Research Article

Mycoplasma pneumoniae and *Legionella pneumophila* co-infection with SARS-CoV-2 infections in COVID-19 patients living in Abadan, Iran

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Abstract

The co-infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections with the bacterial disease has been reported from the other regions of Iran, and Abadan is no exception. Atypical pathogens infections can cause a major burden on health and treatment services. Also, they may share similar radiographic findings and clinical features with SARS-CoV-2 that making a thorough differential diagnosis essential. In this study, we have found ten cases of a patient diagnosed with *Mycoplasma pneumoniae* (*M. pneumoniae*) and *Legionella pneumophila* (*L. pneumophila*), and concomitant SARS-CoV-2 pneumonia, highlighting the need for an etiological investigation of this pandemic.

Keywords: SARS-CoV-2; COVID-19; *M. pneumoniae*; *L. pneumophila*; *C. pneumonia*

Introduction

The pandemic coronavirus disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), was originated from Wuhan, Hubei Province, China in December 2019.[1,2] It rapidly spread worldwide and became a global threat.[3] The major route of transmission for SARS-CoV-2 infection is the delivery of respiratory droplets from symptomatic and asymptomatic individuals to healthy people.[4] Previous studies on respiratory infection ensure that co-infections with a diversity of co-pathogens can occur.[5] Furthermore, co-infections and superinfections are common complications in respiratory viral infections. Some viral infection-related bacterial species include *Staphylococcus*, *Streptococcus pneumoniae*, *Enterococcus*, *Klebsiella pneumoniae*, Enterobacter, Escherichia coli, *Legionella pneumophila*, *Acinetobacter*, *Mycoplasma pneumoniae*, *Chlamydia pneumonia*, and

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Pseudomonas.[6-8] We aimed to assess the rate of bacterial and SARS-CoV-2 co-infections and their outcomes among COVID-19 patients are living in Abadan, Iran.

Material and Method

Sample collection

We have collected respiratory samples from forty RT-PCR-confirmed COVID-19 cases with upper respiratory system symptoms, which live in the city of ABADAN, after patients' approval for participating in the study. The inclusion criteria are mild, moderate, and severe symptoms and patients with ARDS. All of clinical and laboratory manifestations have been collected carefully.

Detection of Community-Acquired Pneumonia (CAP) in COVID-19 patients

We used polymerase chain reaction (PCR) as the detection method for bacterial causing agent of Community Acquired Pneumoniae (CAP) among COVID-19 patients, which are comprise of *mycoplasma pneumonia*, *legionella pneumophila*e and *chlamydia pneumoniae*, in this study. Bacterial DNA was extracted due to kit's manufacturer instruction (Wizard® Genomic DNA Purification Kit Technical Manual) from the collected upper respiratory samples. Then, the quality of extracted DNA was tested using spectrophotometry method by Nanodrop device. For molecular detection of causing agents of CAP in COVID-19 patients, we used the specific primers for following genes, P1 adhesion gene (for detection of *mycoplasma pneumoniae*), macrophage infectivity potentiator (MIP) (for detection of *legionella pneumophila*), 16S rRNA (for detection of *chlamydia pneumoniae*) (Table 1). Then, we applied the production of polymerase chain reaction on the 2% agarose gel for further assessments.

Statistical analysis

Data were analyzed with Stata version 11. Quantitative and qualitative data are presented as mean ± SD and percentages, respectively. Also, confidence interval (CI) was calculated using binomial distribution. The t test, chi-square test and Fisher's exact test were used to compare of data between groups with/without CAP coinfections. P values of less than 0.05 were considered significant.

Results

Demographic and baseline characteristics of COVID-19 patient

Among the 40 participated patients in this study, 27 patients were male. The mean age was 52.6(50.6 to 54.7) years old. Other than fever, which was detected in all of the patients,

headache was reported to be the most abundant symptom (77.5%), followed by dry cough which was appeared in 26 of 40 (65%) COVID-19 patients. Also, 15/40 (37.5%) patients were suffering from shortness of breath. Diarrhea, muscle pain and chill were other appeared symptoms COVID-19 patients. Based on the hospitalization status of patients, 23 (57.5%) patients were outpatients. In addition, in this study we also considered COVID-19 patients' comorbidities as well. The results showed that 35% of patients suffered from cardio vascular diseases, 27.5% of patients have diabetes, and also 35% had blood pressure. Asthma and allergy were other reported comorbidities. Complete demographic and baseline characteristics are available in Table 2.

