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RESEARCH ARTICLE

EVIDENCE OF SARS-COV-2 RNA VIRUS IN CHRONIC WOUNDS OF ACTIVE COVID-19 PATIENTS: A SINGLE-CENTRE RETROSPECTIVE STUDY

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Abstract

Purpose: This studyinvestigated the presence of SARS-CoV-2 RNA virus in the chronic wounds of confirmed COVID-19 positive patients.
Methods: This is a single-centre retrospective observational study conducted at a dedicated COVID-19 tertiary centre. COVID-19 positive patients with chronic wounds (traumatic/infective) were included in this study. The specimens from wounds were collected while doing the dressing and analyzed with real-time reverse tran-scriptase polymerase chain reaction (rRT-PCR) to know the presence of the virus.
Results: A total of eleven patients were included in this study. Eleven samples were collected safely from wound swabs and analyzed. None of the samples from the wound tested positive for the virus while they were tested positive from nasopharyngeal swab which was taken simultaneously or within 3 days prior.
Conclusions: The wounds of COVID-19 patients were considered negative with least risk of transmission of the disease. Thus, these patients were managed safely using proper wound precautions and strict COVID 19 protocols and guidelines.

Introduction:-

Coronavirus disease 2019 (COVID-19) which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was first detected in Hubei Province, Wuhan, China. Within a short span of time, it spread quickly throughout China, and became pandemic in the entire world, causing an alarm among the people, all over the world [1]. It has caused large number of fatalities and mortalities which became a major concern amongst the population [2].SARS-CoV-2 has proved to be highly contagious, because of its progressing reproduction number in most of the countries [3]. Various studies proved that SARS-CoV-2 is a kind of the RNA virus (subgenus sarbecovirus, β-genus, orthocoronavirinae subfamily and order Nidovirales) which is very susceptible to human beings [2,4]. Therefore, most people affected, presented with diverse clinical signs and symptoms, seriously threatening the health and life of the population affected [5,6]. In the early stage of the disease, symptoms are usually mild, comprising dry cough, fever, malaise, myalgia, and fatigue [5,7,8]. If the patient is not adequately treated and isolated, then the virus spread is rampant, while the disease progresses to acute respiratory distress syndrome, respiratory failure and finally death [9,10]. SARS-CoV-2 is mostly transmitted by droplet spread and close contact through person to person contact especially when the person coughs or sneezes and at the same time, blood borne routes and faecal-oral routes are still under study [11,12]. The real-time reverse transcriptase polymerase chain reaction (RT-PCR) of the nasal swabs is the most reliable method of detection. A qualitative real-time reverse transcriptase polymerase chain reaction (RT-

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PCR) of the nasopharyngeal swab testing has been found to be the most reliable and best alternative method to oropharyngeal swabs or detection by blood or stool [13,14]. Health care workers (HCWs) are at a much higher risk of acquiring the disease due to their direct exposure, their involvement in patient care and handling of tissues [14]. Therefore, World Health Organization (WHO) and US Centre for Disease Control (CDC) have implemented strict and protective guidelines for the protection of HCWs, ensuring constant use of protective face masks (e.g., surgical masks, KN95, FFP-2, FFP-3) to gain source control [15].

Chronic wounds are defined as wounds which have failed to achieve anatomical and functional integrity through orderly and timely repair within 3 months or more and healing in such cases is multifactorial [1,16]. As of now, there are very few reports in the literature on the wound swab culture usage for the detection of SARS-CoV-2 virus. We studied the chronic wounds of COVID-19 patients to detect the presence of the virus and the risk of transmission through these patients.

Methods:-

This single center, retrospective observational study, was conducted in the Department of Burns and Plastic Surgery in collaboration with the Department of Microbiology at All India Institute of Medical Sciences, Bhubaneswar, which is an apex tertiary center and specialized COVID-19 hospital. In our study, wound specimen samples were collected during full outbreak of COVID 19 from September 2020 to July 2021, from the active COVID 19 confirmed patients detected by rRT-PCR assay, to study the presence of SARS-CoV-2 RNA virus from open chronic wounds and their possible risk of transmission of disease. All the patients included in the study were vaccinated with the first dose of COVID 19 vaccine. Proper hand hygiene, mandatory usage of face masks and shield along with the appropriate personal protective equipment (PPE) kit were used before collection of the sample.

