SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: 10.36346/sarjpm.2023.v04i01.001

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Cytological Changes in Sputum Sample among Sudanese COVID-19 Patient among Different Town in Sudan 2021

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Article History: | Received: 05.12.2022 | Accepted: 14.01.2023 | Published: 18.01.2023 |

Abstract: ***Background*:** Coronaviruses are large groups of viruses that cause illness in humans and animals. This virus has a higher degree of lethality than other endemic viruses Covid-19 diagnosis is based on viral detection, isolation, or serological changes. For cytopathic effects, sputum cytology is routinely performed for nearly all patients with chest symptoms. ***Objective*:** The study aimed to find Cytological change in sputum samples of COVID -19 patients in Shendi town. ***Materials and Methods:*** This study includes 45 subjects. Thirty were known as Covid 19 positive cases by PCR, and 15 were healthy subjects. Two sputum smears were collected from the study group then fixed in (95% Ethanol alcohol) and stained with Papanicolaou and Giemsa stains, then screening for any cytomorphological changes and microbes. ***Result*:** In cytology screening, intranuclear inclusion and inflammatory cells were observed in all sputum samples (100%) of patients infected with covid-19, other cytomorphological changes that appeared were nuclear atypia (96.6%), cytolysis (64.4%) and destructive change (fibrocytes) in (30%). None of the cytomorphological changes mentioned above were observed in the control sample. Co-infection with bacteria and fungi (aspergillus & Candida) exists in (66.6%) of Covid-19 patients. There was a statistically significant correlation between infection with covid-19 and the cytomorphological changes characterized in their sputum *P. value* was less than (0.05). Intranuclear inclusion in this study was a specific and sensitive indicator for Covid-19. There was no correlation between the period of infection and distractive change appearance or invasion with micro-organisms of the study group (*P. value* more than 0.05), But confections with microorganisms were statistically correlated to the age of the study group (*P. value* less than 0.05). ***Conclusion:*** Cellular changes and consistency of Sputum and saliva samples can provide inexpensive, rapid diagnostic, minimally invasive techniques for Covid-19, particularly in densely populated developing countries and areas.

Keywords: Cytology, Coronavirus, Sputum, Papanicolaou stains, Covid-19.

INTRODUCTION

Coronaviruses are massive organizations of viruses that purpose infection in human beings and animals. Rarely, animal coronaviruses can evolve and infect human beings after which unfold among human beings consisting of has been visible with MERS and SARS. The outbreak of Novel coronavirus disease (COVID-19) became to start with observed in a seafood marketplace in the Wuhan metropolis in Hubei Province of China in mid-December, 2019, Current available evidence for COVID-19 suggests that the causative virus (SARS-CoV-2) has a zoonotic source closely related to bat- origin SARS-like coronavirus. It is an enveloped RNA beta coronavirus associated with the Severe Acute Respiratory Syndrome (SARS) virus, and the virus has been proven to apply the angiotensin-changing enzyme 2 (ACE2) receptor for cell entry [1]. COVID-19 is a disease caused by the virus SARS-CoV-2. The disease was previously referred to as a 2019 novel coronavirus (2019-nCoV), but in February, the

Citation: Mariam Faez Habib, Asma Alameer M. Zeen, Safia M. Hussain, Tibyan Abd Almajed ALtaher, Ghanem Mohammed Mahjaf, Mosab Nouraldein Mohammed Hamad (2023). Cytological Changes in Sputum Sample among Sudanese COVID-19 Patient among Different Town in Sudan 2021. *SAR J Pathol Microbiol*, 4(1), 1-6.