Table 1: *Primers and their targets were used in this study.*

Target gene	length	R	F	Organism
P1	450	TGGCCTTGCGCTACTA-AGTT	AAAGGAAGCTGACTC-CGACA	*M. pneumoniae*
PstI	283	GGTGTGTTTCTAATACCT-GTCC	CGGCTAGAAATCAAT-TATAAGACTG	*C. pneumoniae*
Mip	487	GGGATAACTTGT-GAAACCTG	CAATGGCTGCAACCGAT-GC	*L. pneumophila*

Table 2: *Demographic and baseline characteristics of COVID-19 patients*

Variables		N	Prevalence% (95% CI)
Gender	Female	13	32.5 (17.3 to 47.7)
	Male	27	67.5 (52.3 to 82.7)
Hospitalization Status	Outpatient	23	57.5 (41.5 to 73.5)
	Inpatient	17	42.5 (26.5 to 58.5)
Blood Pressure	No	26	65.0 (49.5 to 80.5)
	Yes	14	35.0 (19.55 to 50.5)
Diabetes	No	29	72.5 (58.1 to 86.9)
	Yes	11	27.5 (13.1 to 41.9)
CVD	No	26	65.0 (49.5 to 80.4)
	Yes	14	35.0 (19.5 to 50.4)
Asthma	No	34	85.0 (73.4 to 96.6)
	Yes	6	15.0 (3.4 to 26.6)
Allergy	No	35	87.5 (76.8 to 98.2)
	Yes	5	12.5 (1.8 to 23.2)
Fever	No	0	0
	Yes	40	100
Diarrhea	No	37	92.5 (79.6 to 98.4)
	Yes	3	7.5 (1.6 to 20.4)
Shortness of breath	No	25	62.5 (46.8 to 78.2)
	Yes	15	37.5 (21.8 to 53.2)
Headache	No	9	22.5 (8.9 to 36.1)
	Yes	31	77.5 (63.9 to 91.1)
Muscle pain	No	38	95.0 (83.1 to 99.4)
	Yes	2	5.0 (0.6 to 16.9)
Dry cough	No	14	35.0 (19.5 to 50.4)
	Yes	26	65.0 (49.5 to 80.4)

Chills	No	38	95.0 (83.1 to 99.4)
	Yes	2	5.0 (0.6 to 16.9)
Age (mean and 95% CI)		40	52.6 (50.6 to 54.7)
N: Number; CI: Confidence Interval; CVD: Cardiovascular Disease			
CI calculated using binomial distribution			

Prevalence of CAP coinfections in overall and based on the different bacterial types

The bacterial causing agents of Community Acquired Pneumonia (CAP) are comprised of *mycoplasma pneumoniae*, *legionella pneumophila* and *chlamydia pneumoniae*. The results of our polymerase chain reaction demonstrated that 9/40 (22.5%) cinfirmed COVID-19 patients were also infected by *mycoplasma pneumoniae*, simultaneously. 2/40 (5%) patients were infected by *legionella pneumophila*. However, *chlamydia pneumoniae* was not detected in any of COVID-19 patients. 10/40 (25%) of COVID-19 patients were infected by at least one of these bacteria. While, one patient was infected by both of *mycoplasma pneumoniae* and *legionella pneumophila* (Table 3).

