Research article

Cytopathological study of pleural effusion in SARS-CoV2 RTPCR positive subjects during pandemic at tertiary care hospital

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ABSTRACT

Introduction and Aim: Respiratory system is the most common system affected by severe acute respiratory syndrome coronavirus 2(SARS-CoV-2).Review of literature reveals that pleural effusion were seen in one third of cases with Middle East Respiratory Syndrome(MERS) but were rarely reported in SARS –CoV2.This study aims to study the cytomorphological changes in Pleural effusion seen in SARS CoV-2 positive cases, to correlate the cytological findings with radiological CT COVID-19 Reporting and Data System(CORADS)scorings and to categorize pleural effusion as isolated cause of SARS CoV-2 or aggravation of underlying other etiologies.

Materials and Methods: Pleural fluid samples of SARS CoV-2 RT PCR positive cases, obtained from patients of all age groups were included in the study. The samples were predominantly from Covid19 wave 1 (March 2020-February 2021) and few from Covid wave 2(March 2021-April 2021). Biochemical analysis, hematological parameters, cytomorphology and associated comorbidity was recorded, and analysis done.

Results: Male predominance was observed. Most common cytological and biochemical finding in pleural fluid analysis was Exudative pleural effusion. Predominant cytological features showed lymphocytic effusion. Radiology showed maximum left sided pleural effusion (42%). CORADS score was 6 in majority of cases and effusion as an isolated cause was seen in few cases.

Conclusion: Pleural effusion is a rare finding in SARS COV-2 induced bronchopneumonia. Effusion is seen with a high CORADS score, and they carry a high risk of mortality. This study suggests having SARS CoV-2 infection as a differential diagnostic entity when we find the mentioned morphological and biochemical findings as discussed in the present study.

Keywords: Covid19; exudative effusion; lymphocytic effusion.

INTRODUCTION

SARS CoV-2 was first identified in Wuhan China, which the World Health Organization declared as pandemic on 30.01.2020 (1). Respiratory system is the most common system affected by severe acute respiratory syndrome coronavirus 2[SARS-CoV-2] (2,3). Review of literature reveals that pleural effusions were seen in one third of cases with Middle East Respiratory Syndrome (MERS) but were rarely reported in SARS –COV2 (2). Common symptoms of Coronavirus infection include flu-like symptoms like fever, cough, fatigue, headache, nausea and in some cases, patients have septic shock, metabolic acidosis (2,3). SARS CoV-2 is diagnosed based on contact history, clinical features, imaging results and RT PCR tests. Symptoms such as pleural effusion, cavitation, calcification, and lymphadenopathy are rarely noticed in SARS CoV-2 patients (4). Chest radiographic findings in SARS COV-2 are typically non-specific. Common computed tomography (CT) findings include ground glass opacities, often with consolidation, typically in bilateral and peripheral distribution (4). A patient with pleural, pericardial effusion and other extra pulmonary lesions indicates that the patient has severe ongoing infection (4). Pleural effusion in SARS CoV-2 patients has been associated with increased severity and high risk of mortality in comparison with patients without pleural effusion. Severe cases of SARS CoV-2 infection have been linked to abundant release of proinflammatory cytokines termed as "cytokine storm" (5). Epidemiology and clinical features of SARS CoV-2 patients have been well delineated, but there are very few studies which have correlated SARS CoV-2 patients with pleural effusion. This study aims to study the cytomorphological changes in Pleural effusion seen in SARS COV-2 positive cases, to correlate the cytological findings with radiological CT COVID-19 Reporting and Data System and to categorize pleural effusion as isolated cause of SARS CoV-2 or aggravation of underlying other etiologies.

MATERIALS AND METHODS

Institutional ethical clearance was obtained for this study numbered as SDMCDS IEC. No. 2021/Medical/Pathology/PG/05. All patients who came to our tertiary hospital and got tested for SARS CoV-2 RT PCR, positive status with pleural effusion were included in the study. Patients with RAT negative but RT PCR positive status were also

included in the study (Flow chart for selection of cases shown in Fig. 1).

