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The Detection of Nasopharyngeal Cytological smears Among Positive COVID-19 Test Patients in Omdurman Medical Military Hospital (Sudan).

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Abstract: Background: *The role of the cytology laboratory in a patient with known COVID-19 is limited.* ***Objectives:*** *The study aimed to detect the nasopharyngeal cytological smears changes among positive COVID-19 test patients in Omdurman medical military hospital in Sudan.* ***Materials and methods:*** *This study was carried in Sudan.Nasopharyngeal and oropharyngeal cytological smears were adopted. The total number of samples were 150 samples. The nasopharyngeal and oropharyngeal swabs were centrifuged, smeared and parallel numbers fixed in 90% ethyl alcohol and other air dried fixed.* ***Results:*** *In terms of nasopharyngeal cytological smears, the microscopic examination of the nasopharyngeal smears did not reveal important cytological and pathological alterations. The observation of the nasopharyngeal smear also revealed an abnormally high number of lymphocytes (Lymphocytosis) and a lot of dry and wet mucous amount in different population which made shedding for a lot of inflammatory cells. Therefore, the cytopathic alterations were of little significance. A possible explanation could be the cytological nasopharyngeal patterns associated with nasal or throat scraping method not nasal swap which is a non-invasive method to take a sample of nasal mucosa. Each of the nasopharyngeal swabs did not reveal epithelial cells or glandular cells. A careful and thorough search failed to detect any viral inclusions and this due to the absence of squamous cells. This suggests that claims of viral inclusions with covid-19 disease may, rather, represent nonspecific cytopathic effect in nasopharyngeal swabs such as lymphocytosis.* ***Conclusion:*** *The smears were revealed a lot of dry and wet mucous amount in different population.*

Keywords: Nasopharynx, cytological smears, COVID-19, Omdurman.

Introduction:

The role of the cytology laboratory in a patient with known COVID-19 is limited [1]. In analogy to the role of the cytology laboratory in SARS, it is mainly to rule out superimposed pulmonary infections in sputum and other respiratory specimens [2]. The cytologic features seen in nasopharynx are nonspecific and reflect the underlying acute pulmonary injury pattern.[83] They consist of the presence of increased number of macrophages, forming loose macrophage aggregates [3]. The macrophages may also show cytoplasmic changes, including the presence of foamy cytoplasm or larger cytoplasmic vacuoles or nuclear changes, including multinucleation and ground glass appearance of nuclei [4]. Because bronchoalveolar lavage (BAL) fluid is sometimes obtained for viral identification [5, 6, 7, 8] and is occasionally positive when nasopharyngeal and oropharyngeal swab samples are negative [9], an aliquot may also be sent to the cytology laboratory. However, the cytologic findings of BAL samples of patients with COVID-19 are not yet reported

[10]. In patients with SARS-CoV-19, cytological examination of nasopharyngeal swabs reportedly showed high numbers of neutrophils and macrophages [11]. Based on the histopathology of SARS, MERS, and COVID-19, nasopharyngeal swabs specimens may also show squamous metaplasia, and features of repair, together with the presence multinucleated cells and highly atypical alveolar type 2 pneumocytes showing cellular and nuclear enlargement, prominent nucleolim and chromatin clearing. These cyto-morphologic features may represent a potential diagnostic pitfall [11].

