http://iahsj.kaums.ac.ir/
International Archives of Health Sciences. 2023; 10(4): 164-170
https://doi.org/10.48307/IAHSJ.2023.183013

Research Article Open Access

Mycoplasma pneumonia and *Chlamydia pneumonia* infection in patients admitted to Beheshti Hospital in Kashan with community-acquired pneumonia

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Received: 12 December 2022 **Revised:** 10 January 2023 **Accepted:** 12 October 2023 **e-Published:** 30 November 2023

Abstract

Objectives: The objective of this study was to investigate the prevalence of *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* infections in patients with community-acquired pneumonia (CAP) admitted to Beheshti hospital in Kashan.

Methods: A descriptive cross-sectional study was conducted on 160 CAP patients admitted to Beheshti hospital in Kashan. Serological tests were performed using the ELISA method to evaluate IgG and IgM antibodies for *Mycoplasma* and *Chlamydia pneumoniae*. A questionnaire was also completed, which included demographic data, hospitalization time, and clinical and paraclinical findings. The data were analyzed using SPSS software (version 20).

Results: The study found that 19 (11.9%) cases tested positive for *M. pneumoniae* IgM antibodies, while 132 (82.5%) cases tested positive for *M. pneumoniae* IgG antibodies. For *C. pneumoniae*, 16 (10%) cases tested positive for IgM antibodies, and 151 (94.4%) cases tested positive for IgG antibodies. There was no significant association between *M. pneumoniae* and *Chlamydia pneumoniae* infections with sex, underlying illness, pneumonia severity, ICU admission, hospital death, hospitalization time, CRP, hematocrit, and platelet count. However, a significant relationship was observed between *M. pneumoniae* and chief complaint ($p<0.001$), as well as age ($p = 0.122$). Additionally, a significant relationship was found between *C. pneumoniae* and white blood cell count ($p = 0.001$), as well as changes in chest radiography ($p = 0.001$).

Conclusion: Given the significant incidence of atypical infections in CAP patients and the difficulties in laboratory detection, effective antibiotics targeting *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* are strongly suggested in the empirical therapy of CAP.

Keywords: Community-acquired pneumonia, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*.

Introduction

Pneumonia is a lung infection caused by viral, bacterial, or other infectious organisms. It is a significant health issue worldwide, with high mortality and morbidity rates across all age groups.[1] Pneumonia is classified based on the clinical background in which a patient develops symptoms of infection. There are four categories of pneumonia: community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), ventilator-associated pneumonia (VAP) and health care-associated pneumonia (HCAP). CAP is a common infectious disease caused by a variety of pathogens, including viruses, bacterial agents such as Streptococcus pneumoniae and *Haemophilus influenzae*, and atypical pathogens such as Chlamydia pneumoniae, *Mycoplasma pneumoniae*, and Legionella pneumophila.[2] Atypical pathogens play a significant role in community-acquired pneumonia and are considered one of the main causes of CAP in many countries.[3,4]

Mycoplasmas, being the tiniest prokaryotic free-living microorganisms, have the potential to cause various clinical manifestations.[5] Among them, *M. pneumoniae* occurs worldwide and is responsible for 20–40% of all CAP cases.[6,7] It is also the most common cause of CAP in the age group of 5 to 20 years. Several laboratory tests are available for diagnosing *M. pneumoniae* infection,

including cold agglutinins, culture, serology, and PCR-based detection of specific nucleic acids. The most reliable diagnostic method is the one that detects Mycoplasma-specific nucleic acids.[8]