WHO announced a new official name that is the coronavirus disease (COVID-19). COVID-19 has spread and involved more than 198 countries in the world. As of May 26, 2020, the World Health Organization reported 5,404,512 confirmed cases and 343,514 deaths [2]. This virus has a higher degree of lethality than other endemic viruses, and it is also more lethal to humans compared to the earlier emerging outbreaks of SARS-CoV-1 in 2003 and Middle East respiratory syndrome Coronavirus (MERS-CoV) in 2012 [3]. Sputum cytology is automatically accomplished for almost all patients with chest symptoms, and a cognizance of the cytological functions suggestive of intense acute respiration syndrome is of the maximum significance for case recognition [4]. Real-time-PCR (RT-PCR) from nasopharyngeal swabs is routinely used for the detection of COVID-19. The positive rate of RT-PCR for nasopharyngeal samples was reported to be 63% in the early stage of COVID-19; therefore, a significant number of patients may have false negative results. Previous data from SARS-CoV infections showed that RNA levels first peaked in upper the respiratory tract but remained higher in lower respiratory tract specimen's weeks after the onset of the illness. The same may be also expected with COVID-19 infections. Detection of early cytomorphological changes in the lung from bronchial fluid or BAL may, therefore, help with the timely start of COVID-19 surveillance and supportive therapy [5].

MATERIALS AND METHODS

Study Design

This is a hospital-based descriptive cross-sectional study aimed to detect cytopathic effects on the sputum of COVID-19 patients among Sudanese patients during the period from June to November 2021.

Study Area

This study was held in River Nile State-Shendi town. Shendi locality- River Nile State- Sudan. Shendi is a town in northern Sudan on the east bank of the River Nile 150 km northeast of Khartoum (16°41'N 33°25'E). The area is inhabited by the Ga'aleen Tribe.

Study Population

This study includes 45 individuals classified as 30 Covid-19 patients as the case group and 15 healthy individuals as control confirmed by PCR.

The Study Sampling

This study with thirty patients having one sample. Of these, two slides from each patient were selected for our study. And 15 samples for control. Stained by pap and Giemsa stain.

Specimen Collection

Thirty smears from a patient with covid-19, and fifteen control (from a healthy individual).

Sample Processing

A sputum sample was taken from a patient and smeared in 2 microscopical slides (one wet and one dry) then fixed in 95% ethanol alcohol. Then the smear will is stained with PAP and Giemsa stain then mount with DPX and examine under the light microscope.

Data Collection Tools

Data was collected by using a questionnaire.

Data Analysis

Data were analyzed with a chi-square test using the SPSS version 16.5 computer program.

Ethical Considerations

The study will be approved by the department of Histopathology and Cytology in Medical Laboratory Sciences at Shendi University, the study will be matched to the ethical review committee board. Sample collection will be done after a verbal agreement with the participants. Permission for this study will be obtained from the local authorities in the area of study. The aims and the benefits of this study will be explained with the assurance of confidentiality.

RESULTS

Table 1: Show distribution of age group among study group

Age group	Frequency	Percentage (%)
Less than 50 years	22	48.9%
More to 50 years	23	51.1%
Total	45	**100%**

Table 2: Show Distribution of sex among the study group

Age group	Frequency	Percentage (%)
Male	39	86.7%
Female	6	13.3%
Total	45	**100%**

Table 3: Show distribution of fibrocyte among the study group

Variables		**Covid-19**				**Total**
		Negative		***Positive***		
		No	**%**	**No**	**%**	
Fibrocyte	*No*	15	33.3	21	46.7	36(80%)
	Yes	0	0	9	20	20%)(9
Total		**15**	**33.3**	**30**	**66.7**	**45(100%)**

P. value= (0.02)

Table 4: the correlation between the period of infection and fibrocytes

Variables		**Period of infection**		**Total**
		Less than15	**More than 15days**	
Fibrocyte	*Not exist*	21	12	9
	Exist	9	5	4
Total		**30**	**17**	**13**

P. value = (0.9)

Table 5: Show distribution of microorganism among the study groups

Variables		**Covid-19 Presence**		**Total**
		Negative	***Positive***	
Micro-organism	*Not exist*	15	10	25
	Exist	0	20	20
Total		**15**	**30**	**45**

P. value= (0.000)

Table 6: Correlation between types of microorganism's period of infection among study groups

Variables		**Period of Covid-19 infection**		**Total**
		Less than 15 days	***More than 15 days***	
Micro-organisms	*Negative*	4	6	10
	Bacteria	4	5	9
	Candida	3	6	9
	Aspergillosis	2	0	2
Total		**13**	**17**	**30**

