anti-inflammatory drugs. In general, leukocytosis, neutrophilic leukocytosis, lymphopenia, and increased level of C-reactive protein indicate involvement of the immune system in these patients, and indeed, the longer the hospitalization duration, the greater the risk of nosocomial infections. The inconsistency in the results of previous studies might be related to factors such as length of hospital stay, type of ward studied, study location, time of the study, ventilation of the study place, age of subjects, and presence of underlying disease [30]. Indeed, patients with chronic diseases, cancers, and COVID-19 are more susceptible to infectious diseases due to reduced neutrophils, levels of C4, percentage of lymphocytes, and platelet count [31]. The limitations in the present study were the lack of standard bronchoscopy in evaluating IA in ICU patients due to the patients' critical conditions and the coincidence of the study with COVID-19.

Regarding the high rates of NIs in our ICU, the present study showed that ICU patients are vulnerable to fungal and bacterial colonization in the lung. Given the prevalence of this infection in patients, it is essential to implement control measures such as managing the length of hospital stay and examining the patient's immune factors to reduce the risk of fungal and bacterial infections in ICU patients. Patients should be prescribed authorized antibiotics to prevent respiratory infection and interrupt transmission routes.

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Saeedi et al.

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