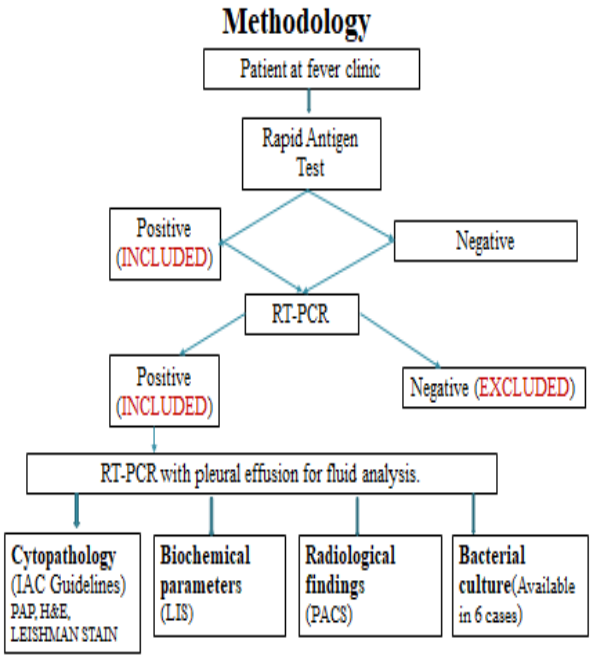

Fig.1: Methodology

Total 31 pleural fluid samples were received. Fluids were sampled and processed using laboratory standard operating procedure (SOP) and slides prepared were stained with Hematoxylin & Eosin, Papanicolaou, Leishman and Ziehl Neelson stains when indicated. Patients demographic data (age and sex), history of present illness, past medical history, volume of fluid collected, hospital course and patient outcome were recorded. Biochemical parameters such as fluid protein, fluid sugar, Lactate Dehydrogenase (LDH), Adenosine deaminase (ADA), Procalcitonin were recorded. Also associated microbiological culture findings were recorded from the laboratory information system. Hematological parameters such as total leucocyte count, differential count were noted. Radiological findings were retrieved from picture archiving and communication system (PACS) for COVID-19 Reporting and Data System score (4,6).

RESULTS

The age distribution of patients ranged from 20 -90 years, the most common age group affected with pleural effusion is 50-60 years. Pleural effusion SARS CoV-2 was found to be predominant in males (61%) with male to female ratio of 1.6:1. Out of a total 31 cases, unilateral pleural effusion was the predominant presentation with 21 cases and bilateral pleural effusion seen in 10 cases.

Biochemical analysis of pleural fluid was done. Protein range was found to be high (> 3g/dl) in all effusion cases, but Fluid glucose was in the normal range. LDH (>200U/l) and ADA (>18U/l) were in the higher range in both transudative and exudative pleural effusion but much higher in exudative pleural effusion (Table 1). In total 31 cases, 18 cases were exudative pleural effusion and 13 cases transudate pleural effusion. CORADS score range for exudative pleural effusion was between 4-6, While for transudative pleural effusion, it ranged between 2-6 (Fig. 2a, 2b). This proved that exudative pleural effusion has higher CORADS scoring than transudate pleural effusion.

Table1: Pleural fluid- biochemical and hematological parameters

Pleural fluid	**Transudate (13 cases) Range and mean**	**Exudate (18 cases) Range and mean**
Protein range <3g/dl: Transudate >3g/dl: Exudate	2.0-5.4 (3.86)g/dl	4.1-6.3 (4.71)g/dl
Sugar range (70-110 mg/dl)	61-128 (96.44) mg/dl	32-190 (115.8)mg/dl
Predominant fluid CELL TYPE	Lymphocytes	Lymphocytes
Fluid cell count (1000 cells/cumm)	60- 350(158.2)cells/cumm	120-90,000 (11,008.88) Cells/cumm
LDH (200 IU/L)	32-868 (241.9)IU/dl	152-1358(402.9)IU/dl
ADA (18 IU/L)	4.83-22.82(11.03)IU/dl	5.91-88(48.64)IU/dl
Total Leucocyte Count (4,000 – 11,000 cells/ cumm)	6830-31830 (11,952 cells/cumm)	9000-14081 (11,660 cells/cumm)
Blood Neutrophils (60-70%)	60-92 (77.5)%	60-93(79.9)%
Blood Lymphocytes (20-30%)	2.5-26.5 (15.2)%	6.6-22(11.6)%
Serum Procalcitonin (0.05) ng/ml	0.01-2.0 (0.4) ng/ml	0.03-0.4 (0.13) ng/ml
Serum C-Reactive Protein (<10 mg/dl)	13.90-208.44(69.79) mg/l	16.83-214.40(78.06) mg/l

