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TO ANALYSE BACTERIAL AND FUNGAL INFECTIONS IN POST CORONAVIRUS DISEASE PATIENTS

Medicine

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ABSTRACT

BACKGROUND: Post COVID-19 disease patients are immunocompromised due to intake of high doses of steroids during covid treatment. In these patients opportunistic infections are very common. The infections of bacteria and fungi has a great influence on the progression and prognosis of the disease, especially in severe patients, which can lead to increased needs for intensive care, antibiotic treatment and increased deaths. Therefore, these infections should be explored in post covid-19 patients.

OBJECTIVE: To analyse respiratory sample (sputum,/BAL) for various bacterial and fungal infection in post Coronavirus Disease patients.

METHODS: The present study was conducted on 50 patients of all age groups and sex who attended Department of Respiratory Medicine, association with Department of Microbiology. Sputum/induced sputum were collected in sterile vials and sent to Microbiology for examination for bacterial and fungal species.[2] In microbiology sputum samples were cultured on Saboraud dextrose agar, macConchey and blood agar. Culture were observed daily for 7 days bacterial and for 28 days for fungal growth.

RESULT: The Out of 50 a total of 32% sputum samples were positive for pyogenic organisms. A total of 4% cases were found to be Psuedomonas aeruginosa, 2% had Acinetobacter baumannii, 8% had Klebsiella pneumoniae, 2% Escherichia coli, 4% Steptococcus pneumonie and 12% had Mycobacterium tuberculosis. Out of 50 a total of 34% sputum samples were positive for fungus, 12% cases had Mucormycosis, 12% cases had Candida sp., 6% cases had Aspergillus fumigatus followed by 2% cases each of Aspergillus flavus and 1 Penicillium respectively.

CONCLUSIONS: This study highlights the prevalence of bacterial and fungal infection in Post coronavirus Disease patients. Thus sputum for fungal and pyogenic culture should be done in post covid symptomatic patients.

KEYWORDS

Post covid, Pyogenic infections, Fungal infections, Sputum

INTRODUCTION

Coronavirus Disease - 2019 (COVID-19) is a very contagious disease mainly involving the respiratory system.[1] It has become a pandemic since the first report in December 2019 in Wuhan, China. It is a huge strike to human health and draws much attention from countries all over the world. Post COVID-19 disease patients are immuno compromised. In these patients opportunistic infections are very common. The infections of bacteria and fungi has a great influence on the progression and prognosis of the disease, especially in severe patients, which can lead to increased needs for intensive care, antibiotic treatment, and increased deaths. Therefore, these infections should be explored in post covid-19 patients.

OBJECTIVES:

To analyse respiratory sample (sputum,/BAL) for various bacterial and fungal infection in post Coronavirus Disease patients.

MATERIAL AND METHODS

The cross sectional study was conducted in the Department of Respiratory Medicine in association with Department of Microbiology, Pt. B.D. Sharma P.G.I.M.S, Rohtak after obtaining the ethical clearance from the institutional Ethical committee. Informed and written consent was taken from patients before including them in the study. In the study post COVID-19 patients attending Respiratory Medicine OPD or emergency at PGIMS Rohtak, 50 patients who volunteer for the study were selected using a table of random numbers. Following microbiological tests were performed –

Sputum - Gram stain, Ziehl Neelsen stain, Fungal culture, Pus culture, CBNAAT Bronchoalveolar lavage (BAL) as and when required – Cytology, Gram stain, Ziehl Neelsen stain, Fungal culture, Pus culture, Cartridge based nucleic acid amplification Test (CBNAAT).

Post COVID case: is defined as a patient beyond 28 days of negative RT-PCR for COVID-19 who was COVID positive in past.

INCLUSION CRITERIA FOR STUDY SUBJECTS:

1 50 Previously COVID - 19 positive patients who have currently completed 28 days after their COVID negative report by RT-PCR test.

EXCLUSION CRITERIA FOR STUDY SUBJECTS:

1 Patients who does not have any COVID exposure.
2 Patients who does not give consent.

PROCEDURE:

Sputum Sample

- Sample collection - patients will be asked in early morning to gargle with plain water and thereafter sputum samples will be taken after coughing. If patient does not expectorate, induction of sputum will be done by inhalation of 3% hypertonic saline for 15 minutes with an ultrasonic nebuliser. 3-5 ml sputum and induced sputum samples will be collected in sterile, wide-mouthed, unbreakable, leak proof container and tightly closed lid.
- Adequacy of Sample - the quality of the expectorated sputum will be assessed both by macroscopic and microscopic examination. Any sample that will be thin watery and with no purulent matter will be considered unsuitable for further processing. Bartlett's scoring method will be used for microscopic evaluation of the expectorated sputum. Sputum will be considered unsuitable if it has a final score of 0 or less. All unsuitable specimens will be discarded and a repeat specimen will be collected. If gram staining

shows > 25 pus cells and <10 epithelial cells/low power field, sample will be considered adequate for culture. The isolates which will be identified as contaminants or commensals will be excluded from the study. Bartlett's score is calculated as[2]:

	Criteria	Score
Neutrophils (pus cells) count (Score A)	< 10 neutrophil/10x field	0
	10-25 neutrophils/10x field	+1
	>25 neutrophils/10x field	+2
Macroscopy (Score B)	Mucoid, Mucopurelent, Purelent, or Blood stained	+1
Squamous epithelial cell count (Score C)	< 10 Squamous epithelial cell/10x field	0
	10-25 Squamous epithelial cell /10x field	-1
	>25 Squamous epithelial cell /10x field	-2

- Storage and Transport conditions - Sputum will be transported to the laboratory as soon as possible. If a delay of a few days cannot be avoided, specimens will be kept cool (refrigerated but not frozen). If the delay exceeds 3 days, an equal volume of cetyl pyridinium chloride (CPC; solution of 1% CPC in 2% sodium chloride) would therefore be added to sputum. Sputum containing CPC can be kept for up to 7 days but must be kept at room temperature (>20 °C since CPC crystallizes at lower temperatures).
- Sample packaging - The basic packaging system for local surface transport of all specimens consists of three layers that are primary receptacle (the specimen container packaged with enough absorbent material to absorb all fluid in case of breakage), secondary packaging (a second durable, watertight, leakproof packaging to enclose and protect the primary receptacle), outer packaging (to protect their contents from external influences during transit).
- Analysis - Sabouraud dextrose agar will be used for fungal culturing; plates will be incubated at 28◦C for 7 days. Identification of fungi will be based on macroscopic and microscopic characteristics using standard mycological methods. The growth of cream pasty opaque colonies >30 colonies on SDA slant is considered significant.
- Sputum Gram stain test will be to distinguish and classify bacterial species into two large groups: gram positive bacteria and gram negative bacteria. For bacteria growth blood agar plate and MacConkey Agar palate will be used. Sample will be incubated at room temperature and will be examine growth at 72-hour intervals.
- Bronchoalveolar lavage (BAL) sample
- Sample collection - It will be taken in patients who are not expectorating sputum. 30 – 40 ml fluid will be obtained by bronchoscopy. The patient will be put fasting for 6 hours before the procedure. Fentanyl and Midazolam will be given intravenously for relaxation. Lignocaine spray will be given to anaesthetise before the procedure. Bronchoscope will be put through mouth or nose and moved down the throat and into the airways. A small amount of saline will be put through the scope. After washing the airways the saline solution will be obtained by negative suction in sterile containers made of polypropylene or other plastics made for suspension tissue culture.
- Adequacy of sample - A BAL sample is considered representative if the volume is more than 20ml, total cell count more than 60 cells per ml, alveolar macrophages more than 10 cells per high power field, presence of less than 1 percent squamous epithelial cells, presence of less than 5 percent bronchial epithelial cells.[14]
- Storage and Transport conditions – BAL Fluid will be transported to the laboratory as soon as possible. If a delay of an hour cannot be avoided, specimen will be kept cool (at 4 degree celsius). For more than one hour delay, the cells would be centrifuged at 250 to 300 for 10 minutes and then resuspended in a nutrient supplemented medium (eg. HEPES) and stored at 4 degree Celsius for 24 hours.
- Analysis - The total cell count will be obtained by haemocytometer, Differential cell counts will be performed by cytocentrifugation with staining. Further culture will be done like the sputum sample

OBSERVATIONS

Observations of the study are shown in form of tables. Table 1 shows the pyogenic infections in sputum of study population and table 2 sputum for fungal culture of study population.

Table 1 prevelance of pyogenic infections

Parameters	No. of cases	Percentage
Pseudomonas aeruginosa	2	4
Acinetobactor baumanni	1	2
Klebsiella pneumonie	4	8
Escherichia coli	1	2
Streptococcus pneumonie	2	4
Mycobacterium tuberculosis	6	12
Sterile	34	66
Total	50	100

Table 2 prevelance of fungi in sputum

Parameters	No. of cases	Percentage
Candida sp.	6	12
Aspergillus fumigatus	3	6
Aspergillus flavus	1	2
Mucormycosis	6	12
Aspergillus niger	0	0
Penicillium	1	2
Sterile	33	66
Total	50	100

Out of 6 patients who were detected positive for Mycobacterium Tuberculosis on Ziehl Neelsen stain, 2 Patients found Rifampicin resistance on CBNAAT.

DISCUSSION

A total of 50 samples were collected from both inpatients and out patients, of all age groups and both sexes, In the present study, majority of patients were in the age range of 51-60 years followed by31-40 and 61-70 years i.e. 15(30%), 8(16%) and 8(16%) respectively. In elderly age group above, only 4(8%) patients were found. Mean age of the study population was 51.92 years. The range of population 18-85 years. Sex distribution of the patients showed that males were more than females i.e. 34(68%) males and 16(32%) females. Majority of patients were labourer i.e. 30% followed by housewife and farmer i.e. 24% each. Out of 50 a total of 26(52%) patients found to be currently chronic smoker. In the present study, we found that out of 50 a total 9 (18%) patients were diabetic, 4 (8%) patients were on antihypertensive and 3(6%) patients had taken anti-tubercular treatment in the past. Out of 50 patients, there were 19 (38%) chronic smokers and 4 (8%) were ex-smokers. Compared with normal patients admitted to general ward, the immune function of post covid patients was more greatly suppressed by receiving high dosage of corticosteroids and more types of antibiotics. The risk of Mucormycosis, candida infection for patients was significantly increased.

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Conflicts of interest
There are no conflicts of interest.

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