



Correlation of cytomorphology of exfoliated mucosal cells present in Viral transport media of COVID 19 positive cases with biochemical and radiological findings.

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ABSTRACT

INTRODUCTION: WHO declared a catastrophic pandemic in form of COVID-19 infection which spread all across the globe in no time ¹. It was observed that this virus was associated with high morbidity and mortality rate ²

Many papers have been published on the morphology of viral cytopathic effects on blood smears or even on saliva samples but to the best of our knowledge, no paper from our region (west UP population in India) has commented upon cytopathic effects of the viral particles on exfoliated cells (from swabs) present in the viral transport media (VTM)

MATERIAL AND METHODS

We planned a retrospective study with the aim of analysing cytomorphological findings of exfoliated mucosal cells present in Viral transport media of COVID-19-positive cases as well as finding the correlation between cytomorphological findings and Biochemical and Radiological parameters. 35 samples were taken from Microbiology Department SMSR and smears made from viral transport media in which nasal and oropharyngeal swabs had been preserved for routine testing of COVID 19 samples.

The smears made were stained by giemsa and PAP stains and observed by 4 independent pathologists to eliminate observer bias and findings recorded. Patients details were collected from Medical records department. All samples were collected under proper COVID protocols and disposed of accordingly with no contamination.

RESULTS

Smears showing marked cytological changes like cytolysis, cytoplasmic grooving etc showed raised Interleukins and d dimer levels indicating more damage than those with preserved architecture. A comparison of findings is given in tables in the text.

CONCLUSION: We can conclude from the above study that viral transport media can serve as an important reserve for Covid-19 infected mucosal cells which show cytopathic changes and hence can be utilized in basic research going on in the field of COVID-19 to ascertain the exact disease pathogenesis

INTRODUCTION

WHO declared a catastrophic pandemic in form of COVID-19 infection which spread all across the globe in no time ^[1]. It was observed that this virus was associated with high morbidity and mortality rate ^[2] The viral composition of this group of virus is quite intriguing and consists of an 3 D enveloped large single stranded RNA genome and does not require a functional nucleus for replication. ^[3]

It has been widely noted and documented that cornerstone for diagnosis of this infectious entity is RT-PCR test and probability of getting best results is when sample is collected from nasopharyngeal or oropharyngeal region or aspirates like sputum and BAL. ^[4] Many papers have been published on the morphology of viral cytopathic effects on blood smears or even on saliva samples but to the best of our knowledge, no paper from our region(west UP population in India) has commented upon cytopathic effects of the viral particles on exfoliated cells(from swabs) present in the viral transport media(VTM).⁵ Hence, we at our hospital lab tried to do exactly that by analysing viral cytopathic effects of exfoliated cells lying in the VTM media(using cyospin technique on VTM material) as well as correlating the cytopathic effects with Interleukin 6 and d-dimer levels along with Chest X-ray findings.

The study was planned with the aim of analysing cytomorphological findings of exfoliated mucosal cells present in Viral transport media of COVID 19 positive cases and to find correlation between cytomorphological findings and Biochemical and Radiological parameters.

MATERIAL AND METHODS

It was a Retrospective study of 35 samples from August 2021 to December 2021 (Delta wave of Covid)The data for IL-6 and D-dimer levels as well as Chest X-ray data for all the cases under study was collected.Morphological changes were analysed separately and cases showing those changes tabulated(Table1).We also correlated biochemical and clinical findings of these cases, specifically Interleukin 6 and d-dimer levels along with Chest X-ray findings.

We used cyospin machine(closed machine) wearing full PPE kit to make cell buttons on slides and stained with MGG and PAP to view findings.Most of the cytomorphological cases were overlapping in the cases recorded. We recorded our findings under the headings of Apoptosis, necrosis, Inflammation, cytolysis, cytoplasmic grooving, Nucleomegaly, and mummified cells. Any other finding was also recorded. Complete confidentiality regarding the subject's information was maintained through all the phases. IEC clearance was applied for.

