Original research article

A Study on Bronchoalveolar Lavage Fluid Cytology in Symptomatic Covid-19 Patients

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Abstract

Background: Coronavirus disease 2019 (COVID-19) is an infectious respiratory disease caused by novel coronavirus SARS-CoV-2 predominately involving the respiratory system. Bronchoalveolar lavage (BAL) is a safe, easily performed, minimally invasive and well tolerated procedure which explores large areas of the alveolar compartment providing cells as well as non-cellular constituents from the lower respiratory tract. It opens a window to the lung.Alterations in BAL fluid cellular and non-cellular components reflect pathological changes in the lung parenchyma. The information obtained from BAL fluid analysis, if considered carefully can have a reliable diagnostic criterion.

Keywords: Bronchoalveolar,Covid-19,Fluid

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious respiratory disease caused by novel coronavirus SARS-CoV-2 that emerged in Wuhan, China at the end of 2019, resulting in a worldwide pandemic. This disease can be asymptomatic or can range from influenza-like illness, pneumonia to severe acute respiratory syndrome with multi-organ involvement leading to increased morbidity and mortality among all populations. The disease spread rapidly and became a pandemic with ~ 216 million confirmed cases and over 4.5 million deaths worldwide and 2,088,611confirmed cases and over 42,518 deaths in India by end of August 2021. Coronavirus is an enveloped single-stranded ribonucleic acid and has 9-12 nm-long surface spikes and hence named as solar corona [(1)]. The corona viral genome encodes four major structural proteins on the envelope. One of the protein spike (S) proteins is responsible for viral entry into the host cell. Spike (S) protein binds to angiotensin-converting enzyme 2 (ACE2) receptor and mediates fusion between the envelope and host cell membranes [(2,3).] The Covid-19 pathophysiology is complicated and involves hematologic systems, epithelial cells, and vascular systems [(4)].As a result of hyperactive host immune response to the SARS-CoV-2 virus, an excessive inflammatory reaction is exerted known as "Cytokine storm" where a large amount of pro-inflammatory cytokines are released. Several studies suggest that cytokine storm is directly correlated to lung injury, multi-organ failure, and unfavourable prognosis of severe COVID-19 [(5)].However, there are no specific Covid related cytomorphological findings in BAL fluid that have been described so far in a larger study group. Bronchoalveolar lavage (BAL) is a routine bronchoscopy procedure that can provide significant information which is helpful in the management of Covid pneumonia and also in severe acute respiratory distress syndrome (ARDS). Bronchoscopy has become a need in patients with COVID-19–associated ARDS requiring mechanical ventilation mainly for clearing the secretion-(broncho aspiration) followed by BAL fluid for microbiological and cytopathological examination. BAL fluid is also used to confirm COVID-19 positivity in case of previous negative nasopharyngeal swab(s) in patients, intubated or not, with clinical

concern for this diagnosis. Broncho alveolar lavage (BAL) samples of Covid-19 positive patients with respiratory distress and/or on mechanical ventilators are often submitted for cell count and differential in conjunction with coagulation study and microbiology testing for evaluation of SARS-CoV-2 infectious status and diagnosis of secondary infections. COVID-19 infection may lead to secondary bacterial and /or fungal infection which results in poor clinical outcomes, especially among critically ill patients. Bacterial and fungal infections found in BAL fluid typically showed high cellularity, with neutrophilic alveolitis in patients with moderate to severe COVID-19–related ARDS [6].Candida, Pseudomonas aeruginosa, Enterobacter aerogenes, Staphylococcus aureus, and Klebsiella pneumoniae were the common coinfections noted in ICU admitted patients [7]. BAL fluid is used for microbiological analysis for early detection of these bacterial /fungal elements in Covid-19 infection which helps in treating the patients for a better prognosis.

Materials and Methods:

Study area: The study is conducted in the Dept. Pathology, Integral institute of medical science and research, Lucknow

Study duration: Patients were enrolled in the study in the period from June 2021 to Oct 2022

Study population: Symptomatic Covid-19 patients (RT-PCR positive) admitted in Integral institute of medical science and research, Lucknow

Inclusion criteria:

- Symptomatic (cough, fever, breathlessness, loose stools, myalgia, tiredness) RTPCR positive Covid-19 admitted patients.
- Age 18 years and above
- Both male and female gender.