Sample collection

The COVID-19 confirmed patients with open chronic wounds were included in the present observational study. Nasal swabs using the RT-PCR assay were collected from all the patients who presented to the department and emergency as a part of routine screening for SARS-CoV-2. Patients after being confirmed with SARS CoV -2 virus were admitted in the isolation ward. Specimens from the open wounds were collected within 3 days of admission in the isolation ward. Written informed consent was obtained from each patient prior to the start of the study. A total of eleven samples were collected in viral transport media (VTM) prior to performing any intervention. Tested specimens included swabs collected from the open wound sites, either infective or non-infective. The samples were double sealed, packed in a black cover, kept safely in a vial box, and carried by a dedicated COVID-19 duty personnel to the Microbiology department for sample testing. All the samples were maintained in the cold chain of specimen transport. Adequate measures were taken to ensure safe delivery of samples and prevent the delay between the collection time and processing time. The wound swab was also sent for laboratory RT-PCR RNA assay testing (Fig1).

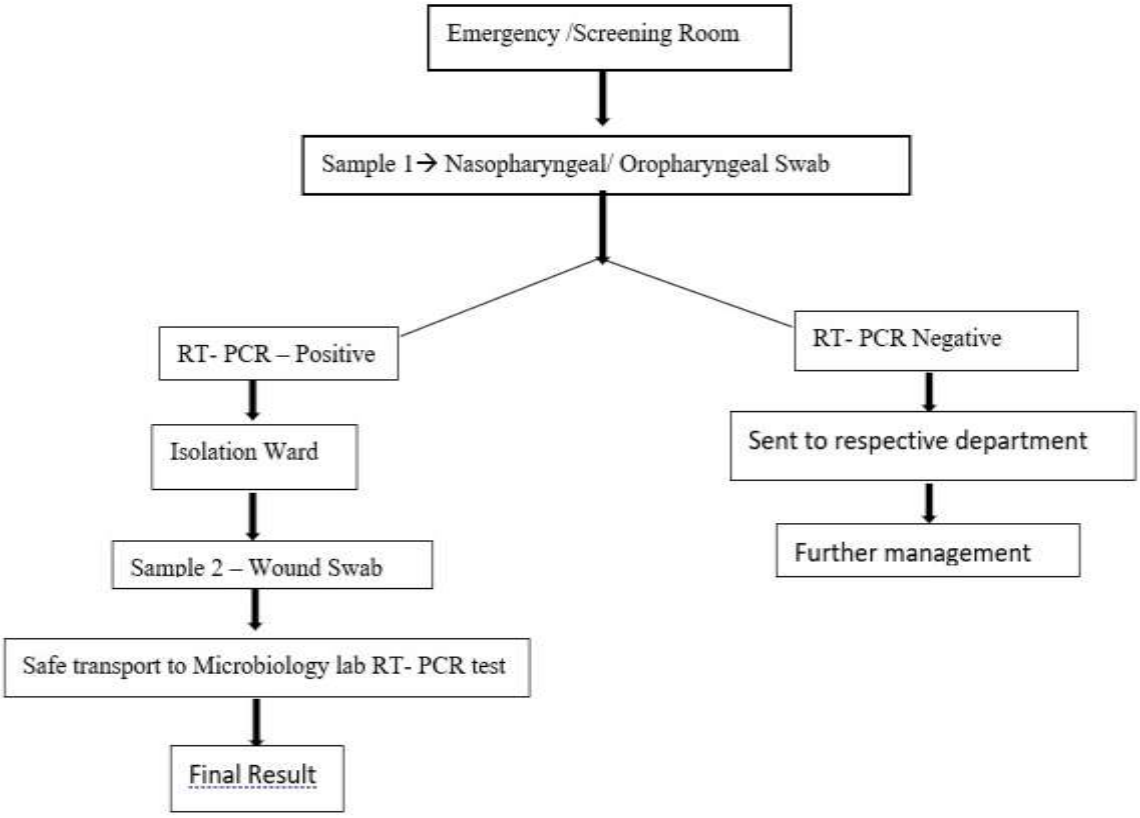

Fig 1.:- Protocol for collection of samples from wound.

RNA Extraction and Real time Reverse Transcriptase Polymerase chain reaction (rRT-PCR)

Manufacturer's instructions were followed strictly without any modifications in case of the kit used in the lab. All the eleven samples were processed in the Biosafety cabinet class II using aseptic measures. The total RNA was extracted ranged from 140µL – 250µL of a sample using QIAamp Viral RNA extraction kit (Qiagen, Germany). Finally, the RNA was eluted in 60µL of elution buffer and stored at –20°C till further use.

TaqPath™ COVID-19 Combo Kit (Applied Biosystems, Foster City, California, USA) was intended for the qualitative detection of nucleic acid from SARS CoV-2 RNA virus isolated from nasopharyngeal and wound specimens, where nucleocapsid (N), spike (S), open reading frame (ORF-1ab) genes, and internal control were targeted by rRT-PCR. Milli Q Water and extracted viral nucleic samples were also used along with the combo kit.