C. pneumoniae has the potential to induce numerous respiratory infections that may either be asymptomatic or present with mild symptoms. This bacterial pneumonia is difficult to distinguish clinically from other types of pneumonia. Serologic testing, which detects the presence of IgM and IgG antibodies against the bacteria, has traditionally been used as the standard diagnostic approach to diagnose *C. pneumoniae* infection.[9,10] The occurrence of *C. pneumoniae* and *M. pneumoniae* varies significantly across different studies conducted on patients with CAP. For instance, a study conducted by Bozzoni et al., on 177 adult hospitalized patients with CAP revealed that 6.8% tested positive for *M. pneumoniae*.[11] A serologic test undertaken by Banzal in India, on the other hand, revealed that *M. pneumoniae* infections were found in 15% of CAP cases in adults.[12] Furthermore, another study found that *M. pneumoniae* was responsible for 24% of pneumonia cases in hospitalized children.[13] Chaudhry et al. reported a high prevalence of *M. pneumoniae* infection among children with CAP, with a serological positivity rate of 27.4%.[14] Meloni et al., utilized molecular and serology methods and reported a 17.5% rate of *M. pneumoniae* infection in adult patients,[15] while Chambers et al. reported a rate of 16%.[16] In Trinidad, the seroprevalence of *M. pneumoniae* infections in patients with pneumonia was found to be 66.7%.[17] In a study of 70 hospitalized CAP patients in Jordan, Al-Hajaya discovered that the overall seroprevalence of C. pneumoniae IgG was greater in CAP patients than in controls, with a detection rate of 44.3% versus 30.2%. Additionally, Chlamydial IgM antibodies were detectable in 27.1% of CAP patients, whereas only 3.2% of the controls showed the presence of these antibodies.[18] Using PCR techniques, the frequency of CAP caused by *C. pneumoniae* in Germany was 0.9% (5 out of 546 cases).[19] Similarly, in Brazil, out of 66 patients admitted to the hospital Estadual Sumaré with CAP, 8.2% tested positive for *C. pneumoniae*.[20] Furthermore, *C. pneumoniae* was identified in 6% (8 out of 133) of adult CAP cases admitted to three hospitals in Kuwait.[21]

Despite being well-known pulmonary pathogens worldwide, the prevalence of *M. pneumoniae* and *C. pneumoniae* in our hospital remains scarce due to the lack of reliable and rapid diagnostic methods. Given that these atypical pathogens possess intrinsic resistance to beta-lactams and can lead to severe complications in certain patients, it becomes crucial to ascertain their frequency within our hospital.

Objectives

The objective of this study was to utilize ELISA methods in order to determine the frequency of *C. pneumoniae* and *M. pneumoniae* infections in patients with CAP.

Methods

This study was conducted at Beheshti hospital in Kashan, Iran, and included 160 cases ranging in age from 10 to 95 years. The study took place from March 2017 to December 2018. The sample size was determined based on the census. An infectious diseases expert identified CAP in patients who satisfied the inclusion criteria based on clinical symptoms and chest X-ray findings. Patients with certain conditions were excluded from the study. After conducting history-taking and physical examinations, a questionnaire was completed, which included demographic information, clinical findings, underlying diseases, hospital stay duration, ICU admission, severity score (CURB 65), and results of CBC, CRP, and CXR.

Following the explanation and consent form, 5 cc of blood was collected from patients to conduct serology tests for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. The serum samples were then sent to the hospital laboratory for analysis. The enzyme-linked immunosorbent assay was used to measure IgM and IgG antibodies against *M. pneumoniae* and *C. pneumoniae*. The Vircell kit from Parque Technologic de la Salud, Avecina, Spain, was used to study specific IgM and IgG antibodies to *M. pneumoniae* in serum samples of patients. The titers above 0.9 were considered positive, and those below 0.5 were negative. The sensitivity and specificity for IgG were 98% and 97%, respectively, while for IgM, they were 97% and 92%.

Similarly, specific IgM and IgG antibodies to C. pneumoniae were tested in patient blood samples using the EUROIMMUN kit from Medizinische Labordiagnostika AG, UK. The titer above 1.1 was considered positive, and the titer below 0.8 was negative. The sensitivity and specificity for IgG were 97.9% and 95.1%, respectively, while for IgM, they were 100%. The detection of IgM was considered an acute infection, and its relationship with age, sex, clinical symptoms, underlying disease, length of hospital stay, ICU admission, severity score (CURB65), WBC, CRP, and final outcome was determined.

The data acquired from this study underwent analysis using SPSS (version 20.0, SPSS Inc, Chicago, IL, USA).

Mean and standard deviation were calculated for quantitative variables, while frequency percentage (absolute and relative) was determined for qualitative variables. Moreover, frequency tables were generated. Data analysis involved the utilization of the chi-square test and Fisher's exact test, with a significance level of $P < 0.05$.