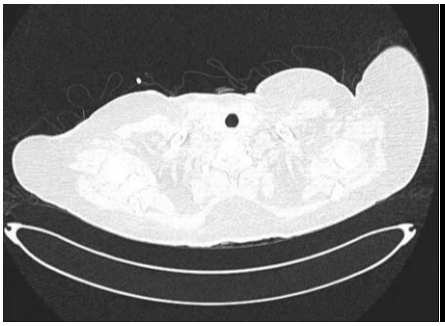

Fig. 2a: CT Chest: CORADS 6

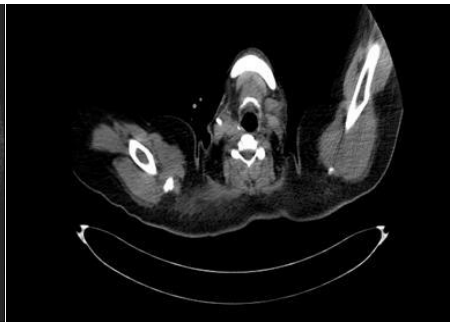

Fig. 2b: CT Chest: CORADS 6

The underlying cause for pleural effusion was studied and observed that only 6 patients had isolated pleural effusion due to SARS CoV2 infection with 4 cases of males and 2 females. Isolated cause of pleural effusion had exudative effusion predominance finding, CORADS score was variable ranging from 2-6, remaining cases were associated with underlying existing morbidities as shown in Table 2.

Table 2: Secondary causes of pleural effusion

Pleural effusion secondary to other comorbidities	Number of patients
Diabetes mellitus Type I	5
Hypertension	6
Acute kidney Injury	1
Thyroid disorders	4
Pancreatitis	1
Nephrotic Syndrome	1
Focal segmental glomerulosclerosis	2
Systemic Lupus Erythematosus	1
Pancreatic pseudocyst	1
Anemia	1
Subacute Intestinal Obstruction	1
Membranous Glomerulonephritis	1

Associated hematological parameters and biochemical assay showed rise in total leucocyte count in all cases, mainly neutrophilia and relative or absolute lymphopenia and thrombocytopenia. Procalcitonin (0.05ng/ml) and C reactive protein(<10 mg/l) were increased in all the cases as shown in Table 1.

Morphological evaluation of all cases revealed predominance of lymphocytes, followed by neutrophils, histiocytes and mesothelial cells. Background showed RBCS and proteinaceous background (Fig. 3a, 3b, 3c and 3d). There was no evidence of hemophagocytosis in the cytological evaluation in any of the cases. Morphology of lymphocytes displayed mild increase in nuclear cytoplasmic ratio with euchromatic nucleus, occasionally single prominent nucleoli noted and basophilic to vacuolated cytoplasm. These lymphocytes did have reactive changes which are seen in viral infection induced effusion. Neutrophils and mesothelial cells had normal morphology. Few cases had more histiocytes infiltrated in slides.