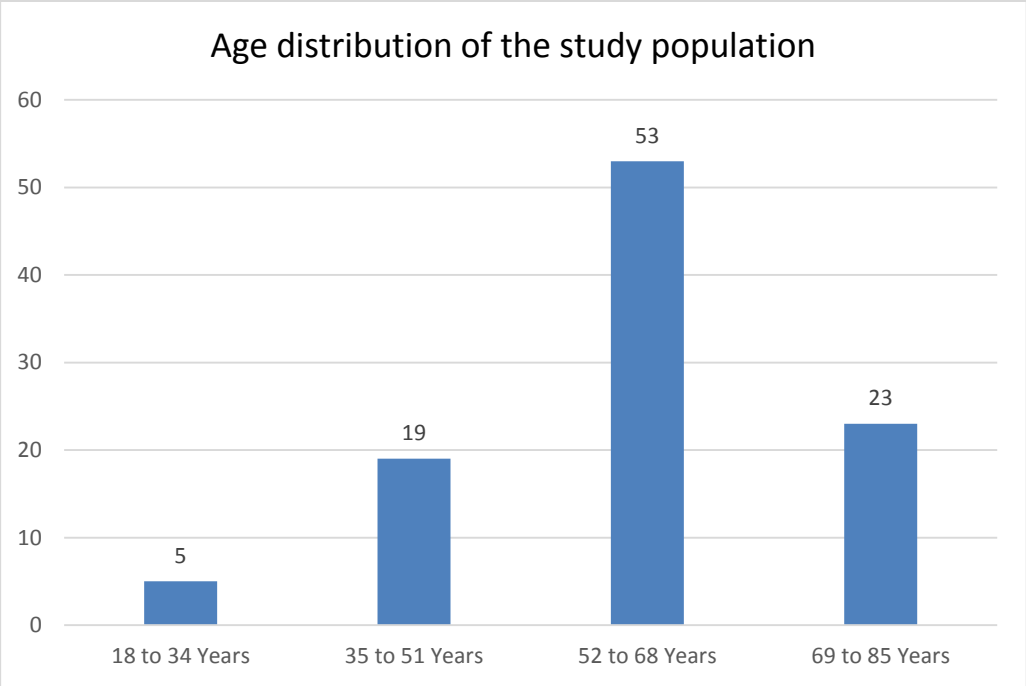
Exclusion Criteria:

- Known cases of interstitial lung disease, Chronic obstructive lung disease, Tuberculosis, Asthma, pneumonia (other than covid -19) and Chronic smoker.
- Inadequate BAL fluid sample - volume less than 10ml
- BAL fluid samples sent to the laboratory after 24 hours of the procedure.
- Sample received twice of the same patient was not considered.

Results:

In our study population predominate age group was in the range of 52-68 years and male patients were more (81%) compared to females. 76% of the patients were found to be severely ill ,followed by 10% moderately ill and 14% had mild illness. BAL fluid differential count showed predominate neutrophilic distribution (87%) followed by lymphocytes (13%), Eosinophils and plasma cells (0%). Neutrophil and lymphocyte in association with severity of disease showed p value (0.111) individually. Reactive type II pneumocytes 53(53%), Reactive bronchial epithelial cell22 (22%) multinucleated giant cells 18(18%) and Viropathic changes10 (10%) and 39(39%). Cytomorphological changes showed no significant p value in association with severity of the disease. Oxygen saturation, respiratory rate and chest x-ray findings were correlating with severity of the disease and showed significant p value (<0.05). Di-dimer and serum ferritin (p value 0.490 and 0.670 respectively) though increased in all the study population did not show significant association with the severity of the disease.

A total of 100 patients who were clinically symptomatic and RTPCR Covid-19 positive who underwent bronchoalveolar lavage and the same BAL fluid sent for analysis were included and data was analysed prospectively.