RESULTS

Morphological Features(n-35 cases)	Degree of change	Number of cases showing changes	Percentage of cases showing changes(%)
Mummified cells	Mild degree	2	5.7
Cytolysis	Mild	22	62.9
Cytoplasmic grooving	Mild	12	34.3
	Moderate	3	8.6
Nucleomegaly	mild	6	17.1
Inflammation			

Neutrophil predominant	Mild(1-2/hpf)	2	5.7
Macrophage predominant	Mild(1-2/hpf)	5	14.3
	Moderate(4-5/hpf)	1	2.9
Apoptosis	mild	6	17.1
Necrosis	Mild to moderate	10	28.6
Fungal elements	Moderate to severe	2	5.7

Table 2: A comparative analysis of Morphological findings in exfoliated cells in Viral Transport media with D-dimer levels .

Features in cases/investigation	Mummified cells	Cytolysis	Cytoplasmic grooving	Nucleomegaly	Inflammation	Apoptosis	Necrosis
D-dimer levels(ng/ml)							
Less than 0.50	0	2	2	0	1	3	4
More than 0.50	0	13	6	0	7	1	6

Table 3 Comparison of Biochemical parameter IL-6 with cytomorphological findings.

Features in cases/investigation	Mummified cells	Cytolysis	Cytoplasmic grooving	Nucleomegaly	Inflammation	Apoptosis	Necrosis
IL-6							
<16.0pg/ml	0	2	0	0	0	1	2
>16.0pg/ml	0	10	6	0	5	1	8

Table 4: Comparison of radiological findings with cytomorphological profile of slides examined

Features in cases/investigation	Mummified cells	Cytolysis	Cytoplasmic grooving	Nucleomegaly	Inflammation	Apoptosis	Necrosis
Chest Xray changes(consolidation and hilar lymphadenopathy)							
Mild	0	2	2	0	0	0	0
Moderate to severe	0	6	6	0	7	2	7

Figures 1 to 7 highlight all the morphological findings as mentioned above.

DISCUSSION

Very few studies have been carried out on mucosal smears to study Covid-19 infection's viral cytopathic effects. We did not find any Indian study done on exfoliated cells in the viral transport media of oropharyngeal, and nasopharyngeal swabs done for routine testing of Covid-19 cases.

The stratified squamous epithelium of the oral mucosa contains ACE2 receptors ^[6-7] Hence this mucosa is target for the initiation and progression of COVID-19 infection. Once the virus makes an entry it causes replication in the host cell and importantly causes reduction in ACE2 receptors which become a fertile soil for development of morbidities. ^[8,9] It has been documented in the literature that in order to obtain oral epithelial cells, two widely used modalities used are exfoliative cytology and brush biopsy. We know that these brush cytology swabs are carried in VTM (Viral transport media) in labs for testing and it is but natural that some of these cells will fall off in the VTM while being transported. These brushed-off cells were our study target.

Exfoliative cytology is effective screening tool and can be used to collect samples for other investigative techniques such as reverse transcription PCR, Western blot analysis, and immunofluorescence.

Very few studies of such nature have been done. Exfoliated cells of oral mucosa have been studied in one study for morphological changes and in cytological analysis the authors found that superficial squamous cells, non-keratinizing type were round or oval, larger sizes with pyknotic nuclei, with low N:C ratio. The intermediate cells were smaller in with larger nuclei. ^[8-11]

Few studies depicted the cytomorphology of infected cell changes due to cytolysis and contributes to cell damage as result of viruses' virulent pathogenesis. ^[12-13] We too found various cytopathic effects as listed in table 1 which we attribute to similar pathogenesis. Some other studies done on non-Covid viruses have shown to have viral cytopathic effects of saliva and exfoliated cells in the mucous. ^[14]

Morphological studies on the cytopathic effect of exfoliated cells in VTM in particular have not been conducted so far, although there are differences in the mechanism of cytopathic effects in all these viral infections.

A study in the literature explained cytomorphology of damaged cells taken from saliva of infected covid patients. where the authors hypothesized the degree of damage to mucosal epithelium leading to symptoms like sore throat, etc. ^[15] They tried to define apoptotic and necrotic activity in light microscopy.

It is the need of the hour to have more such studies based on cytomorphology of exfoliative cells in a viral transport media. This will help us to segregate effects like Apoptosis, Necrosis and Necroptosis.

In the study done on saliva from clinically healthy, it was observed that the morphology of exfoliative cells appear as necrotic cell ^[16]. We in the VTM found exfoliated cells with cytopathic changes which were well recognized in light microscopy.