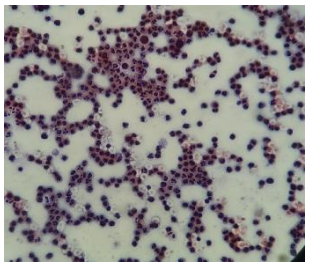

Fig. 3a: 40X PAP Stain: Neutrophil predominance

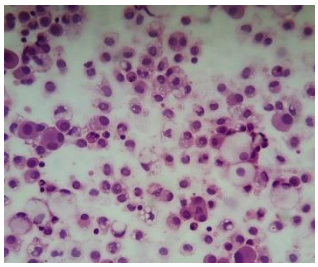

Fig. 3b: 40X H & E Stain. Histiocyte predominance

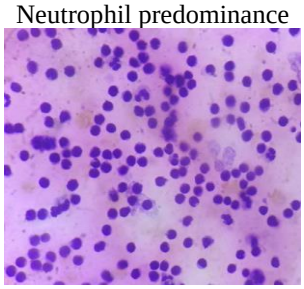

Fig. 3c: 40X Leishman Stain. Lymphocyte predominance

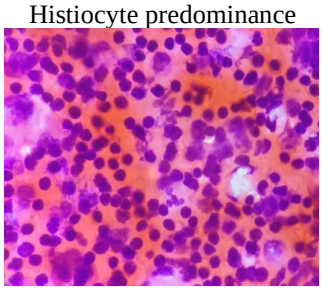

Fig. 3d: 40X H & E stain. Few atypical lymphocytes

Fluid subjected to microbiological examination revealed Gram positive cocci, pus cells, Gram negative bacilli, budding yeast cells, in one patient *Klebsiella pneumoniae* was isolated. Out of 31 cases, 1 death was recorded due to complications of SARS CoV2 infection and rest recovered subsequently with adequate and accurate treatment.

DISCUSSION

As SARS CoV-2 is a new entity and very few studies found in literature could support our findings. In our knowledge there is no Indian study on pleural effusion in SARS CoV-2 infection. This study tried to understand and discuss the pathogenesis and pathological findings in pleural effusion.

Pleural effusion is very common amongst all pleural diseases. Most common symptom of pleural effusion is dyspnea. The more the volume of pleural effusion, more is the severity of dyspnea (6). Pleural effusion is accumulation of fluid between parietal and visceral pleura known as pleural cavity. Pleural fluid acts as a lubricant between two pleural surfaces. The amount of fluid constantly being produced and exchanged is around 0.1mg/kg to 0.3mg/kg. Pleural fluid is produced by parietal pleura vasculature and is

removed by lymphatics present in mediastinal and diaphragmatic pleura. Effusion is caused due to increased production or decreased excretion or any imbalance between normal hematopoietic system. It is due to imbalance between oncotic pressure and hydrostatic pressure, increased capillary permeability or impaired lymphatic drainage (6,7). Pleural effusion is classified into transudate or exudate based on Modified Light's criteria. Most common causes of transudative pleural effusion are left heart failure, nephrotic syndrome, liver cirrhosis due to mechanism of altered hydrostatic or oncotic pressure. Infections, Inflammatory disorders like pneumonia, TB, pancreatitis, rheumatoid arthritis, malignancy cause exudative pleural effusion. Chylothorax due to lymphatic obstruction can also cause pleural effusion. Other rare causes of pleural effusion are due to esophageal rupture, post radiotherapy, due to drug intake and ovarian hyperstimulation syndrome (8).

Spectrum of SARS CoV2 infections ranged from asymptomatic to critical patients and even death, with majority of patients being mild to moderate disease severity cases (9). Pleural effusion, lung cavitation, lymphadenopathy and calcification are other rare findings of pleural effusion. Critical SARS CoV-2 patients with findings of pleural effusion, pericardial effusion, and lymph node enlargement are considered to have increased infection (4).

Common pleural abnormality in SARS CoV-2 patients is pleural thickening followed by pleural effusion (2). Only a minority of patients with SARS CoV-2 develop pleural effusion, but it is more common in patients with severe diseases and co-morbidities (1-4). Diagnostic approach of SARS CoV-2 virus includes RT-PCR, Next generation sequencing, serological diagnosis combined with immunochromatography, CRISPR/Cas13a System and imaging technology like Chest radiography or CT (9). There are not many studies which have compared the cytopathology and CORADS CT scoring. The present study highlights these findings.