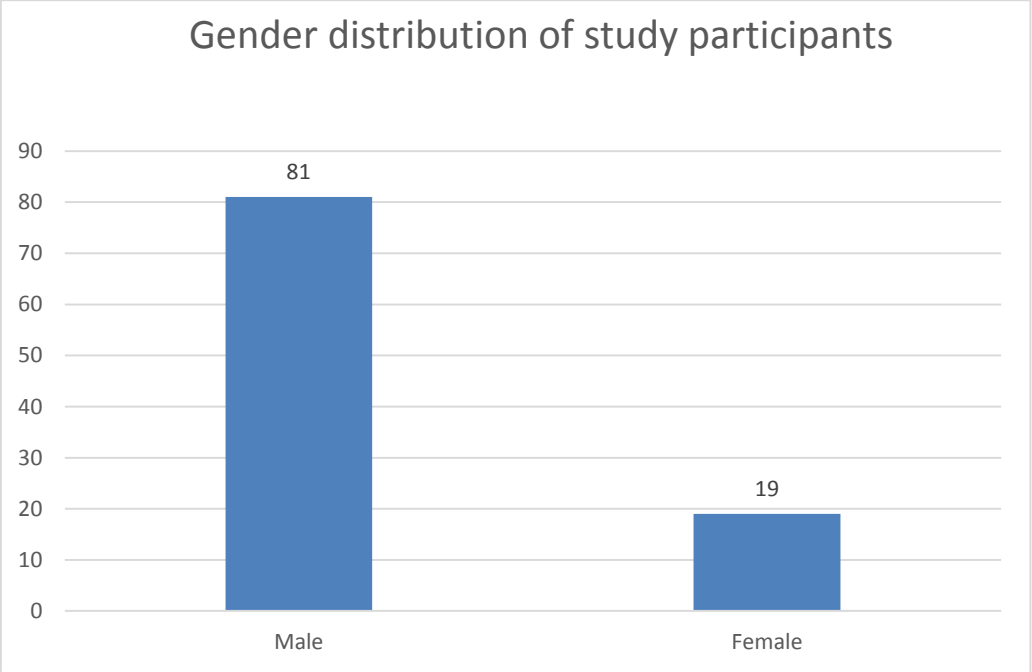
Graph 1: Age distribution of the study population.

The predominate age group of COVID 19 positive patients in our study were in the range of 52-68 years.

Table No 1: Association between Age and Severity

Age	Severity			P Value
	Mild	Moderate	Severe	
18 to 34 Years	0(0.0)	0(0.0)	05(6.6)	0.662
35 to 51 Years	02(14.3)	02(20.0)	15(19.7)	
52 to 68 Years	07(50.0)	07(70.0)	39(51.3)	
69 to 85 Years	05(35.7)	01(10.0)	17(22.4)	

Though majority of the study population were in the range of 52-68yrs and 69-85yrs there is no association between the age and severity of the disease.



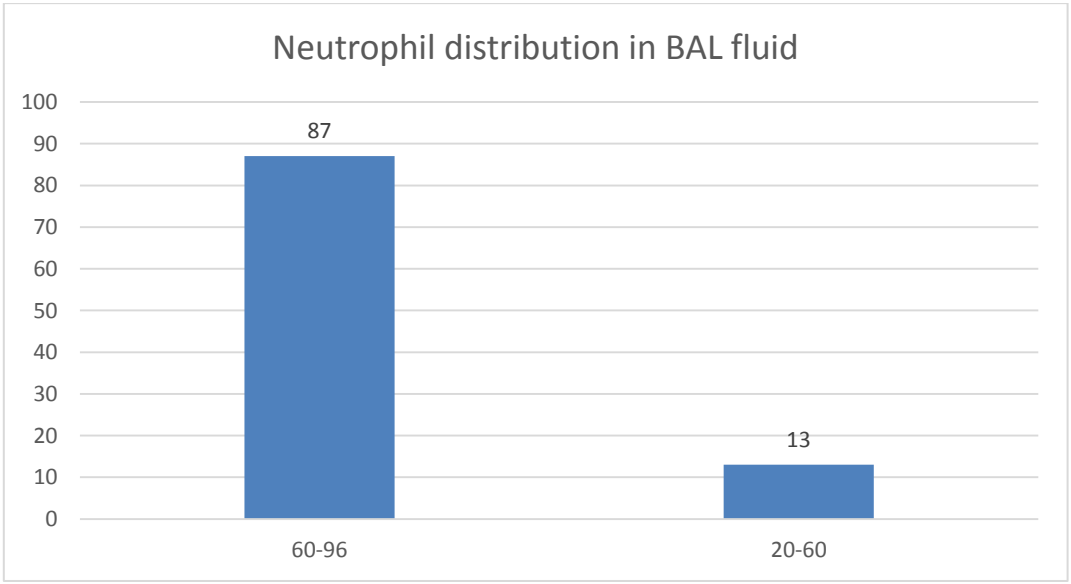
Graph 2: Gender distribution of study participants