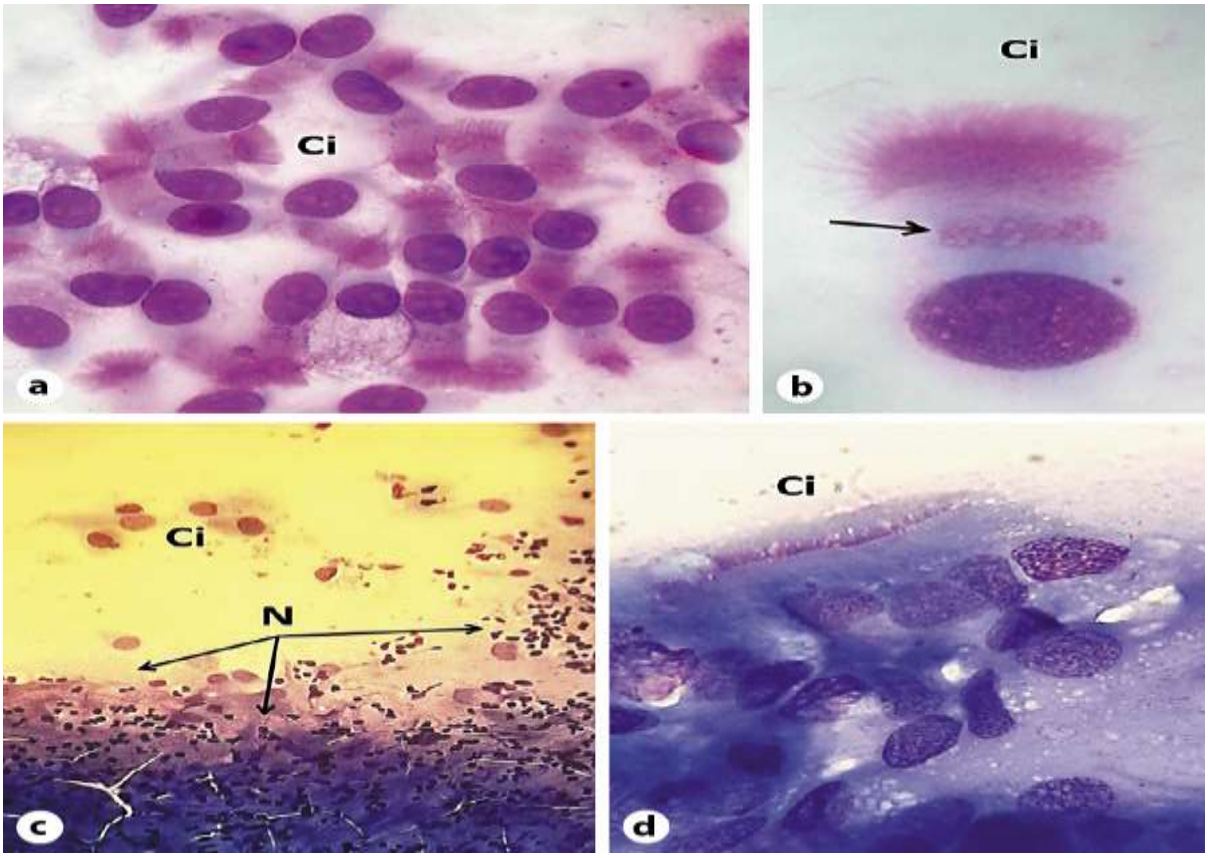

Fig.1.2: **a** Nasal cytology within normal limits (May-Grünwald-Giemsa, ×800× magnification). **b** Normal ciliated cell. The arrow indicates the "hyperchromatic supranuclear stria" (SNS; May-Grünwald-Giemsa, ×2,000 magnification). **c** Nasal cytology in a COVID-19 patient (May-Grünwald-Giemsa, ×400 magnification). **d** Nasal cytology in a COVID-19 patient evidencing absence of the hyperchromatic SNS (May-Grünwald-Giemsa, ×1,000 magnification). Ci, ciliated cell; N, neutrophils.

While the immune response to SARS_COV_2 is still not fully understood, most of individuals suffer from uncontrolled viral replication resulting in multi-organ failure in the most severe cases. The lack of informative cytological changes and the efficiency of some immunosuppressive therapies in severe or non-severe cases of the disease give an excuse for this research. Currently, the evidence base for the clinical management of COVID-19 is mostly limited to case series and other relatively small observational studies. Despite the limitations, this research will provide useful insight into the manifestations of the disease.
The study aimed to detect the nasopharyngeal cytological smears changes among positive COVID-19 test patients in Omdurman medical military hospital in Sudan.

Material and Methods

Study Area & population
The study was conducted at Omdurman. It is a city in Sudan.
Study design:
This was an analytic prospective study, designed for the detection of nasopharyngeal cytological smears among positive COVID-19 test patients in Omdurman medical military hospital in Sudan.
Study location:
The study was performed in Omdurman medical military Hospital, Department of Histopathology & Cytology.
Sample size:

Total usage of nasopharyngeal and oropharyngeal cytological smears was adopted. The total number of samples were 150 samples. The nasopharyngeal and oropharyngeal swabs were centrifuged, smeared and parallel numbers fixed in 90% ethyl alcohol and other air dried fixed.

Sample collection:

Cytological smears

Nasopharyngeal and oropharyngeal swabs from 150 PCR-confirmed COVID-19 patients collected from patients in viral transport medium were used for detection of SARS COV2 by qRT-PCR technique. RNA was extracted using vitrogen kit, and qRT-PCR was performed using Taq Path kit (Thermo Fisher Scientific) on ABI 7500-Fast, Thermo-fisher machine. The cycle threshold (Ct) value for each sample was recorded. We collected the positive test swabs, centrifuged by Hettich centrifuge (Benchtop centrifuge, MIKRO 220 classic, France) and smeared in frosted slide (Microscope slides, white color frosted, 25*75, 50PCS/Box, China) for analysis using Diff-Quick stain (RAPI-STAIN, MS 408-2, Saudi Arabia) and Hematoxyline stain (HEMG1-OT, South Eastern Europe) and Eosin 1% stain (Eosin G, Ref: 15405/0025, South Eastern Europe). All cytological smears examined under microscope (Olympus CH-2 Binocular, Japan) for analysis.