Throughout this research, the utmost importance was given to honesty and trustworthiness. Valid and innovative methods were employed to ensure accurate results. The budget and tools were used accurately, and the analysis of the results was conducted with honesty. Additionally, the confidentiality of participants' information was strictly maintained, and their consent was obtained before their inclusion in the study and collection of blood samples. These measures were taken to ensure that the obtained results could be utilized for future research purposes. The ethical approval code for this study is IR.KAUMS.REC.2016.13. The study was conducted in accordance with the Declaration of Helsinki.

Results

In this study, a total of 160 patients with CAP were included. Among them, 98 patients (61.25%) were female, resulting in a sex ratio of 1.6 females to 1 male. The majority of cases (51.87%) were under the age of 60. The average age of the patients was 57.20±21.69 years, ranging from a minimum of 10 years to a maximum of 95 years.

The mean length of hospital stay for CAP patients was 7.38 ± 2.81 days, with the majority (61.3%) staying in the hospital for less than 7 days. The most commonly reported complaint and clinical symptom was cough, observed in 68 patients (42.5%). The majority of patients (38.8%) got a score of 1 when the severity of pneumonia was assessed using the CURB 65 scoring system, followed by scores of 2 (30%). A total of 11 patients (6.9%) required admission to the intensive care unit (ICU). The hospital mortality rate was 5 (3.1%). The most frequent finding on chest x-rays was one-sided involvement, accounting for 51.9% of cases [Table 1].

Out of the total cases, leukocytosis was observed in 70 cases (43.75%), thrombocytopenia in 23 cases (14.38%), anemia in 84 cases (52.5%), and increased CRP in 144 cases (90%). The study did not find any significant correlation between acute *M. pneumoniae* infection and factors such as sex, underlying disease, pneumonia severity, ICU admission, hospital mortality, chest radiography findings, length of hospital stay, CRP levels, hematocrit, platelet count, and white blood cell count. However, a significant relationship was observed between clinical signs and age. In the case of *C. pneumoniae*, a significant association was only found in the number of white blood cells and changes in chest radiography [Table 2,3].

Table 1. Characteristics of hospitalized patients with Community-Acquired Pneumonia

		Frequency	Percent
Sex	Male	62	38.8
	Female	98	61.2
Age	<60	83	51.9
	>60	77	48.1
Underlying disease	yes	80	50
	no	80	50
Hospital stay duration	<7 day	98	61.3
	>7 day	62	38.8
Clinical symptoms	Cough	22	13.8
	Sputum	46	28.7
	Dyspnea	34	21.3
	Chest pain	13	8.1
	Fever	45	28.1
Severity score (CURB 65)	0	40	25
	1	62	38.8
	2	48	30
	3	7	4.4
	4	3	1.9
ICU admission	Yes	11	6.9
	No	149	93.1
Outcome	Death	5	3.1
	Survived	155	96.9
CXR	One sided	83	51.9
	Two sided	42	26.3
	Multi lobar	26	16.3
	Cavity	9	5.6
	Total	**160**	**100**

Discussion

In this research, a total of 160 patients diagnosed with acute community-acquired pneumonia were examined. Among them, 11.9% of the patients were found to have an acute *M. pneumoniae* infection based on serology and positive IgM. Additionally, 17.5% of the patients tested positive for the *M. pneumoniae* IgG antibody, indicating a previous infection. Chambers et al. discovered a 16% frequency of *M. pneumoniae* infection in their investigation. They further described that this infection was more commonly of mild to moderate severity (95%) and occurred in a younger age group, often accompanied by myalgia and headache. When we compared our findings to those of previous studies done in other Iranian cities, we discovered that the prevalence of *M. pneumoniae* infection in our research was lower than that reported in

Tehran (43%), but greater than that reported in Rasht (1%), Tabriz (5%), and Ardabil (6.3%).[22-26] Furthermore, when comparing the frequency of acute mycoplasma infection in our study with other countries, we found that it was lower than in India (15%), Trinidad (66.7%), Korea (40%), Japan (24.2%), Iraq (19.4%), and Turkey (16.2%).[12,17,27-30]

Table 2. Distribution of *M. pneumonia* IgM in hospitalized patients with community-acquired pneumonia