In our study COVID 19 positive patients were predominately male (81%) than females (19%).

Table No 2: Association between Gender and Severity

Gender	Severity			P Value
	Mild	Moderate	Severe	
Male	11(78.6)	10(100.0)	60(78.9)	0.272
Female	03(21.4)	0(0.0)	16(21.1)	

Though majority of the study population are male there is no association between the gender and the severity of the disease in our study. P value is not significant.



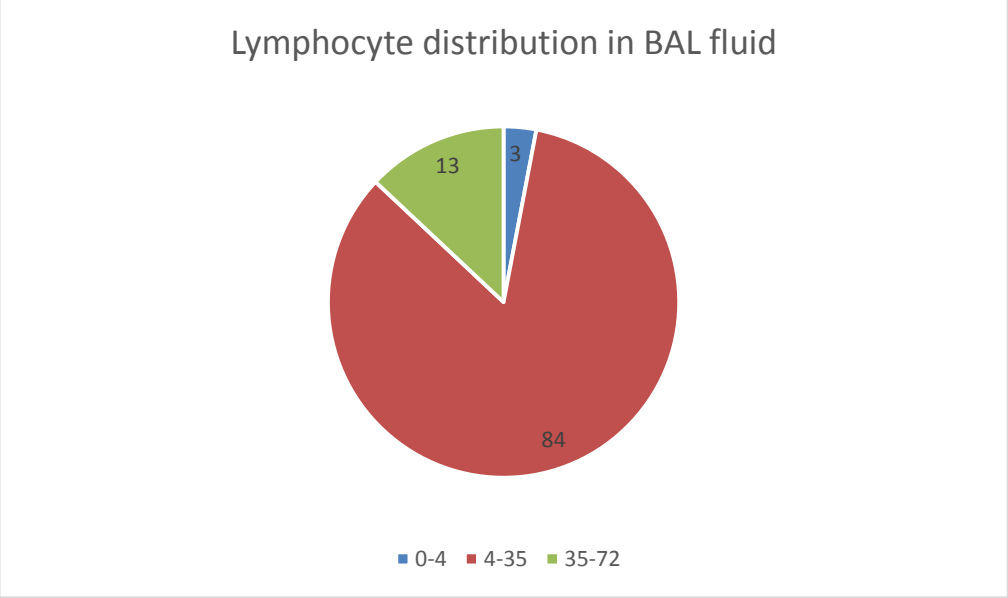
Graph 3: Neutrophil distribution in BAL fluid.

The neutrophil distribution in the range of 60-96% were around 87% and in the range of 20-60 % is 13%

Table No 3: Association between neutrophil and Severity

Neutrophil	Severity			P Value
	Mild	Moderate	Severe	
20-60	03(21.4)	03(30.0)	07(9.2)	0.111
60-96	11(78.6)	07(70.0)	69(90.8)	

Neutrophils are predominate cell type in 87% of population .No association was found between neutrophilic distribution and severity of the disease. P value is not significant.



