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ORIGINAL ARTICLE

Clinical significance of viral loads in patients infected with SARS-CoV-2 in Fayoum University Hospitals

[1]Hossam Eldin M. Abdelaziz*, **[2]Radwa A. Elhefny**, **[1]Noha K. Abdel-Ghafaar**, **[3]Wafaa Y. Abdel-Wahed**, **[1]Fadwa Abdel Reheem**

[1]Department of Clinical and chemical pathology, Faculty of Medicine, Fayoum University, Egypt
[2]Department of Chest Diseases, Faculty of Medicine, Fayoum University, Egypt
[3]Department of Public health and Community Medicine, Faculty of Medicine, Fayoum University, Egypt

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Corresponding Author:
Hossam Eldin Mahmoud AbdelAziz Ali
[1]Department of Clinical and chemical pathology, Faculty of Medicine, Fayoum University, Egypt
Tel.: 01554043143
hma05@fayoum.edu.eg
ORCID ID:
0000-0002-8748-3126

ABSTRACT

Background*: Throughout unexplained cases of pneumonia, a new human coronavirus first found in Wuhan, China in December 2019 had spread worldwide. Viral loads from respiratory samples were measured and considered an indication of active virus replication and used for monitoring severe viral respiratory tract infections routinely.* ***Objective:*** *is to evaluate if the nasopharyngeal viral load has any link with known clinical parameters at disease progression in cases infected with (SARS-CoV-2) during the early three months (May, June, July /2020) of the epidemic in Fayoum University Hospitals.* ***Methodology:*** *Nasopharyngeal swabs were taken from cases with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and viral loads were detected by real-time Reversed transcriptase polymerase chain reaction (RT-PCR) according to cycle threshold (Ct) where high viral load means Ct value <25, moderate viral load; Ct value is from 25 to 35 and low viral load means Ct value >35.* ***Results:*** *Moderate and high NP viral load were significantly higher in patients with fever, upper respiratory tract symptoms, bone aches, and vomiting. High levels of both CRP (p=0.021) and CT findings (p=0.005) were significantly associated with moderate and high viral load. There were significant differences between viral loads groups as regards the occupation of HCWs (p=0.005).* ***Conclusion:*** *SARS-CoV-2 viral load were high in the nasopharynx at the early phase of infection; also high viral load were noticed more in HCWs.*

INTRODUCTION

The novel 2019 coronavirus [severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)] was first announced in December 2019 in Wuhan, China, and has led thus far in remarkable worldwide morbidity and mortality [1]. In spite of coronavirus disease 2019 (COVID-19) has affected around 78,998,403 cases globally, there is still broad gaps remaining in our understanding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pathogenesis which include the link between levels of replication of virus and severity of disease [2].

Additional viral studies of SARS-CoV-2 are needed that will be performed not only using respiratory samples but also blood samples [3]. The initial cause of death is respiratory failure in COVID-19 patients, but complications appearing from a hyperactivity of immune response and vascular damage are in addition an important presentation in both pulmonary and extra-pulmonary systems. In addition, it was suggested that viremia which is detected in plasma utilizing a qualitative viral detection assay might be linked to severity of disease, in spite of updated studies have been handicapped by the shortage of viral load quantification[4].

Together, these detections suggest that the systemic SARS-CoV-2 viral circulation and expanse of plasma viremia is important in COVID-19 outcomes prediction but there is small known data concerning it [4].

Moreover, Yang Liu et al, 2021 reported that relative SARS-CoV-2 RNA load in the nasopharynx is closely related to COVID-19 severity [5].

The current study aimed to evaluate the link between nasopharyngeal viral load and clinical parameters during disease progression in cases infected with (SARS-CoV-2) during the early months of the epidemic in Fayoum University Hospitals (May, June and July 2020).