The rapidity of cell turnover leads to accumulation of enucleated cells and which depicted as squames in the smears examined from oral mucosa ^[17]

An inflammatory reaction is the result of interaction between the host and virus and affects health status. We saw inflammatory cells in exfoliated material or VTM signified intense pharyngitis and

the role of mononuclear cells(macrophages>neutrophils) in our case in the pathogenesis of the Covid-19 cytokine storm. In another study mapping of inflammation in the oral cavity of Covid 19 cases(by taking oropharyngeal swabs) they saw that in COVID 19 positive cases amount of inflammation was lower when compared to uninfected cases. However, further research is needed on these findings.^[18-19]

In keeping with the inflammation and cytokine storm in mind we also tried correlating biochemical and radiological findings with cytomorphological findings and found that neutrophils and macrophages were inevitably seen in VTM smears of cases with increased d dimer,IL6, and chest x-ray changes hence proving the pathogenetic mechanism of Covid-19 is cytokine-mediated.(Table 2-4)

In one study where the authors compared various biochemical and CBC findings in COVID they saw that 75.81% patients showed higher than normal values of D Dimer while 24.19% patients had a normal value. Similar findings were observed in another study where D-dimer was markedly higher among COVID patients.^[20]

However, we could not find studies correlating X ray , cytomorphological findings of cells in Viral transport media, and biochemical parameters IL6 and D dimer together.

Only a few studies have been done on cytomorphological changes in oral mucosa in covid let alone exfoliated cells in VTM media hence the only limitation of this study is inability to define the role of VTM media itself in causing morphological changes(although media are generally chemically inactive) as well as we need bigger sample size to confirm our findings.

CONCLUSION

We can conclude from the above study that viral transport media can serve as an important reserve for Covid-19 infected mucosal cells which show cytopathic changes and hence can be utilized in basic research going on in the field of COVID-19 to ascertain the exact disease pathogenesis of COVID-19 by molecular studies, which in turn may aid in formulating good therapeutic and prognostic modalities for this currently unknown monster killing people acutely as well as through the Long Covid syndrome.

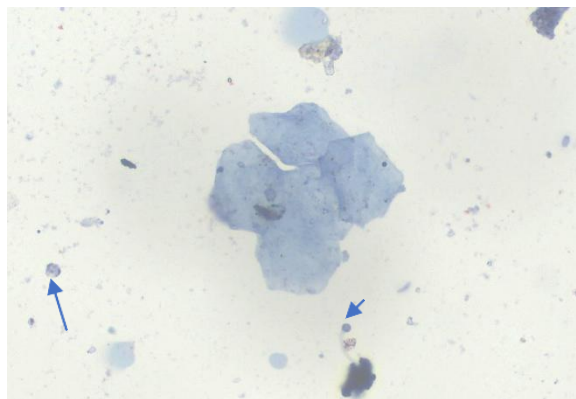


Figure 1:Anucleated squames with occasional degenerating ?? inflammatory mononuclear cell (arrow)seen in smear prepared from viral transport media

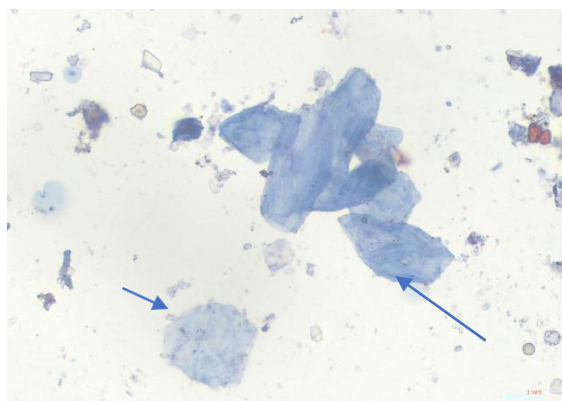


Figure 2:Anucleated superficial squamous epithelial cells with cytoplasmic folds(arrows) in giemsa stained smears