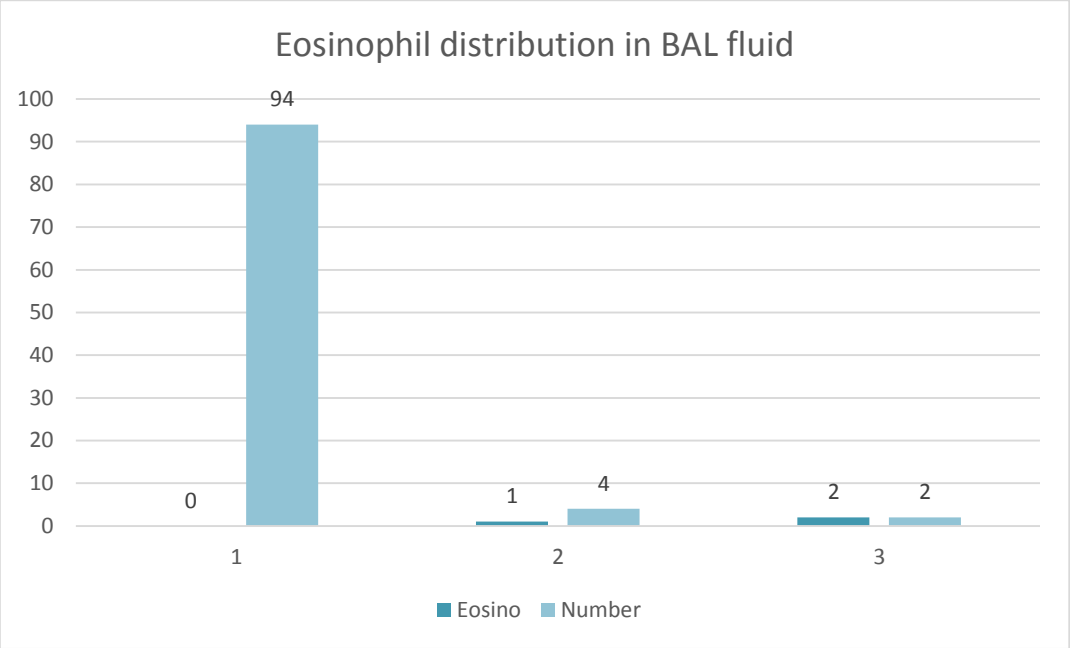
Graph 4: Lymphocyte distribution in BAL fluid

The lymphocytic distribution in the range of 4-35 % were around 84% and in the range of 35-72% were around 13%

Table No 4: Association between Lymphocytic and Severity

Lymphocytic	Severity			P Value
	Mild	Moderate	Severe	
0-4	0(0.0)	0(0.0)	03(3.9)	0.274
4-35	11(78.6)	07(70.0)	66(86.8)	
35-72	03(21.4)	03(30.0)	07(9.2)	

The lymphocytes being predominate cell type in 13% of population .There is no association between lymphocytic distribution and severity of the disease. P value is not significant



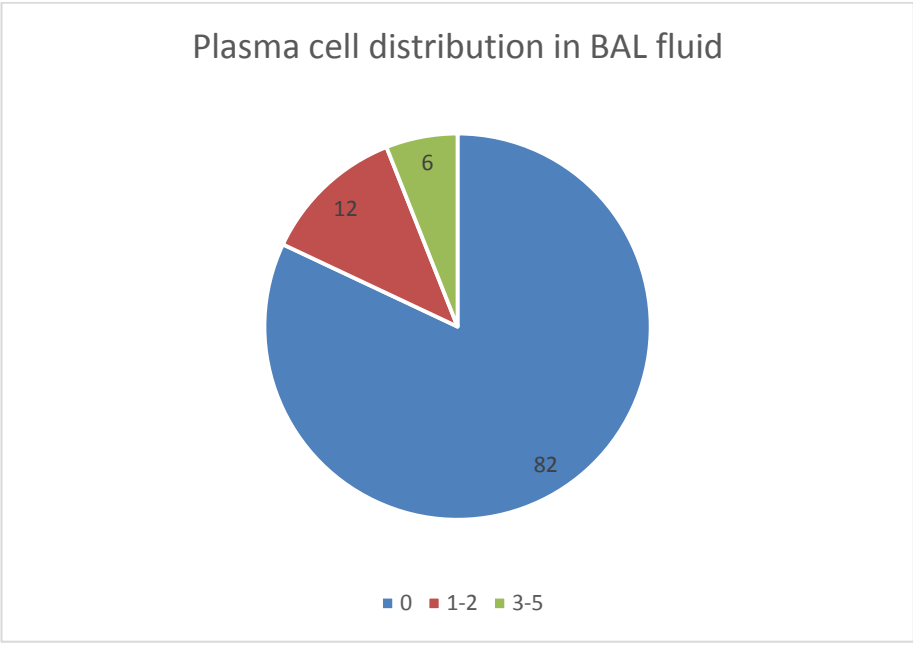
Graph 5: Eosinophil distribution in BAL fluid