Characteristics		Positive	Negative	Total	P Value
		N (%)	N (%)	N (%)	
Sex	Male	6(3.75)	56 (35)	62(38.75)	0.494
	Female	13(8.12)	85953.12)	98(61.25)	
Age (year)	≤60	15(9.37)	68(42. 5)	83(51.87)	0.012
	>60	4(2.5)	73(45.62)	77(48.12)	
Underlying disease	Yes	8(5)	72(45)	80(50)	0.463
	No	11(6.87)	69(43.12)	80(50)	
ICU admission	Yes	0(0)	11(6.87)	11(6.87)	0.207
	No	19(11.87)	130(81.25)	149(93.12)	
Hospital stay	≤7	12(7.5)	86(53.75)	98(61.25)	0.856
	>7	7(4.37)	55(34.37)	62(38.75)	
Clinical symptom	Cough	0(0)	22(13.75)	22(13.75)	0.001
	Sputum	18(11.25)	28(17. 5)	46(28.75)	
	Dyspnea	1(0.62)	33(20.62)	34(21.25)	
	Chest pain	0(0)	13(8.12)	13(8.12)	
	Fever	0(0)	45(28.12)	45(28.12)	
Severity CURB65	0	8(5)	32(20)	40(25)	0.347
	1	7(4.37)	55(34.37)	62(38.75)	
	2	4(2.5)	44(27. 5)	48(30)	
	3	0(0)	7(4.37)	7(4.37)	
	4	0(0)	3(1.87)	3(1.87)	
Outcome	Death	0(0)	5(3.12)	5(3.12)	0.449
	Survived	16(10)	139 (86.87)	155 (96.87)	
CXR Fining	One lateral	10(6.25)	73(45.62)	83(51.87)	0.879
	Bilateral	6(3.75)	36(22.5)	42(26.25)	
	Multilubar	2(1. 25)	24(15)	26(16.25)	
	Cavity	1(0.62)	8(5)	9(5.62)	
WBC	4000-10000	9(5.62)	81(50.62)	90(56.25)	
	>10000	10(6.25)	60(37.5)	70(43.75)	0.406
CRP	<10	2(1.25)	14(8.75)	16(10)	0.935
	>10	17(10. 62)	127(79.37)	144(90)	
Total		19(11.88)	141(88.12)	160(100)	

Table 3. Distribution of *C. pneumoniae* IgM in hospitalized patients with community-acquired pneumonia

Characteristics		Positive	Negative	Total	P value
		N (%)	N (%)	N (%)	
Sex	Male	5(3.13)	57 (35.62)	62(38.75)	0.516
	Female	11(6.87)	87(54.38)	98(61.25)	
Age (year)	≤60	10(6.25)	73(45.62)	83(51.87)	0.370
	>60	6(3.75)	71(44.38)	77(48.13)	
Underlying disease	Yes	6(3.75)	74(46.25)	80(50)	0.292
	No	10(6.25)	70(43.75)	80(50)	
ICU admission	Yes	0(0)	11(6.88)	11(6.88)	0.252
	No	16(10)	133(83. 12)	149(93.12)	

Hospital stay	≤7	12(7.5)	86(53.75)	98(61.25)	0.856
	>7	7(4.37)	55(34.37)	62(38.75)	
Clinical symptom	Cough	1(0.63)	21(13.12)	22(13.75)	0.136
	Sputum	5(3.13)	41(25. 62)	46(28.75)	
	Dyspnoea	7(4.37)	27(16.88)	34(21.25)	
	Chest pain	0(0)	13(8.12)	13(8.12)	
	Fever	3(1.87)	42(26.26)	45(28.12)	
Severity score	0	6(3.75)	34(21.25)	40(25)	0.647
CURB65	1	5(3.12)	57(35.62)	62(38.75)	
	2	5(3.12)	43(26. 87)	48(30)	
	3	0(0)	7(4.37)	7(4.37)	
	4	0(0)	3(1.87)	3(1.87)	
Outcome	Death	0(0)	5(3.12)	5 (3.12)	0.449
	Survived	16 (10)	139 (86.87)	155 (96.87)	
CXR	One lateral	10(6.25)	67(41.87)	83(51.87)	0.001
Fining	Bilateral	0(0)	42(26.25)	42(26.25)	
	Mutilubar	0(0)	26(16.25)	26(16.25)	
	Cavity	0(0)	9(5.62)	9(5.62)	
WBC	4000-10000	3(1.87)	87(54.37)	90(56.25)	
	>10000	13(8.12)	57(35.62)	70(43.75)	0001
CRP	<10	2(1.25)	14(8.75)	16(10)	0.725
	>10	14(8. 75)	130(81.25)	144(90)	
Total		19(11.88)	141(88.12)	160(100)	

