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Research Article

POST COVID INFECTIONS –A THREAT TO HEALTHCARE INSTITUTIONS

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ABSTRACT

Background: COVID19 disease caused by SARSCoV2 virus is an acute respiratory tract illnessassociated with multiorgan involvement in many cases. This corona virus has caused pandemicall over the world.**Aim and objectives**: This study was undertaken to determine different post COVID infections,risk factors associated and clinical outcome of patients.**Material and methods:** Clinical samples from 42 post COVID patients were collected duringour study period of 6 months who showed sudden deterioration in recovery period from COVIDand the samples were processed in our laboratory using conventional and automated techniques.Different risk factors associated and laboratory parameters were studied.**Results:** Male patients were more common to develop post COVID infections than females.Maximum numbers of patients were of 41-60 years age range. Fungal infections includingmucormycosis(11), candidiasis(8), aspergillosis(1); bacterial infections including Acinetobacterspecies(3), Klebsiellapneumoniae(8), Escherichia coli(2), Burkholderiacepacia(1), MethicillinResistant Staphylococcus aureus(3), Enterococcus species(1), Pseudomonas aeruginosa(1),Tuberculosis(1) ,viral infection including 2 influenza virus infected cases. 6 patients died andrest recovered with appropriate treatment. Most common risk factor was use ofimmunosuppressive drug especially Corticosteroids (72%). C-reactive protein, ferritin, totalleukocyte count, creatinine and D-dimer showed a rising trend during development of post-COVID infections as compared to values at admission. Diabetes mellitus (15%) was the mostcommon post-COVID non-infectious complication, followed by hypertention, myocardial
infarction and cerebrovascular accident.**Conclusion:**COVID19 is altering the landscape of hospital acquired infections and severalopportunistic infections. Clinicians must be vigilant whenever a recovering COVID patientsuddenly deteriorate or turns symptomatic, as often the post-COVID infections increase themortality manifold.

INTRODUCTION

COVID19 disease caused by SARSCoV2 virus is an acute respiratory illness with or without multiorgan involvement which has caused a dangerous pandemic allover the world[1]. Infection is transmitted by infective respiratory droplets and contact transmission[1]. COVID19 disease commonly showed presentations like- respiratory symptoms, fever, diarrhea, loss of taste and smell; however, rare symptoms like- abdominal pain, dehydration, jaundice, convulsions, encephalopathy, nephritis, arrhythmias, conjunctivitis, myocarditis, etc. are also seen[2].Though there is no specific therapy, multiple drugs have been tried and many of them are FDA approved[3].Many patients suffer from infections and non-infectious complications in post-COVID period[4]. People who suffered from severe illness with COVID19 may experience organ damage affecting the heart, kidneys, skin, brain as well as residual lung damage[5].Many patients in post-COVID period may develop secondary bacterial ,mycological and viral infections which further complicate the natural course of the disease and increases both morbidity and mortality in such patients[6].There have been surprisingly lesser studies on post-COVID bacterial, mycological and viral infections among COVID positive patients.Therefore,an observational study was undertaken to determine the different post-COVID infections in the Microbiology Department,R.G.Kar Medical College and Hospital, Kolkata.

OBJECTIVES

To determine different types of infections including bacterial , mycological and viral infections among post COVID patients and their clinical outcome; to determine the demographic profile and risk factors of post COVID patients developing different types of infections; to determine the average levels of different laboratory markers and their comparison between the levels at admission and levels at the time of developing different post COVID infections and to determine the rates of some of the common post COVID non- infectious complications

MATERIAL AND METHODS

An observational study was conducted for 6 months from January 2021 to June 2021 including clinical samples from 42 COVID patients who suddenly turned symptomatic during their recovery period and later found to develop different

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bacterial, mycological and viral infections. Sputum, tissue from sinonasal mass, biopsy from nasal turbinates, deep tracheal aspirate, bronchoalveolar lavage, central line tip, blood, urine and nasopharyngeal swabs collected were processed in our laboratory with conventional and automated laboratory techniques and RTPCR was done for nasopharyngeal swabs. Susceptibility testing were performed on Mueller Hinton Agar (MHA) plates and MHA with 2% glucose with 0.5 microgram/ml methylene blue for bacteriological and mycological isolates respectively by disk diffusion method as per CLSI guidelines[7].However, VITEK2 was also used for few highly resistant isolates. CBNAAT of a sputum sample from a suspected pulmonary tuberculosis case was performed both for detection of *Mycobacterium tuberculosis* complex and Rifampicin resistance status. One case of esophageal candidiasis was detected by upper GI endoscopy. LPCB (Lactophenol cotton blue) mount and culture on Sabouraud's dextrose agar (SDA) were done for moulds.Dalmau plate technique and CHROM agar were done to speciate Candida isolates. Though susceptibility testing was performed for *Candida species*, susceptibility testing were not performed for the moulds but confirmatory diagnosis were made and patients were treated as per the current guidelines[8,9]. Influenza patients were treated with Oseltamivir and symptomatic management was done as per guidelines[1]. The clinical outcomes of the patients were observed.