Majority of the population in our study group (94%) has 0 eosinophils in the BAL fluid.

Table No 5: Association between Eosinophils and Severity of the disease

Eosinophils	Severity			P Value
	Mild	Moderate	Severe	
0	12(85.7)	10(100.0)	72(94.7)	0.264
1	02(14.3)	0(0.0)	02(2.6)	
2	0(0.0)	0(0.0)	02(2.6)	

No association was noted between eosinophils and severity of the illness.



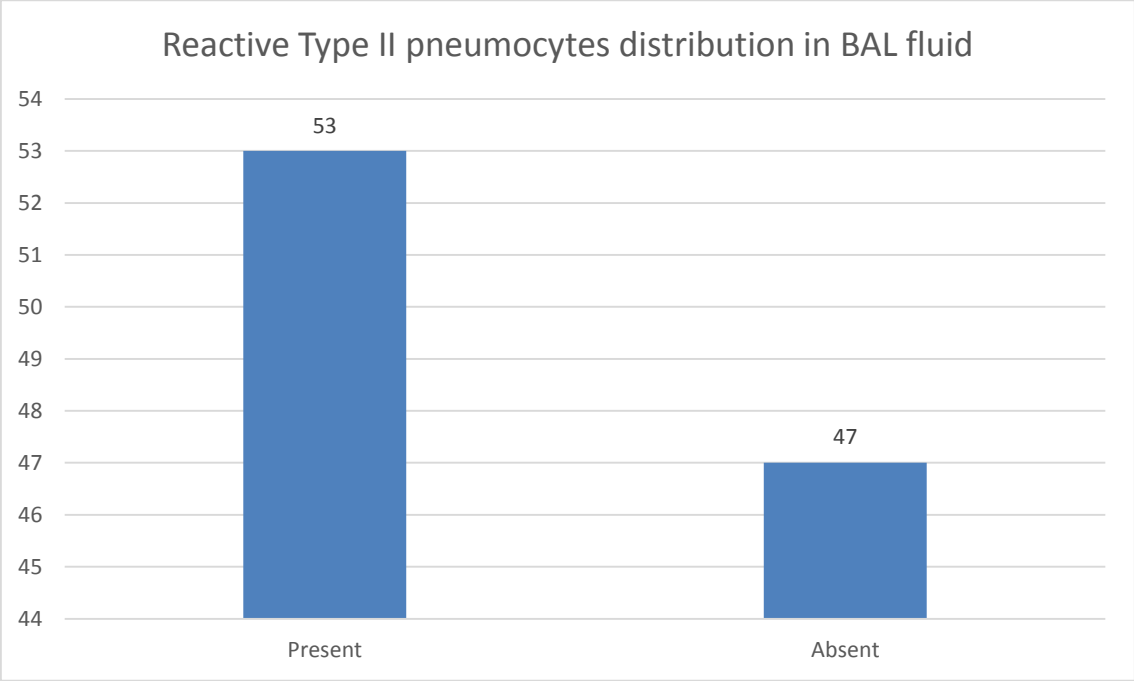
Graph 6: Plasma cell distribution in BAL fluid

- 82% of population show (0) plasma cells in BAL fluid of the participants.

Table No 6: Association between Plasma cells and Severity of the disease.

Plasma	Severity			P Value
	Mild	Moderate	Severe	
0	10(71.4)	09(90.0)	63(82.9)	0.403
1-2	02(14.3)	0(0.0)	10(13.2)	
3-5	02(14.3)	01(10.0)	03(3.9)	

No association was noted between plasma cells and severity of the disease. P value is not significant.