Ethical statements

We conducted this study in compliance with the principles of the Declaration of Helsinki. The study protocol was revied and approved by the Institutional Ethical Committee.Written informed consent was obtained from each patient prior to the start of the study.

Results:-

Eleven specimens were studied from eleven patients. There were eight male and three female patients with the mean age being 60.5 years. Most of the patients had mild symptoms of COVID-19 infection while rest of them were asymptomatic. Symptomatic patients were managemed with oral antibiotics and antihistamines. Asymptomatic patients were just kept for observation in the isolation ward for the testing of COVID 19 virus. Proper dressing and wound care were managed using safety precautions and PPE kit. Almost eight patients had diabetes mellitus as comorbidity, two patients had gangrene due to vascular occlusion and only one patient presented with a post traumatic chronic wound. The samples included eleven wound swabs collected directly from the wound bed which were then sent to the Microbiology department for laboratory testing with rRT-PCR assay (Table1). None of the samples tested positive for the SARS-CoV-2 virus which have been elicited in the table.

Table 1:- Characteristics of the study by number of samples taken.

SAMPLE NO.	AGE (YEARS)	SEX	DIAGNOSIS	NASAL SWAB TEST CONFIRMED POSITIVE	DATE OF WOUND SWAB TAKEN	WOUND SWAB RT-PCR RESULT
1.	75	Male	Right diabetic foot ulcer	17.09.2020	18.09.2020	Negative
2.	50	Female	Left diabetic foot ulcer	01.05.2021	03.06.2021	Negative
3.	70	Male	Bilateral lower limb cellulitis with ulcer	07.06.2021	09.06.2021	Negative
4.	62	Male	Left diabetic foot ulcer	09.06.2021	10.06.2021	Negative
5.	65	Female	Right diabetic foot ulcer	10.06.2021	11.06.2021	Negative
6.	35	Male	Right leg traumatic crush injury with non-healing ulcer	15.06.2021	16.06.2021	Negative
7.	51	Male	Right diabetic foot ulcer	27.06.2021	29.06.2021	Negative
8.	67	Female	Left diabetic foot with vasculitis	29.06.2021	30.06.2021	Negative
9.	70	Male	Right lower limb gangrene	02.06.2021	03.07.2021	Negative
10.	61	Male	Right diabetic foot ulcer	01.07.2021	03.07.2021	Negative
11.	60	Male	Left foot diabetic ulcer with dry gangrene	02.06.2021	03.07.2021	Negative

Discussion: -

In our retrospective study, we found that the SARS CoV-2 RNA virus was not detected in the wound swabs whereas the patient's nasal swabs still tested positive for the virus especially during the active stage of the disease. The risk of transmission of the disease was least when taken from wound swabs as compared to high infectivity rate when obtained from nasal or throat swabs. The pilot study conducted at the premium institute of PGIMER, Chandigarh by Gaba S et al., first published in their article, on the negative wound swab tests in a confirmed positively tested COVID 19 patients, projecting that disease transmission was least through open wounds in those eight patients [14]. Zhang et al. in their study found the virus through various routes including oral swabs, anal swabs, and blood, which suggested a possible fecal-oral and body fluid transmission [11]. The presence of coronavirus in ascitic fluid was first published in their article by Culver et al., [17] whereas coronavirus was not detected from the peritoneal fluid aspirated from abdominal wound in the study conducted in PGIMER, Chandigarh [14]. Wang et al. did their clinical study on the inpatients who were suspected to have SARS-CoV virus infection. The samples were collected from various specimens including nasopharyngeal, feces, blood, sputum, and urine. Out of the 205 patients, maximum positive detection for SARS CoV- 2 virus was obtained from nasal swabs while small percentage of positive results were obtained from feces and blood samples, thereby ascertaining their route of transmission of the virus [18]. Similar study in Beijing Research Center reported the presence of SARS CoV-2 virus isolated from sputum and stool samples [19]. Sharma et al. did a comparative analysis in their study based on various specimens to detect

SARS-Cov-2 RNA virusby rRT-PCR along with combined swab test (nasopharyngeal and oropharyngeal swab) for both diagnostic and monitoring COVID-19 patients [20].