P. value= (0.38)

Table 7: The correlation between microorganisms and age

Variables		**Age**		**Total**
		Less than 15 days	***More than 15 days***	
Micro-organisms	*Not exist*	18	7	25
	Exist	4	16	20
Total		**22**	**23**	**45**

*P. value= (*0.01)

Table 8: The correlation between the covid and cellular atypia among study the group

Variables		**Covid**				**Total**	
		Negative		***Positive***			
		No	**%**	**No**	**%**	**No**	**%**
Cellular atypia	*No*	15	33.3	1	2.2	16	35.5
	Yes	0	0	29	64.5	29	64.5
Total		**15**	**33.3**	**30**	**66.7**	**45**	**100**

P. value= (0.000)

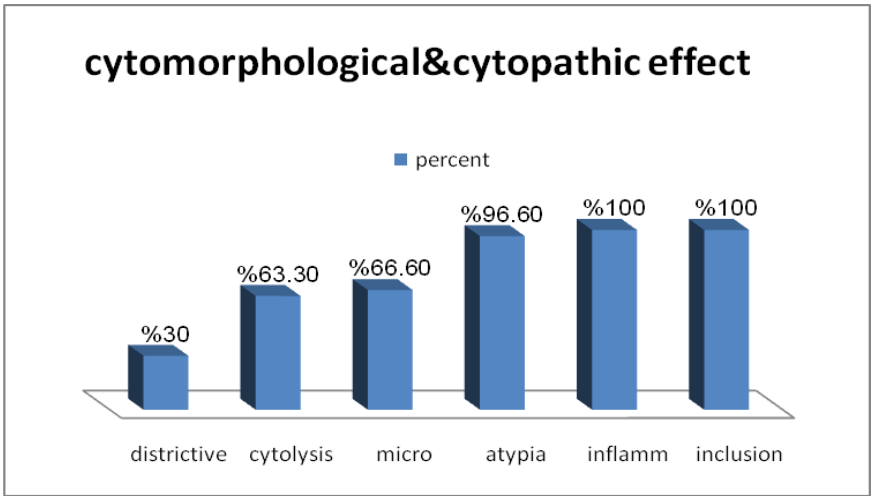


Figure 1: The percentage of cytomorphological change and cytopathic effect in the covid-19 Patient.