Inclusion criteria: Patients showing sudden clinical worsening during recovery from COVID19 disease.

Exclusion criteria: Patients who did not give consent were excluded from the study.

Ethical clearance: For the present study, the ethical approval was taken from the Institute Ethics Committee, R.G.Kar Medical College and Hospital, Kolkata.

STATISTICAL ANALYSIS

The data obtained were analysed with the statistical tool R. The different percentages were calculated. Fisher's exact test/one-way Chi-square test was used for comparative analysis. The tests were evaluated at a confidence level of 95% and $p<0.05$ was considered statistically significant.

RESULTS

During 6 months (January 2021-June 2021) samples were collected from 42 post COVID patients after informed consent who showed sudden clinical worsening during recovery period from COVID and were later found to develop several bacteriological, mycological and viral infections. They were randomly selected and an observational study was conducted. The age distribution range was between 0 and 80 & above. **Table1**shows the age range and gender distribution among the post COVID patients developing different infections including 57.14% male and 42.85% female patients.**Table2.** shows different postCOVID infections and their clinical outcomes. **Figure1(a) and (b)** shows different antimicrobial agents and their percentage of susceptibility among different clinical isolates. **Table3.** Shows average value of different laboratory markers at the time of admission with COVID positive status and at the time of developing different post COVID infections among patients. **Table4.** Shows different risk factors implicated in the development of different post COVID infections. **Figure2.**Shows percentages of different post COVID non-infectious complications.

Table1 Age and gender distribution of patients developing different post COVID infections

Age group (years)	No. of male patients	No. of female patients	Total patients in each age group
0-20	2	2	4 (9.52%)
21-40	3	5	8 (19.04%)
41-60	9	5	14 (33.3%)
61-80	6	4	10 (23.9%)
81&above	4	2	6 (14.28%)
Total paients	24 (57.14%)	18 (42.85%)	42

shows age and gender distributions among patients developing post COVID infections

Table2 Patient with different post COVID infections and their clinical outcomes

Post COVID infections	No. of patients/samples and types of samples taken	Clinical outcome of patients
Mucormycosis (4 *Rhizopus arrhizus*,4 *Mucor species*,3 *Rhizomucorpusillus*)	8 Rhinocerebral (from sinonasal mass) 3 Pulmonary (from BAL fluid)	9 recovered and 2 died
Candidiasis (4 *Candida albicans*,2 *C.tropicalis*,1 *C.guilliermondii*)	2 pulmonary (1 BAL fluid,1 Sputum) 4 UTI (4 mid-stream urine) 1 oral candidiasis (swab) 1 esophageal candidiasis(detected by Upper GI endoscopy, speciation not done)	All recovered
Pulmonary Aspergillosis (*Aspergillusfumigatus*)	1 BAL fluid	Recovered
Acinetobacter species	3 sepsis (Central line tip)	1 recovered and 2 died
Klebsiellapneumoniae	4 sepsis (Blood) 4 pneumonia (Sputum)	7 recovered and 1 died
Escherichia coli	2 pneumonia (Deep tracheal aspirate)	Both recovered
Burkholderiacepacia	1 sepsis (Blood)	Recovered
MRSA	1 carrier detected (biopsy from nasal turbinate) 2 pneumonia (sputum)	Recovered
Enterococcus species	1 sepsis (Blood)	Recovered
Pseudomonas aeruginosa	1 sepsis (central line tip)	Died
Influenza virus	1 patient with flu-like features and 1 pneumonia (nasopharyngeal swabs)	Both recovered
Mycobacterium tuberculosis(DSTB)	1 pulmonary TB (sputum)	Recovered with ATT
Total patients	42	

shows patients with several post COVID infections and their clinical outcomes. Abbreviations:BALfluid-Bronchoalveolar lavage ;MRSA-Methicillin Resistant Staphylococcus aureus; DSTB-Drug sensitive tuberculosis i.e., Rifampicin sensitive; GI-Gastrointestinal; UTI-Urinary tract infections; ATT-Antitubercular therapy.