METHODOLOGY

Study design:

A retrospective cohort study was conducted in Chest Department in collaboration with Clinical and Chemical Pathology Department, Faculty of Medicine, Fayoum

University, over three months in the period from April 2020 to June 2020. The study included 84 hospitalized patients with suspected COVID 19 infection. The Ethical Committee of Fayoum University approved this study (Approval number: R 149; Session: 78; Date: 15-11-2020).

Nasopharyngeal swabs were obtained from all cases with suspicious COVID-19 infection, during the first three days of appearance of symptoms.

Trained medical personnel collect the nasopharyngeal swabs. Samples were sent in viral transport media (VTM)/PerkinElmer pipeline and to the Clinical Pathology Laboratory at 4 ^{0}C for further testing.

Samples:

Nasopharyngeal (NP) swabs were obtained using flocked NP swabs and transported to the laboratory in universal viral transport media (UTM-RT® System, Copan Diagnostics, Murrieta, CA, USA) at 4 ^{0}C within 1 – 2 hours of collection. All samples were transported to the lab in VTM.

RT-PCR for SARS-CoV-2 virus:

Extraction was done according to manufacture instructions using (Prepito Viral DNA/RNA300) kit, Perkin Elmer, in LabTurbo 48C instrument, Taiwan; which is based on fully automated sample preparation and nucleic acid extraction and purification.

That was followed by RT-PCR amplification and detection of two target regions in SARS-CoV-2 genome (ORF and N gene) using SARS-CoV-2/SARS-CoV Multiplex real time PCR detection kit (COVID-19 genesig® Real-Time PCR assay), Primerdesign, in DNA Technology RT-PCR DTlite 4 instrument, Russia.

We used Ct (cycle threshold) values of ORF gene as semi-quantitative measures for viral load, and accordingly we divided the patients into three groups. Patients with high viral load; having Ct value <25, with moderate viral load; Ct value from 25 to 35 and those with low viral load having Ct value >35 [6].

Statistical analysis

Data was collected, coded, and analyzed using the Statistical Package for Social Science (SPSS) software version 28. A descriptive analysis in the form of means and standard deviations was calculated for numerical data, number, and percent for qualitative data, comparing between groups that was done using (chi-square) test for qualitative data. The level of statistical significance was ≤ 0.05

RESULTS

Eighty-four hospitalized patients with COVID-19 (diagnosis of COVID-19 based on testing of nasopharyngeal swabs) were incorporated in the study, the mean age was 26.6 ± 4.7, 39% (33 cases) were females and 60% (51 cases) were males. Regarding their occupation, 64% were health care workers. The duration of hospital stay was 6.5 (3-18) days (**table 1**).

Table 1: Demographic data of patients and duration of hospital stay

Patients data	Number (n)=84	
Age	26.6±4.7	
Sex [Female]	33	(39.3%)
[male]	51	(60.7%)
Occupation [HCW]	54	(64.3%)
Hospital stays (days)	6.5	(3-18)

Table (2) showed the clinical symptoms, signs and radiological affection of the enrolled patients at time of admission. Symptoms were fever, sore throat, cough, dyspnea, fatigue, anosmia, bone ache, upper respiratory and vomiting and signs was hypoxia.

Table 2: Clinical symptoms, signs and radiological affection of patients at time of admission

Symptoms	(n=84)
Fever	36 (42.9%)
Sore throat	9 (10.7%)
Cough	30 (35.7%)
Dyspnea	9 (10.7%)
Fatigue	9 (10.7%)
Anosmia	6 (7.1%)
Bone ache	3 (3.6%)
Upper respiratory	3 (3.6%)
Vomiting	3 (3.6%)
Oxygen saturation 85%	3 (3.7%)
GGO or consolidation	30 (35.7%)

We reported SARS-CoV-2 viral loads analysis in **table (3)**. Among hospitalized individuals, they had detectable SARS-CoV-2 RNA at the time of initial sample collection by nasopharyngeal swabs. Detection of respiratory tract SARS-CoV-2 RNA was generally low (24 patients), moderate (27 patients) and high (33 patients) [6]. Mean age was 28.0 ± 5.47 in low viral loads, 25.7 ± 5.9 in moderate viral loads and 28.6 ± 5.6 in high viral loads. Significant differences were found between viral loads groups as regards health care worker as an occupation (p=0.005).