Analysis of Data :

SPSS Statistics v26.0 was used to analyze the data. Qualitative variables were conveyed as proportions and frequencies. Tests of normality were done and normally distributed continuous data were presented as mean and standard deviation (SD). The association between COVID-19 positivity, cytological changes was tested using *p* values. Statistical significance was demarcated at *p* values of ≤ 0.05.

Ethical Consideration:

Ethical approval was obtained from the hospital administration and the local health authority. Ethical considerations were fulfilled by obtaining verbal informed consent from all the participants who fit the study inclusion criteria. No threat or pressure was imposed on the participants who denied participation in the study. The confidentiality of all the participants was maintained.

Inclusion and Exclusion criteria:

Inclusion criteria:

The inclusion criteria were included all ages of known positive COVID-19 test male or female gender which are attended Omdurman medical military hospital. **Exclusion criteria:**

This research was excluded all known negative COVID-19 test male or female gender which are attended Omdurman medical military hospital.

Methods:

Test procedure:

Cytological smears:

Diff-Quick stain:

1. Fix air dried smears in stabilized fixative (Solution C). Staining can commence after 5 seconds fixation. For routine staining a longer period is recommended.
2. Transfer slides directly to buffered mixture of eosin (Solution A) and dip five times.
3. Transfer slides directly to buffered mixture of Azure/Methylene blue (Solution B) and dip five times.
4. Wash briefly in distilled water and allow to dry.
5. Examine under oil immersion lens of microscope.

Hematoxyline and Eosin stain:

1. Fix smears directly in 95% ethanol for 15 minutes.
2. Rinse in tap water.
3. Stain in Hematoxyline solution for 5 minutes.
4. Rinse in tap water.
5. Stain in Eosin solution for 2 minutes.
6. Dehydrate, clear, cover slip and examine under microscope.

Assessment of outcome of each group:

Five parameters of assessments were forwarded to an experienced pathologist and cytologist with completely blinded technique. They were asked to write their comments on the cytological changes and the accuracy of rapid COVID-19 test.

Results

Table (1) The accuracy of rapid COVID-19 test among positive COVID-19 real time PCR test.

The accuracy of rapid COVID-19 tests among positive COVID-19 real time PCR test.

Standard Q Covid-19 IgM/IgG Combo Test	RT.PCR	IgM	IgG	Covid-19 patients (N=150)	Interpretation
	Positive	Positive	Positive	39	Active infection
	Positive	Negative	Positive	111	Post infection

Table (1) shows that rapid antigen test is most sensitive in all individuals which showed positive. Furthermore, there was a highly significant correlation in status among positive patients real time PCR COVID-19 with rapid COVID-19 test ($P < 0.001$).