Discussion

Wide bacterial communities exist in the healthy airways, therefore, the detection of bacteria in secretions of the oral cavity does not necessarily constitute an infection.[9,10] On the other hand, pathogenic bacterial infection as a part of an underlying chronic disease or a hospital acquired respiratory infection can complicate the patient's condition and transmit another pathogen.[11,12] In our study, the result of PCR test showed that 10 out of 40 patients with SARS-CV-2 infection were positive for *M. pneumoniae* or *L. pneumophila*. The signs and symptoms of COVID-19 disease, *M. pneumoniae*, and *L. pneumophila* are similar, including shortness of breath, fever, and cough. To date, R Chaudhry et al., Blasco et al., Easom et al., Wu et al., Oliva A et al., Zhang et al., Gao et al., Chen et al., and Gayam et al. have reported co-infections of SARS-CoV-2 infection with *M. pneumoniae* and *L. pneumophila* by using molecular and serological assays.[13-15] Legionellae and *Mycoplasma pneumoniae* can be a major health burden in the respiratory disease such as COVID-19 pandemic.[11,16] With regard that our co-infection patients in our study were hospitalized, it suggests that patients who are hospitalized should be screened for a diverse range of common respiratory pathogens for the detection of co-infections or mixed infections to save patient lives during the COVID-19 pandemic.

Conclusion

Detection of respiratory infection and COVID-19 disease is necessary using molecular assays for the causative agent's detection of co-infection to recommend appropriate antimicrobial drugs.

According to the previous studies, Co-infection along COVID-19 disease can be occurring. Co-infection SARS-CoV-2 infection with other respiratory pathogens remains a targeted treatment strategy for COVID-19 patients. Our findings are obtained from a small sample size; therefore this research has thrown up many questions in need of further investigation. Empirical antimicrobial drugs in COVID-19 patients can be useful after obtaining more data regarding co-infection with SARS-CoV-2 infection.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

1. Mona Fani, Milad Zandi, Saeedeh Ebrahimi, et al. The role of miRNAs in COVID-19 disease. Future Virol 2021;16(4):301-306.

2. Navand AH, Saber Soltani, Parastoo Hosseini, et al. Diabetes and coronavirus infections (SARS-CoV, MERS-CoV, and SARS-CoV-2). J Acute Dis 2020; 9(6): 244-247.

3. Mona Fani, Milad Zandi, Saber Soltani, et al. Future developments in biosensors for field-ready SARS-CoV-2 virus diagnostics. Biotech and applied biochem 2020.

4. Velavan TP, CG Meyer. The COVID-19 epidemic. Trop Med Int Health 2020; 25(3):278-280.

5. Saber Soltani, Armin Zakeri, Milad Zandi, et al. The role of bacterial and fungal human respiratory microbiota in COVID-19 patients. BioMed Res Int 2021; 2021: 6670798.

6. Timothy M Rawson, Luke SP Moore, Alison Holmes, et al. Bacterial and fungal coinfection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis 2020; 71(9): 2459-2468.

7. Patel J, V Sampson. The role of oral bacteria in COVID-19. The Lancet Microbe 2020;1(3):e105.

8. Nicolson GL, GF de Mattos. COVID-19 Coronavirus: Is Infection along with Mycoplasma or Other Bacteria Linked to Progression to a Lethal Outcome? Int J of Clin Med 2020;11(05):282-302.

9. Wypych TP, Wickramasinghe LC, BJ Marsland. The influence of the microbiome on respiratory health. Nat Immunol 2019;20(10):1279-1290.

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10. Weronika Barcik, B Brett Finlay, Rozlyn CT Boutin, et al. The role of lung and gut microbiota in the pathology of asthma. Immunity 2020; 52(2):241-255.

11. Nazmul Hoque M, Salma Akteret, Anwar Hossain M, et al. Microbial co-infections in COVID-19: Associated microbiota and underlying mechanisms of pathogenesis. Microb Pathog 2021;156:104941.

12. Liu X, Ni Zou, Dan Wang, et al. Influencing factors analysis and modeling of hospital acquired infection in elderly patients. J of Comb Optimiz, 2019; 37(4):1-23.

13. Rasoul Mirzaei, Sajad Karampoor, Sima Ghiasvand, et al. Bacterial co-infections with SARS-CoV-2. IUBMB life, 2020. 72(10):2097-2111.

14. Musuuza JS, Nasia Safdar, Lauren Watson, et al. Prevalence and outcomes of co-infection and super-infection with SARS-CoV-2 and other pathogens: A systematic review and meta analysis. PloS one 2021;16(5):e0251170.

15. Lai CC, Wang CY, Hsueh PR. Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? J of Microbiol Immunol Infect 2020; 53(4):505-512.

16. Dey R, Ashbolt NJ. Legionella Infection during and after the COVID-19 Pandemic. ACS ES&T Water 2020.