In view of the COVID-19 scenario, it was utmost necessary to pay attention to the contagion among patients, securing oneself from not being a carrier of the contagion and most importantly not neglecting the patients of their treatment while being kept isolated. Whether it is a single or multiple surgical procedure transmission of the SARS-CoV-2 RNA virus is triggered, and when such patients with chronic open wounds come for frequent dressings, risk of COVID-19 transmission is inevitably increased. Hospital stays in such patients mainly focused on avoiding complications and with performing minimal surgical intervention as far as possible. Even though in our results all the wound swabs turned out to be negative, even then strict protocols were maintained, while collecting the samples because transmission risk was still present as the nasal and throats swabs were positive for the virus. Ever since the disease has turned out to be pandemic, there is an increase burden to health care workers, more COVID-19 dedicated hospitals with proper facility for the affected patients.

Limitations

Since, our study was based only on eleven patients, a larger sample size was required to confirm the absence or presence of the virus from wound swabs. Many patients used telemedicine for consultation from the fear of acquiring the disease from hospitals, therefore wound swabs could not be taken from such patients, thus leading to a smaller sample size. Non evaluation of other potential specimens such as urine, blood, feces, saliva were not used for the detection of SARS Cov-2 RNA virus, which again portrayed as a limitation of the study.

Conclusion:-

This study concluded that handling of open raw wounds in active COVID-19 positive patients was otherwise safe. No traces of SARS-Cov-2 RNA virus were detected in any of the sample taken from the patients. These wound swab test from active COVID-19 patients helped in establishing the proper management protocol and taking evidence-based decision before discharging the patients, especially in the need of the hour during the pandemic crisis. Even though nasopharyngeal and oropharyngeal testing are most appropriate for the detection of SARS-Cov-2 RNA virus for monitoring purpose, yet detection made through other specimen samples helped in better prognostic indicator of viral clearance while the patient was under treatment.

References:-

1. Zhou H, Jin Q, Lu H. Exposure risk of patients with chronic infectious wounds during the COVID-19 outbreak and its countermeasures. J of Orthop Surg and Res. 2020 Dec;15(1):1-0. 10.1186/s13018-020-01976-0
2. Zhu N, Zhang D, Wang W, et al; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 2020;382(8):727–733
3. Yuan J, Li M, Lv G, Lu ZK. Monitoring transmissibility and mortality of COVID-19 in Europe. Int J Infect Dis. 2020.
4. "The new scope of virus taxonomy: partitioning the virosphere into 15 hierarchical ranks." Nature Microbiology 5, no. 5 (2020): 668-674.
5. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan. China. Lancet. 2020;395(10223):497–506.
6. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan. Jama: China; 2020.
7. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507–13.
8. Cipollaro L, Giordano L, Padulo J, Oliva F, Maffulli N. Musculoskeletal symptoms in SARS-CoV-2 (COVID-19) patients. J Orthop Surg Res. 2020;15(1): 178.
9. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating personto-person transmission: a study of a family cluster. Lancet. 2020;395(10223): 514–23.
10. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a singlecentered, retrospective, observational study. Lancet Respir Med. 2020.
11. Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 2020;9(1):386–389

12. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic features and clinical course of patients infected with SARSCoV-2 in Singapore. Jama. 2020.
13. Higgins TS, Wu AW, Ting JY. SARS-CoV-2 nasopharyngeal swab testing—false-negative results from a pervasive anatomical misconception. JAMA otolaryngology–head & neck surgery. 2020 Nov 1;146(11):993-4.
14. Gaba S, Kampalli G, Goyal K, Suri V, Chauhan P, Meena SC, Bhatnagar A, Singh MP, Sharma RK. No traces of SARS-CoV-2 in wounds of COVID-19 positive patients: A pilot study. Ind J of Plas Surg. 2020 Dec;53(03):399-401.
15. Impact of lockdown for SARS-CoV-2 (COVID-19) on surgical site infection rates: a monocentric observational cohort study
16. Werdin F, Tennenhaus M, Schaller HE, Rennekampff HO. Evidence-based management strategies for treatment of chronic wounds. Eplasty. 2009;9: e19.
17. Culver A, Arbelot C, Bechis C, Cassir N, Leone M. First description of SARS-CoV-2 in ascites. IDCases 2020;21:e00836to confirm virus absence in the wounds.
18. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA 2020;323(18):1843–1844
19. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis. Published online February 24, 2020.
20. Sharma, K., Aggarwala, P., Gandhi, D., Mathias, A., Singh, P., Sharma, S., Negi, S. S., Bhargava, A., Das, P., Gaikwad, U., Wankhede, A., Behra, A., & Nagarkar, N. M. (2021). Comparative analysis of various clinical specimens in detection of SARS-CoV-2 using rRT-PCR in new and follow up cases of COVID-19 infection: Quest for the best choice. PLOS ONE, 16(4), e0249408.