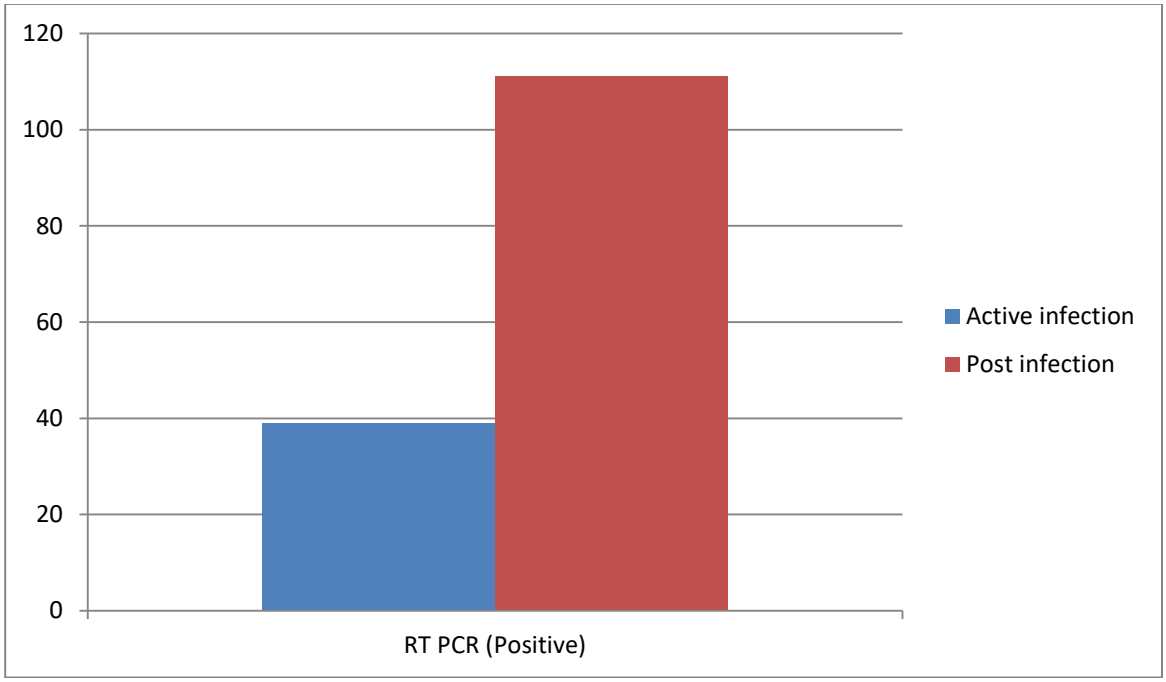

Fig.1: The accuracy of rapid Covid-19 test among positive RT PCR.

Cytological changes:

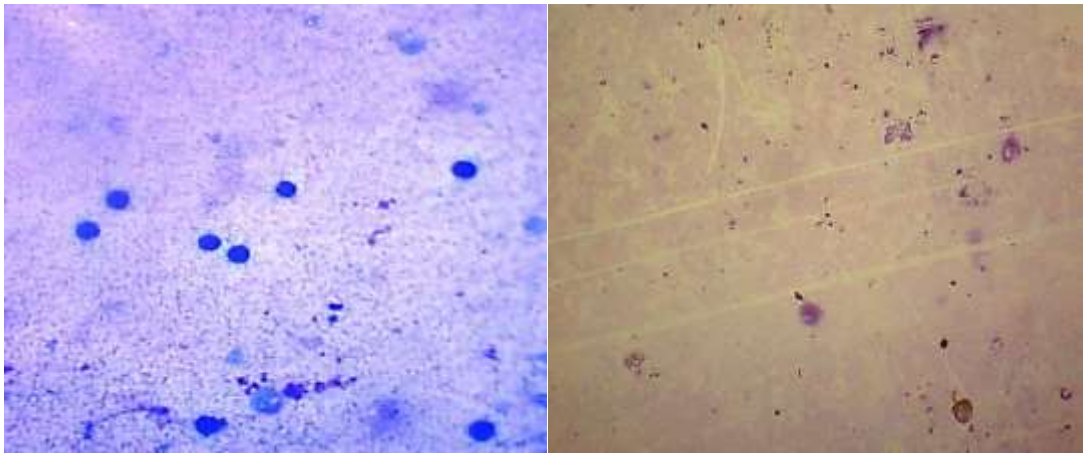

Fig.2: The microscopic examination (x40) of the nasopharyngeal smears stained by Diffquick, revealed lymphocytosis and a lot amount of wet mucus. **Discussion:**

The microscopic examination of the nasopharyngeal smears did not reveal important cytological and pathological alterations. The typical cytopathic changes that are usually found in viral respiratory infections, such as Ciliocytophthoria, multinucleation and viral inclusion bodies were not detected, which disagree with the previous study done by Winichakoon P, The macrophages may also show cytoplasmic changes, including the presence of foamy cytoplasm or larger cytoplasmic vacuoles or nuclear changes, including multinucleation and ground glass appearance of nuclei [5]. The observation of the nasopharyngeal smear also revealed an abnormally high number of lymphocytes (Lymphocytosis), which is not supported with the previous study done by Akinbami LJ, the cytological examination of nasopharyngeal swabs reportedly showed high numbers of neutrophils [11].

The smears were revealed a lot of dry and wet mucous amount in different population which made shedding for a lot of inflammatory cells.

Therefore, the cytopathic alterations were of little significance. A possible explanation could be the cytological nasopharyngeal patterns associated with nasal or throat scraping method not nasal swap which is a non-invasive method to take a sample of nasal mucosa. Each of the nasopharyngeal swabs did not reveal epithelial cells or glandular cells, which disagree with previous study reported by Akinbami LJ, nasopharyngeal swabs specimens may also show squamous metaplasia and features of repair [11].

A careful and thorough search failed to detect any viral inclusions and this due to the absence of squamous cells. This suggests that claims of viral inclusions with covid-19 disease may, rather, represent nonspecific cytopathic effect in nasopharyngeal swabs such as lymphocytosis.

Finally, we would probably expect greater microscopic alterations in the pulmonary alveoli, but for this purpose we recommend to perform a nasal or throat scraping method, which remains the gold standard for Covid-19 cytological assessment.

Conclusion:

The significant cytological changes in Covid-19 patterns associated with nasal or throat scraping method not nasal swap, which remains the gold standard for Covid-19 cytological assessment. The nasopharyngeal smear revealed an abnormally high number of lymphocytes (Lymphocytosis) not Neutrophilia. The smears were revealed a lot of dry and wet mucous amount in different population.

We recommend to perform further research of nasal or throat scraping method, which remains the gold standard for Covid-19 cytological assessment.

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