Pathogenesis of the virus is discussed well in literature under the concept of pathogenesis of MERS viral infection. This virus is transmitted via respiratory droplets and aerosols from person to person. Inside the body virus enters host receptors and enters host cells via endocytosis. SAR-CoV2 virus is made of four proteins S (Spike protein), M (Membrane protein), E (envelope protein), N (Nucleocapsid protein). S protein has 2 subunits, S1 and S2. It is present on the viral cell surface and is important for attachment and penetration. S1 protein binds to host cell receptors and S2 protein helps in viral and host cell penetration. SAR-CoV2 virus has affinity for ACE receptors present on pulmonary epithelial cells, and it acts as a functional receptor. After the virus enters pulmonary alveolar epithelial cells, it releases its content inside. N protein binds to genomic RNA and M protein facilitates the cellular endoplasmic reticulum. Virus replicates and forms negative RNA strands through transcription. Nucleocapsids are transported to the lumen and exocytosed to extracellular space. Newly formed viral particles keep invading the alveolar epithelial cells and ready for transmission. This mechanism of SARS CoV-2 infection could be one of the pathological pathways causing pleural effusion. The most severe complication of SARS-CoV-2 is the development of pneumonia and acute respiratory distress syndrome (10,11). ARDS is the leading cause of death in SARC-COV2 infection (5). In early phases of SARS –CoV2 infection the release of cytokines and chemokines is delayed but in severe and critically ill patients there is delayed release of Interferons which is required for natural immune defense against virus and increased release of proinflammatory cytokines (IL-1B,IL-6,TNF) and chemokines (CCL-2,CCL-3,CCL-5).These proinflammatory mediators attract inflammatory cells like neutrophils and monocytes in lung tissue as well as in peripheral blood and cause lung damage. Excess release of proinflammatory mediators like cytokines and chemokines is considered as a cytokine storm (5). Additionally, pulmonary vascular endothelium may also be affected by SARS-CoV-2, causing endotheliosis and microangiopathy. Parapneumonic effusion and cavitations can also be caused by SARS CoV-2 infection leading to ischemic parenchymal damage, activation of fibroblasts, fibrosis and activation of cytokines leading to cytokine storm, which also leads to exudate in alveoli (10,11).

In the present study, out of 31 cases, male predominance was seen which was also observed by the study done by Chong *et al.,* (3 males out of 4 cases) and Zhan *et al.,* (87 males out of 153 cases) (4-6). Present study as well as when compared with other literature proved that SARS CoV-2 infection causing pleural effusion was found to be more common in males. Laterality of pleural effusion was unilateral in the present study with 13 cases showing left sided pleural effusion out of 31 cases whereas in Chong *et al.,* and Zhan *et al.,* bilateral pleural effusion was consistent. So, the present study finding was inconsistent with studies compared with Chong et al and Zhan *et al.,*(4,6). Cytological study of pleural effusion revealed lymphocytes to be predominant cell type in present study, which was found to be consistent with Richard *et al.,* Woon *et al.,* Zhan *et al.,* study. Second most common cell type is lymphohistiocytic pleural effusion. 'Hemophagocytosis' which is one of the rare findings seen in SARS CoV-2 patients with pleural effusion is found to be due to immune system hyper reactivity and hemophagocytic lymphohistiocytosis. We couldn't find erythro- phagocytosis as seen in study by Richard *et al.,* Body cavity effusions are relatively uncommon but are

Table 3: Comparison of present study with other studies: Hematological and biochemical parameters