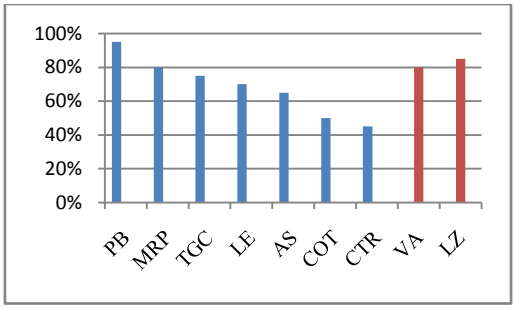

Figure1(a) Percentages of different susceptible drugs in gram negative bacteria (blue) and in gram positive bacteria (red).

Abbreviations: PB-PolymixinB(300 units), MRP-Meropenem(10), TGC-Tigecycline(15), LE-Levofloxacin(5), AS-Ampicillin-sulbactam (20), COT-Cotrimoxazole(25), CTR-Ceftriaxone(30), VA-Vancomycin(30), LZ-Linezolid(30), all disc concentrations are in microgram except PB.

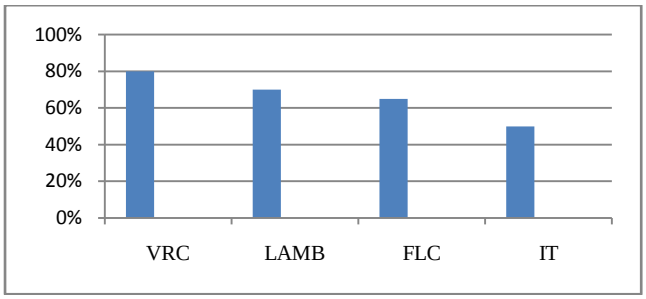

Figure1 (b) Percentages of different susceptible drugs in different Candida species

Figure 1(b). shows percentages of different susceptible drugs in Candida species. Abbreviations: VRC-Voriconazole(1), LAMB-Liposomal Amphotericin B(20), FLC-Fluconazole(25), IT-Itraconazole(10); all disc concentrations are in microgram.

Table 3 Mean value of different laboratory markers at the time of admission of patients with COVID positive status and at the time of development of post COVID infections

Parameters	Mean values at the time of admission with COVID positive status	Mean values at the time of development of post COVID infections
C-reactive protein (mg/dl)	30	100
Ferritin (ng/ml)	1000	1500
Creatinine (mg/dl)	1	1.5
Haemoglobin (gm/dl)	11	11.5
Total leukocyte count (count/microlitre)	12000	14000
D-dimer (ng/ml)	600	700

shows mean value of different laboratory parameters during patient's admission with COVID and after developing several post COVID infections.

Table 4 Risk factors for development of post COVID infections

Risk factors	Percentage of patients
Mechanical ventilation	20%
Pre-existing uncontrolled diabetes mellitus (HbA1c >7gm%)	25%
Immunosuppressive drugs including Corticosteroids	72%
Prolonged duration of hospitalization (>3 weeks)	70%
Obesity (BMI >35)	30.9%
Patients with malignancy	5%

Table 4 Shows different risk factors for developing different post COVID infections. Out of all risk factors use of immunosuppressive drugs (including Corticosteroids) and prolonged duration of hospital stay were statistically significant with p-values: 0.005 and 0.013 respectively. Other risk factors were not statistically significant.

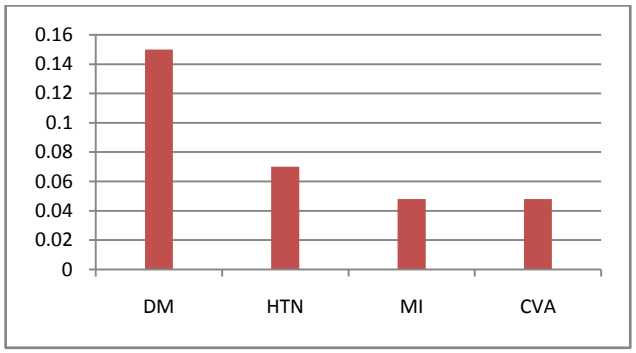

Figure2. Shows post COVID non-infectious complications

Shows rate of different post COVID non-infectious complications including- DM:Diabetes mellitus (15%); HTN: Hypertension (7%); MI: Myocardial infarction (4.8%); CVA: Cerebrovascular accident (4.8%).