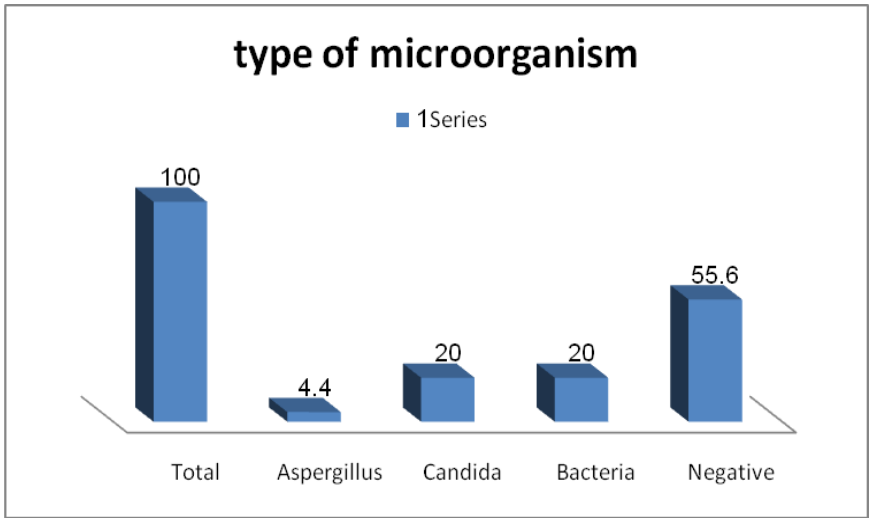
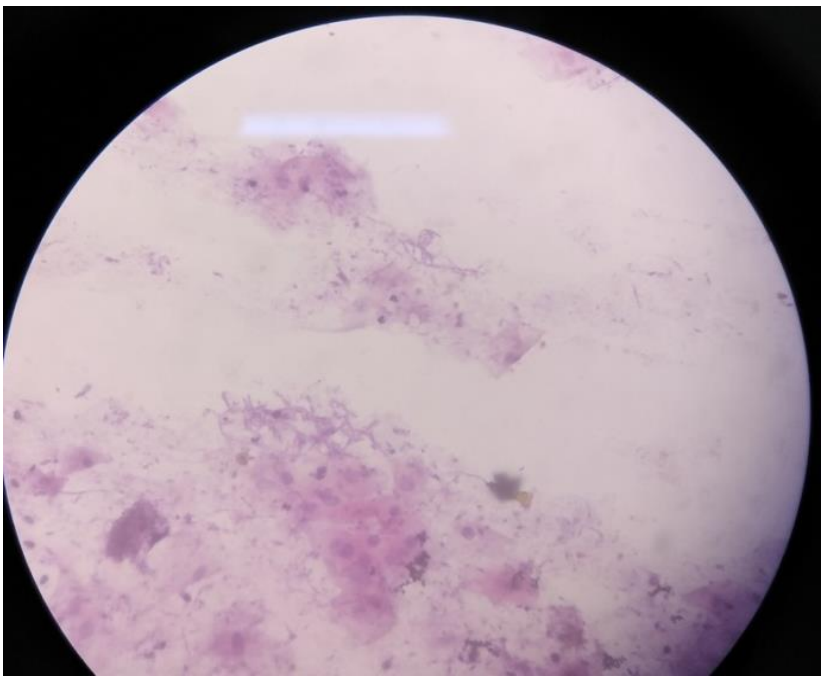
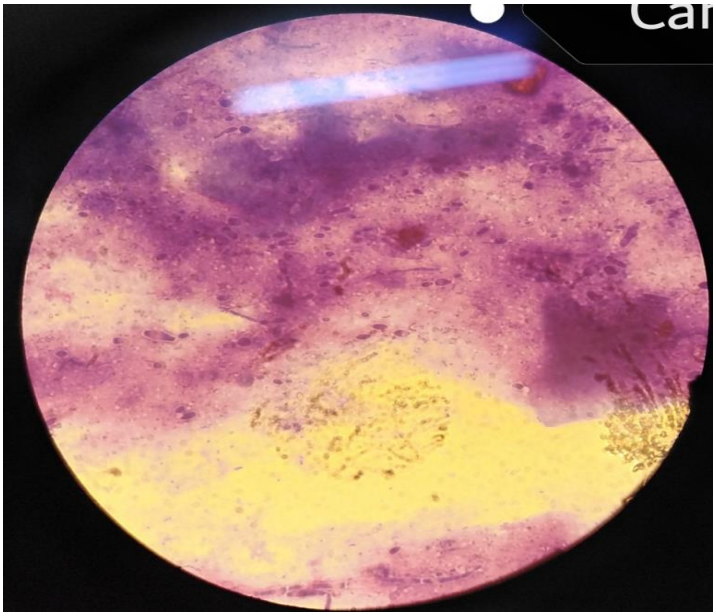


Figure 2: The correlation between the covid-19 and type of microorganism



Photomicrograph 1: show internuclear inclusion in sputum smear stain by Pap stain with (HPF)



Photomicrograph 2: show coinfection candida in sputum smear stain by Giemsa stain with (HPF)