Table 3: SARS-CoV-2 viral loads analysis among hospitalized individuals

		Low (24)	Moderate (27)	High (33)	P value
Sex	**Female**	**9 (27.3)**	**15 (45.5)**	**9 (27.3)**	**0.081**
	Males	**15 (29.4)**	**12 (23.5)**	**24 (47.1)**	
Age	**Mean ±SD**	**28.0 ± 5.47**	**25.7 ± 5.9**	**28.6 ± 5.6**	**0.131**
HCWs	**yes**	**6 (16.7)**	**21 (38.9)**	**24 (44.4)**	**0.005**
	No	**15 (50.0)**	**6 (20.0)**	**9 (30.0)**	

Moderate and high nasopharyngeal viral loads were significantly higher in patients with fever, upper respiratory tract symptoms, bone aches and vomiting. In addition, high levels of both C-reactive protein (CRP) (p=0.021) and CT findings (p=0.005) were significantly higher in patients with moderate and high viral load as shown in table **(4)**. Two cases aged above forty died were reported with moderate and high viral loads at the time of diagnosis.

Table 4: The relation between the viral loads and the clinical presentation

		Low n (%)	Moderate & High n (%)	P value
Fever	yes	6 (16.7)	30 (83.3)	**0.036**
	No	18 (37.5)	30 (62.5)	
Upper respiratory	yes	3 (100%)	0	**0.021**
	No	21(25.9)	60 (74.1)	
Sore throat	yes	0	9 (100)	0.054
	No	24 (32)	51(68.0)	
Cough	yes	9 (30)	21(70)	0.829
	No	15 (27.8)	39 (72.2)	
Dyspnea	yes	3 (33.3)	6 (66.7)	0.710
	No	21(28)	54 (72.0)	
Fatigue	yes	3 (33.3)	6 (66.7)	0.710
	No	21(28)	54 (72.0)	
Bone ache	yes	3 (100%)	0	**0.021**
	No	21(25.9)	60 (74.1)	
Vomiting	yes	3 (100%)	0	**0.021**
	No	21(26.9)	60 (74.1)	
Anosmia	yes	3 (50)	3 (50)	0.346
	No	24 (29.6)	57 (73.1)	
CRP	+ve	9	60	0.021
	-ve	15	0	
Oxygenation <=85%	yes	0	3 (100)	0.554
	No	24 (29.6)	57 (70.4)	
Lung affection by CT	yes	3 (10)	27 (90)	**0.005**
	No	21 (38.9)	33 (61.1)	

DISCUSSION

Coronaviruses can be transmitted primarily by close contact of respiratory droplets, which are caused by coughing, sneezing, talking and breathing. This can explain and lead to the detection of SARS-CoV-2 viral loads with upper and lower respiratory symptoms.

We showed that viral loads were high in hospitalized patients and those having more severe symptoms. These results confirmed that the peak of viral loads was after starting of symptom and assist the initial Asian cohorts' results, which showed that viral load in throat swabs peaks during the pre-symptomatic phase of the disease and drop slowly to reach an undetectable level by day 18 to 21 [7].

We believe that high viral loads are seen in mild symptoms such as vomiting, bone aches and upper respiratory tract symptoms rather than lower respiratory tract symptoms, as they will be a reflection of the time from start of infection. When we perform interpretation within our present understanding of the natural history of COVID-19; these findings will be appealing.

Wölfel et al, [8] reported that cases with COVID-19 infections had peaks of upper respiratory viral loads within the first week of development of symptom.

Liu et al, [9] showed that viral loads of mild cases are significantly lower in comparison with severe cases.

Levels of moderate and high of SARS-CoV-2 loads were linked to the inflammatory marker and the severity of disease, including elevated CRP and CT findings [10].