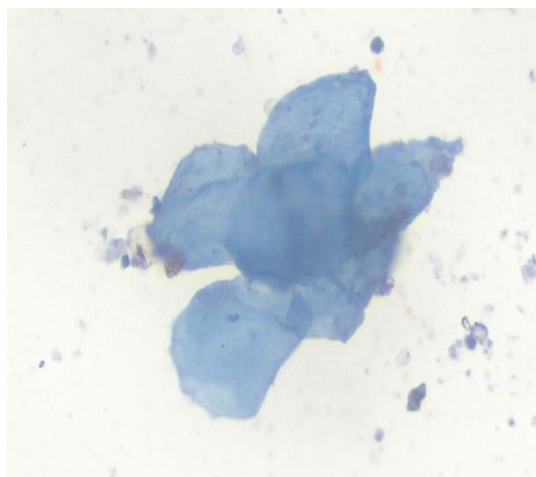


Figure 3: Anucleate squames and cytolysis in smears made from transport media

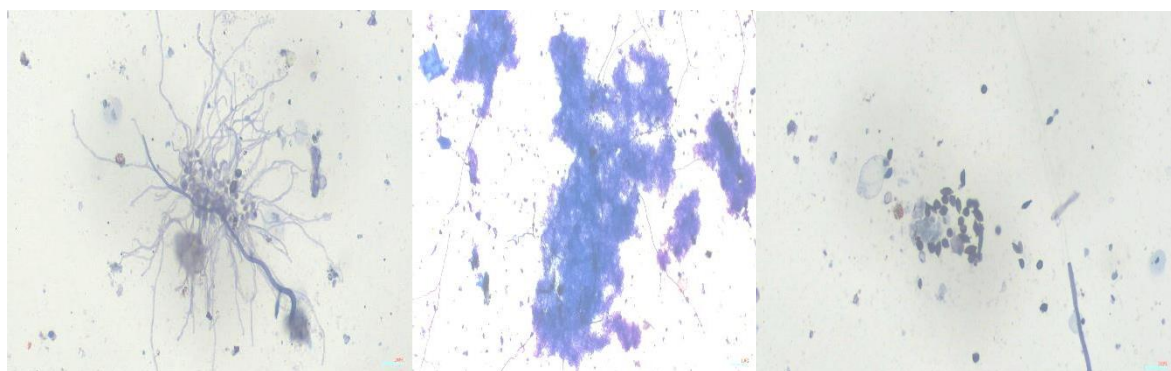


Figure 4:Fungal elements seen in some smears prepared from viral transport media

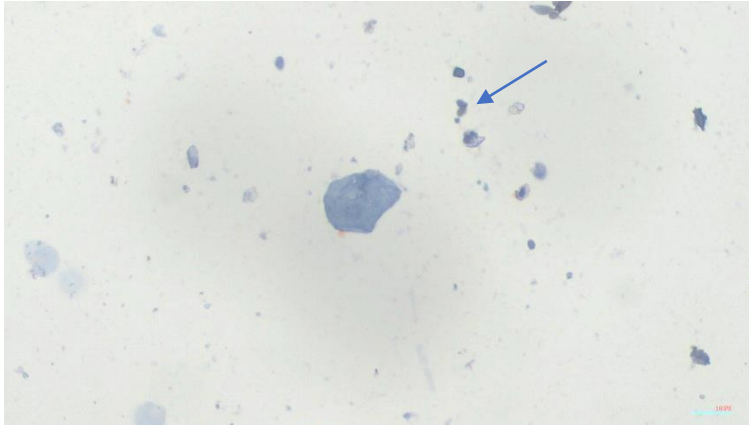


Figure 5:Single squame showing cell degeneration a faint hint of degenerating nuclei, cytoplasmic folding and hint of vacuole formation. Background shows degenerating inflammatory cells(arrow)



Figure 6a and b:Nucleomegaly and cytoplasmic grooving in superficial squamous epithelial cell

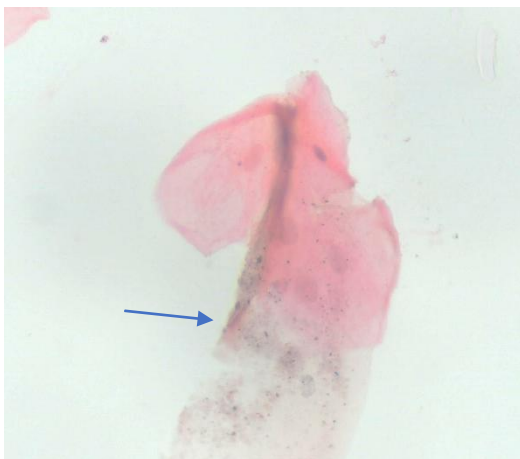


Figure 7:Degenerating cells with nucleomegaly and cytoplasmic pigmentation(arrow,degeneration)

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