The variation in the findings of studies can be attributed to variations in disease prevalence across different geographical regions, as well as disparities in laboratory kits and the accuracy of laboratory techniques. *M. pneumoniae* infections can affect both the upper and lower respiratory tracts and occur both locally and globally in children and adults. Weather and geography are not considered significant factors. Numerous outbreaks of *M. pneumoniae* infections have been identified in community settings or in confined locations such as military bases, hospitals, religious communities, and facilities for individuals with mental or developmental disabilities.[31]

In this particular research, it was observed that 10% of the individuals tested positive for *C pneumoniae* IgM, indicating an acute infection, while 94.4% tested positive for *C. pneumoniae* IgG. Among the adult CAP cases admitted to three hospitals in Kuwait, C. pneumoniae was found in 8 individuals, accounting for 6% of the cases.[28] Another study on 70 hospitalized CAP patients in Jordan by Al-Hajaya found that 27.1% had detectable IgM antibodies. In Germany and Brazil, the frequency of IgM antibodies was 0.9% and 8.2%, respectively.[32–34] Furthermore, Ngeow et al., conducted a multicenter surveillance study in eight countries, including Malaysia, Thailand, China, the Philippines, Taiwan, South Korea, Singapore, and Indonesia. They found that *M. pneumoniae* and *C. pneumoniae* were associated with 23.5% of CAP cases. Generally, studies from the Asia-Pacific region have reported a lower proportion of atypical pathogens in CAP, accounting for less than 10% of the cases.[35]

Our data show that there is no significant relationship between *M. pneumoniae* and *Chlamydia pneumonia* infections and characteristics including gender, underlying disease, pneumonia severity, ICU admission, hospital death, length of hospital stay, CRP, hematocrit, and platelet count. However, we did observe a significant relationship between *M. pneumoniae* and clinical symptoms ($p<0.001$) as well as age ($p=0.122$). Additionally, we found a significant correlation between *C. pneumonia* and white blood cell count ($p=0.001$) and changes in chest radiography ($p=0.001$).

Lui G's research on 1193 patients revealed that 28.6% of the causal organisms were identified as atypical pathogens, with a majority of them affecting elderly patients (63.4%) with comorbidities (41.8%). Additionally, more than one-third of the patients were classified as having 'intermediate' or 'high' risk CAP on presentation with CURB-65 2–5 (42.5%).[36]

The clinical symptoms and laboratory findings did not show any significant differences between the two pathogens in this study. It was shown that no one symptom or test result can be utilized to distinguish *C. pneumoniae* pneumonia from pneumonia caused by other atypical respiratory infections. Therefore, there is a

requirement for more precise and swift laboratory diagnostic methods that can assist in initiating the appropriate treatment. Due to the absence of reliable clinical indicators for fast identification, empirical therapy with atypical pathogen coverage should be explored for hospitalized CAP patients.[36] Despite being a controversial strategy, it has shown potential for reducing mortality rates, shortening hospital stays, and lowering overall hospitalization costs.[4]

Conclusions

M. pneumoniae (11.9%) and *C. pneumoniae* (10%) were responsible for a large number of community-acquired pneumonia patients that required hospitalization. Given the high prevalence of these infections and the challenges associated with laboratory diagnosis, it is strongly advised to include effective antibiotics targeting these two organisms in the empirical treatment of community-acquired pneumonia.

Acknowledgment

Authors declare their sincere thanks to all patients who participated in this study.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

community-acquired pneumonia: CAP; hospital-acquired pneumonia: HAP; ventilator-associated pneumonia: VAP; health care-associated pneumonia: HCAP.

Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

Kashan University of Medical Sciences supported this research (Grant No9260).

Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Institutional Review Board approval (code: IR.KAUMS.REC.2016.13) was obtained.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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How to Cite this Article:
Momen-Heravi M, Sharif MR, Ghasemi Mobarakabadi N. Mycoplasma pneumonia and Chlamydia pneumonia infection in patients admitted to Beheshti Hospital in Kashan with community-acquired pneumonia. Int Arch Health Sci. 2023;10(4):164-170. doi:10.48307/IAHSJ.2023.183013