Association between SARS-CoV-2 viral loads with CRP level, showed that the activity of viral infection could share to the hyper-inflammatory state that is a sign of severe COVID-19 [10].

Since the start of the pandemic of coronavirus 2019 (covid-19), workers at healthcare have appeared an amazing adaptability and professional commitment in spite of hazards of becoming infected and transmitting infection to others[11].

Health care workers at front line who are typically in closest proximity with confirmed COVID19 patients represent 11% of US COVID19 cases according to the first national level data released by the Centers for Disease Control and Prevention (CDC) [12].

Based on this preliminary data, this study showed a significant difference between the low, moderate and high nasopharyngeal viral loads (p=0.005).

CONCLUSION

SARS-CoV-2 viral load were high in the nasopharynx at the early phase of infection; higher nasopharyngeal viral loads were noticed in HCWs.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

Declarations:

Ethics approval and consent to participate:

This study was performed at Fayoum University Hospital and approved by the Fayoum University Research Ethics Committee, which is a member of Egyptian Network Research Ethics Committee (ENREC) (Approval number: R 149; Session: 78; Date: 15-11-2020).

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Author 1 [H. A.]: Conception, Design& Supervision

Author 2 [R. E.]: Conception, Design& Supervision.

Author 3[N. A.]: Funding, materials, Data collection and/or Processing& Literature Review.

Author 4 [W. A.]: Analysis and/or Interpretation.

Author 5 [F. A.]: Literature Review, Writing & Critical Review.

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REFERENCES

1. Nemunaitis J, Stanbery L, Senzer N. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection: let the virus be its own demise. Future Virol. 2020; 10.
2. Li H, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): current status and future perspectives. Int J Antimicrob Agents. 2020; 55(5):105951.
3. Mathuria JP, Yadav R, Rajkumar. Laboratory diagnosis of SARS-CoV-2 - A review of current methods. J Infect Public Health. 2020; 13(7):901-905.
4. Fajnzylber, J., Regan, J., Coxen, K. et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun.2020; 11, 5493.
5. Yang Liu, Wenjian Liao, Lagen Wan, Tianxing Xiang, Wei Zhang. Correlation between Relative Nasopharyngeal Virus RNA Load and Lymphocyte Count Disease Severity in Patients with COVID-19. Viral Immunol 2021; 34(5):330-335.
6. Klinger Soares Faíco-Filho, Victor Cabelho Passarelli, and Nancy Bellei. Is Higher Viral Load in SARS-CoV-2 Associated with Death? Am J Trop Med Hyg. 2020; 103(5): 2019–2021.
7. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, Lau YC, Wong JY, Guan Y, Tan X, Mo X, Chen Y, Liao B, Chen W, Hu F, Zhang Q, Zhong M, Wu Y, Zhao L, Zhang F, Cowling BJ, Li F, Leung GM: Temporal dynamics in viral shedding and transmissibility of COVID-19. Nature Medicine 2020; 26, 672–675.
8. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Muller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brunink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C: Virological assessment of hospitalized patients with COVID-2019. Nature 2020; 581.
9. Liu Y, Yan LM, Wan L, Xiang TX, Le A, Liu JM, Peiris M, Poon LLM, Zhang W: Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis 2020; 20.
10. Fengjuan Shi, Tao Wu, Xiaojuan Zhu, Yiyue Ge, Xiaoyan Zeng, Ying Chi, Xuefei Du, Liguo Zhu, Fengcai Zhu, Baoli Zhu, Lunbiao Cui, and Bin Wua: Association of viral load with serum biomakers among COVID-19 cases. Virology 2020; 546: 122–126.
11. Liu Q, Luo D, Haase JE, et al. The experiences of health-care providers during the COVID-19 crisis in China: a qualitative study. Lancet Glob Health 2020; 8: e790-8.
12. CDC COVID-19 Response Team. Characteristics of Health Care Personnel with COVID-19 — United States. MMWR Morb Mortal Wkly Rep 2020; 69:477–481.