DISCUSSION

Covid-19 can Cause Various Conditions Including respiratory, enteric, and neurological diseases, and led to a pandemic that has affected millions worldwide [6]. Sputum cytology is routinely performed for nearly all patients with chest symptoms, and an awareness of the cytological features suggestive of the severe acute respiratory syndrome is of the utmost. This study includes 45 subjects. Thirty of them were known as Covid-19 positive cases, 13 were healthy subjects and two were suspected cases of corona but confirmed to be negative by PCR. The age range among the study group was 38 to 73 years. Most of the patients were in age more than 50 years 23/45 (51.1%). The sex distribution among the study group was 39/45 (87%) male and 6/45(13%) female. In cytology screening intranuclear inclusion and inflammatory cells were observed in all sputum samples (100%) of the patients infected with covid-19 and cellular atypia (96.6%), there were also cytolysis (64.4%) and destructive change (fibrocytes) in (30%). None of the cytomorphological changes mentioned above were observed in the control sample. Intranuclear inclusion, described by some authors as nuclear chromatin clearing with peripheral margination of the nuclear membranes-, inflammatory cells, multi-nucleated cells, giant cells, high N/C ratio prominent nuclei, and cellular atypia, synthesis appear in this study were in agreement with Stadlmann S, *et al.,* (2020) &Marco Giani (2020) (who found the similar Cytopathological changes in bronchial lavage of Covid-19 patient. Also, the current study results are in line with G M K Tse, *et al.,* (2021) who found in the study of fifteen sputum samples from patients were compared with 25 control samples. In patients with SARS, loose aggregates of macrophages were seen more frequently in the sputum. These macrophages frequently showed morphological changes, such as cytoplasmic foaminess, vacuole formation, and nuclear changes (including multinucleation and a ground glass appearance) when compared with the control samples**.** Conclusion, the Cytopathological detection of virus-infected multinuclear macrophages in bronchial fluid or (BAL) might add substantial early information in COVID-19 [4]. Remarkably, all authors observed multinucleated giant cells which were not further described. In MERS-CoV infections, multinucleate syncytial cells, some of them with prominent eosinophilic nucleoli, were also described [7]. But Stedman s describes more multinucleated giant cells were of histiocytic origin. Thus, macrophages in COVID-19 infection may also be targets for COVID-19 besides pneumocytes as was described in pneumonia caused by measles [5]. Alveolar fibrocytes may originate from the recruitment of circulating fibrocytes to the lung mainly through the CXCR4-CXCL12 axis and from local differentiation of monocytes in alveolar spaces, which is promoted by profibrotic cytokines [IL-4, IL-13, transforming growth factor-b (TGF-b1)] and inhibited by serum amyloid P (SAP) component, interferon-c, and IL-12 [8]. In the current study fibrocyte was observed in 9/30 (30%) of covid patient sputum smears there was a statistically significant correlation between fibrocyte and covid-19 *P. value* (0.02). Liu J, Zheng X, Tong Q, *et al.,* (2020) mention advanced disease, fibroblast proliferation with thickened alveolar septa suggesting a fibrotic process has been reported [7]. This means that covid-19 infection is an acute destructive infection of the lung, but no statistically significant value was found in the correlation between fibrocyte and period of infection *P. value* (0.9). Remarkably In cytology screening a Co-infection 20/30 (66.6%) was observed among Covid-19 patients, types of this infection were bacteria 9/20 (45%) and fungi 11/20 (55%) (Aspergillus & Candida) and there was a statistically significant correlation

between co-infection and covid-19 *P. value* (0.00). None of the microorganisms mentioned above were observed in the control sample. Microorganism observation in this study was in consistent with what was mentioned by Marco Giani *et al.,* (2020) the existence of Aspergillosis in COVID-19 BAL specimen {6} and what was mentioned by Stadlmann S, *et al.,* (2020) in their case report about coinfection with *Staphylococcus aureus* in COVID-19 BAL specimen [5]. No Statistically significant value was found between Co-infections to covid-19 and the period of covid-19 infection *P. value* (0.38), but statistically, a significant value was found with age and covid-19 patients' *P. value* (0.01). In this study, most microorganism invasions appear among the elder 16/20 (80%), more studies need to explain using microbiological techniques to find whether infections among the elder were before or after covid- 19. In this study, a statistically significant correlation was found between cellular atypia and covid-19 infection *P. value* (0.00), this study goes with Marco Giani (2020) who stated Desquamated pneumocytes inside covid-19 patients [9]. But this result not far of what mention with Hanley B, *et al.,* who stated that pneumocyte hyperplasia.

CONCLUSION

Patients who were diagnosed with COVID-19 reviewed cytopathic effects including intranuclear inclusion. There was an acute destructive change in the lung (fibrocytes). There were correlations between co-infection (bacteria & fungi), the age of patients, and the period of infection. There was also nuclear atypia of pulmonary cells. Sputum cytology can provide a rapid diagnosis by minimally invasive techniques for covid 19.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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