Graph 7: Reactive Type II pneumocytes distribution in BAL fluid

The presence or absence of reactive pneumocyte type II is almost similar in the study group. While 53% of them were positive with reactive pneumocyte type II, 47% of them were not.

Table No 7: Association between Reactive pneumocytes Type II and Severity of the disease

RP Type	Severity			P Value
	Mild	Moderate	Severe	
Present	08(57.1)	08(80.0)	37(48.7)	0.166
Absent	06(42.9)	02(20.0)	39(51.3)	

Though reactive pneumocytes Type II were present in 53% of population, no significant association was noted with the severity of the disease. P value is not significant

DISCUSSION

Coronavirus disease 2019 (COVID-19) is an infectious respiratory disease caused by novel coronavirus SARS-CoV-2. This disease can be asymptomatic or can range from influenza-

like illness, pneumonia to severe acute respiratory syndrome with multiorgan involvement leading to increased morbidity and mortality among all populations. The lung is the primary organ involved in the Covid 19 disease.The Bronchoscopy has become a need in patients with COVID-19–associated ARDS requiring mechanical ventilation mainly for clearing the secretion-broncho aspiration followed by BAL fluid microbiological and cytopathological examination. The present study was a prospective observational study conducted at a single centre. We enrolled 100 RTPCR Covid patients who underwent Broncho alveolar lavage and the same fluid was sent for analysis. We aimed to see cytomorphological changes in the BAL fluid and assess the frequency of co-infection and correlate these findings with that of the severity of the disease. Of interest, the biochemical values such as Di-dimer and Serum ferritin value is correlated with that of the severity of the disease.

Age and Gender predilection:

A total of 100 infected patients with Covid-19 disease were enrolled in the study of which 81(81%) of them were male and the rest of them were female. Our findings coincide with the study done by Bwire G. M[85] which suggests that the ability to fight an infection differs in men and women because of differences in biological immune system mediated by several factors including sex hormones. Peckham., et al[8] study suggests that though the rate of infection is equal in both male and female the severity of the disease is more common in males. Because of the extra X chromosome in females, they have a potential protective effect of oestradiol against the development of hyperinflammatory immune responses. Testosterone the male sex hormone or hypoandrogenism suppresses the immune system and is associated with increased inflammatory cytokines, antibody titres, CD4/CD8 ratios, and natural killer cells, and a decrease in regulatory T cells which is the cause for the increased rate of infection in male compared to females. In our study majority of them were in the age ranging from 56-68(53.0%) which concurs to the study done by Mueller AL et al[9], which suggested that age alone is by far the most significant risk factor for death due to COVID-19. The ageing of the immune system which results in the decreased immune response to fight against infections and also various comorbidities such as obesity, cardiovascular disease, hypertension, diabetes, and respiratory system diseases which are common in old age are contributing factors for increased Covid infection in older persons. The immune responses to vaccination are also often weak or defective in aged persons.

Neutrophils, Lymphocytes, Eosinophils & Plasma cells:

The neutrophils (87%) were the predominant cell type in the BAL fluid among 100 samples in our study followed by lymphocytes (13%) eosinophils and plasma cells being 0%. 87% of them showed 60-96% of neutrophils. Our study is similar to the study done by Chiara Dentone., et al[7] on 64 patients showed that neutrophils (70%) were the predominant cells, lymphocytes, and eosinophils being 1% in BALF of Covid -19 patients.Canini V., etal[11] did a study on 20 BAL fluid samples of Covid 19 patients which showed strong negative correlation between neutrophilia and cytomorphological changes.The Covid -19 virus attaches itself to the ACE-2 receptors which are highly expressed on the pulmonary epithelial cells and enter the pneumocytes which release chemokines and cytokines which in turn increase the neutrophils leading to alveolar damage. Hence cytokine storm explains the increased neutrophilic cellularity in BAL fluids of Covid 19 patients.[12]The cytokine storm can directly damage the lymphocytes and the virus also can directly invade the lymphocytes causing decreased lymphocytic count in BAL fluid[14]. But in a study by Ian Gelarden[13], a comparative study was done on BAL fluid findings between covid and non-covid patients showed that predominate cell types were lymphocytes in Covid patients compared to non-