Leucocyte count	**Present study**		**Zhan *et al.*,**	**Chong *et al.*,**
	Transudate	**Exudate**		
Total Leucocyte count (4000-11,000 cells/ cmm)	6830-31830 (11,952 cells/cmm)	9000-14081 (11,660 cells/cmm)	6940 cells/cmm	9563 cells/cmm
Neutrophils (60-70%)	60-92 (77.5)%	60-92 (77.5)%	5350 cells/cmm	-
Lymphocytes (20-30%)	2.5-26.5 (15.2)%	6.6-22 (11.6)%	1020 cells/cmm	-
Biochemical parameters	Present study		Zhan *et al.*,	Chong *et al.*,
	Transudate	Exudate		
LDH(<200U/L)	32-868(241.9) U/L	152-1358(402.9) U/L	225-409 (312) U/L	1810U/L
ADA (18U/L)	4.83-22.82(11.03) U/L	5.91-88(48.64) U/L	-	-
Fluid protein (<3g/dl Transudate, >3g/dl Exudate)	2.0-5.4(3.86)	4.1-6.3 (4.71)	-	24.28
Fluid sugar (70-110 mg/dl)	61-128(96.44) mg/dl	32-190(115.8) mg/dl	-	-
Procalcitonin (0.05 ng/ml)	0.01-2.0 (0.4)ng/ml	0.03-0.4 (0.13)ng/ml		

identifiable on CT imaging in a subset of COVID-19 patients, with pleural effusion present in 5.88% and pericardial effusion in4.55% of cases in 1 meta-analysis. Pleural effusions are more common in patients with severe disease. Pulmonary findings on biopsy have included diffuse alveolar damage, hyaline membranes, and interstitial inflammation composed predominantly of lymphocytes (1). One recent report noted plasmacytosis in a bronchoalveolar lavage specimen. Rare reports of pleural effusion specimens have generally noted reactive mesothelial cells and non-specific mixed inflammation (1). Autopsy studies of the lungs have shown diffuse alveolar damage, often with superimposed bacterial bronchopneumonia (1). Proposed pneumocyte viral cytopathic changes including hyperplasia, multinucleation, and intranuclear inclusion bodies have been reported. Systemic findings at autopsy have included hemophagocytosis, multiple thrombo-emboli, endotheliosis and tissue megakaryocyte recruitment (1).

LDH and fluid protein was increased in present study as well as Zhan *et al.*, and Chong *et al.*, study (4,6). Adenosine deaminase (ADA) increased in present study around 48.64 IU/L in exudative pleural effusion (Normal level: 18 IU/L). Pleural fluid protein increased in present study in both transudate (3.86g/dl) and exudates (4.71g/dl) and is consistent with study done by Chong *et al.*, (24.28 g/dl) (4,6). Pleural fluid sugar increased in present study in exudative effusion 115.8 mg/dl (Normal level : 70-110 mg/dl). Procalcitonin increased in both transudative (0.4 ng/ml) and exudative (0.13 ng/ml) effusion in present study (Normal level: 0.05 ng/ml). Biochemical analysis has been compared with other studies in Table 3.

Hematological parameters in present study suggested neutrophilic leukocytosis and lymphopenia as shown in table 3. Study done by Hui *et al.*, showed predominantly lymphopenia and leukopenia as predominant histological finding (12). Present study did not encounter any pediatric patient with pleural effusion and so we did literature search for the same and found children infected with SARS CoV2 virus have fewer complications as compared to adults (13). Supportive therapy, hydration, calorie intake and oxygen supplementation were included in the basic treatment of children. Children presenting with complications like pleural effusion, deranged hematological parameters and inflammatory markers was a very rare presentation (13). As SARS CoV-2 complicated with pleural effusion is seen in critically ill patients and severe patients it can be used as a prognostic indicator for treatment of patients on early basis (14). Pleural effusion along with parenchymal involvement is also associated with poor outcome (15). Very few studies are available for comparison and discussion, and this is first Indian study with 31 samples been studied in detail during Covid pandemic time.

Limitation of the study

Due to Covid restrictions and ill-defined protocols, few cases of pleural effusion are being studied taking all precautions and considering the risk of exposure of the investigator.

CONCLUSION

Pleural effusion is a rare finding in SARS COV-2 induced bronchopneumonia. Majority of the effusions were exudative with lymphocytic inflammation. Neutrophilia, lymphopenia, and thrombocytopenia are consistent findings in almost all cases. Effusions seen with high CORADS score with abnormal biochemical and hematological parameters are usually critical and carry high risk of mortality.

This study suggests having SARS CoV-2 infection as a differential diagnostic entity when we find the mentioned morphological and biochemical findings as discussed in the present study.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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