covid patients. The BAL fluid flow cytometry showed predominately T lymphocytes cells with a mixture of CD4 and CD8 positive cells indicating that immune response to Covid 19 infections is high in their study population. In our study, 13% of the BAL sample showed lymphocyte as the predominant cell type which can be correlated with the above study.A study was done by Baron A, *et al*. on 24 mechanically ventilated patients ,concluded that moderate to severe COVID-19–related ARDS typically shows high cellularity, with neutrophilic alveolitis that could be linked to bacterial or fungal superinfections which were often observed in their study population and was the hallmark of moderate to severe SARS-CoV-2–related ARDS itself. The eosinophils and plasma cells were present in 6% and 18% respectively in our study population and did not show significant increase among 100 samples received. Though Eosinophils and Plasma cells are a frequent component of chronic inflammatory processes there is no significant increase in covid disease in a study done by Vedder et al[3] which is similar to our study.

Alveolar macrophages, Multinucleated giant cells, Type II pneumocytes ,Reactive bronchial epithelial cells & Viral cytological changes:

Though the increase in the number of alveolar macrophages in BAL fluid is not specific to any disease[15], presence of alveolar macrophages are considered for adequacy of BAL fluid specimen (as mentioned in the methods and materials). Alveolar macrophages are the predominant cell type in BAL fluids of normal healthy individuals[13]. They play a major role in immune reaction by presenting the ingested antigens to the T lymphocytes and these activated macrophages produce cytokines that recruit other inflammatory cells. In our study, the predominant cell type was neutrophils 87% and none of them showed macrophage as the predominate type which is similar to the study done by Chiara Dentone.et al[7].

Activated macrophages and activated bronchial epithelial cells appears as binucleate cells and multinucleated giant cells and can be seen in any inflammatory conditions and their presence is not specific to any disease[15]. In our study, the multinucleated giant cells are present in 18% of the study population which signifies ongoing inflammatory process. Pneumocytes type II are highly reactive cells that respond to various pathologic processes and show significant morphologic change and they are rarely seen in normal bronchoalveolar lavage. In our study, the type II pneumocytes were noted to assess the frequency of appearance in covid19 BAL fluid and they were present in 53% of the study population which signifies a going inflammatory process. Reactive bronchial epithelial cells also signifies ongoing inflammatory processes and were found to be in 22% of our study population. Fibrin strands which are necrotic nuclear material and signifies inflammatory process[17]were found in 33% of our study population. Calabrese F et al[16].

Study suggests that Viral aetiology was said to be suspected based on cytoplasmic and nuclear changes in macrophages and epithelial cells like the presence of cell and nuclear enlargement, epithelial desquamation, foamy cytoplasm, larger para nuclear-cytoplasmic vacuoles, nuclear clearing and intranuclear inclusions which were found in our study. Though these findings are non-specific and can be found in other respiratory viral infections, they consider it to represent a potential diagnostic pitfall. In our study, viral cytopathic changes were noted in 10% of the study population. They also suggest that an increased number of macrophages, type II pneumocytes, reactive bronchial epithelial cells & multinucleated cells signify acute lung injury and repair in COVID-19 patients. They concluded in their review that the BAL fluid was found to give information about the lung injury and repair related to COVID-19 which could carry clinical significance and can impact the therapeutic strategies[16].

Conclusion

Coronavirus disease 2019 (COVID-19) is an infectious respiratory disease caused by novel coronavirus SARS-CoV-2. This disease can be asymptomatic or can range from influenza-like illness, pneumonia to the severe acute respiratory syndrome.The common clinical presentation in our study is fever cough and breathlessness.BAL fluid analysis provides significant information about the pathological changes undergoing in the lung which is helpful in the management of COVID pneumonia and also in severe acute respiratory distress syndrome (ARDS). Bronchoscopy has become a need in patients with COVID-19–associated ARDS.

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