DISCUSSION

The present study was conducted in the Department of Microbiology, R.G.Kar Medical College and Hospital, Kolkata with objectives of determining different post COVID bacterial, mycological and viral infections ;different associated risk factors, measuring the different laboratory parameters such as C-reactive protein (CRP), Ferritin, creatinine, haemoglobin, total leukocyte count (TLC), D-dimer at the time of admission with COVID19 positive status and at the time of development of post COVID infections, determination of development of different post COVID non-infectious complications. The key findings of our study were that–there were 57% male patients and 42.8% female patients. Maximum number of patients were of 41-60 years age range. The different post COVID infections developed were-mucormycosis (11 patients) including 8 rhinocerebral and 3 pulmonary cases; candidiasis (8 patients) including 2 pulmonary, 4 urinary tract infection ,1 oral and 1 esophageal candidiasis cases ;pulmonary aspergillosis (1 case); *Acinetobacter species* (3 cases), *Klebsiellapneumoniae* (8 cases), *Escherichia coli* (2 cases), *Burkholderiacepacia* (1 case) , MRSA (3 cases), *Enterococcus species* (1 case), *Pseudomonas aeruginosa* (1case), 1 case of pulmonary tuberculosis, Influenza virus positive in 2 cases. 6 patients died including 2 rhinocerebralmucormycosis cases, 2 patients with *Acinetobacter species* and 1 patient with *Klebsiellapneumoniae* due to septicaemia and 1 patient with *Pseudomonas aeruginosa*. Out of all patients the percentage of susceptibility to different antimicrobial agents among gram negative bacterial isolates were 95% to PB, 75% to TGC, 80% to MRP, 70% to LE, 65% to AS, 50% to COT, 45% to CTR; among gram positive bacterial isolates VA susceptibility and LZ susceptibility were 80% and 85% respectively.Among candida isolates the percentage susceptibility were -80% to VRC, 70% to LAMB, 50% to IT and 65% to FLC. However, for moulds susceptibility were not put, but confirmatory diagnosis was done and they were treated as per current guidelines[8,9]. Use of immunosuppressive drug especially Corticosteroids was most common risk factor associated with post COVID infections. CRP, ferritin, TLC, creatinine, haemoglobin and D-dimer showed a rising trend in mean values during post COVID infections as compared to values at admission. Diabetes mellitus (15%) was the most common post COVID non-infectious complication followed by

hypertension (7%), myocardial infarction (4.8%) and cerebrovascular accident (4.8%).

In our study,the different age groups involved are 0-20 years, 21-40 years, 41-60 years,61-80 years and 81years & above and the rate of post COVID infections in these age groups were -9.52%, 19.04%, 33.3%, 23.9% and 14.28% respectively. In another study by *Otterson LB et al*, patients among 18-64 years showed 35.4% and more than or equal to 65 years age patients showed 45.4% occurrence of post COVID infections[10]. This discrepancy can be due to difference in demographic patterns. In our study,there were 57% male and 42.8% female post COVID patients developing different infections. In another study by *Jin MJ et al*, male patients with COVID19 disease were more at risk of worse outcomes including post COVID infections and death as compared to females[11]. So, our study results corroborates with this study. In present study, different post COVID infections were seen like fungal infections including mucormycosis (n=11, 26.1%), candidiasis (n=8, 19%), pulmonary aspergillosis (n=1, 2.38%), bacterial infections including *Acinetobacter species* (n=3, 7.14%), *Klebsiellapneumoniae* (n=8, 19%), *Escherichia coli* (n=2, 4.76%), *Burkholderiacepacia* (n=1, 2.38%), MRSA (n=3, 7.14%), *Enterococcus species* (n=1, 2.38%), *Pseudomonas aeruginosa* (n=1, 2.38%), pulmonary tuberculosis (n=1, 2.38%) and viral infection including influenza virus (n=2, 4.76%). In another study by *Pasquier G et al*, in India during COVID19 pandemic, COVID associated mucormycosis (CAMM) incidence was 0.27% to 1.8% among hospitalized and 1.6% among ICU patients[12].So, our study did not corroborate which may be due to higher prevalence of uncontrolled diabetes mellitus in our set up or may be due to smaller sample size in our study.In another study by *Ahmed N et al*, 12.6% of fungal infection was caused by candida species among post COVID patients[13]. This data does not corroborate with our study. In another study by *Egger M et al*, the prevalence rate of COVID associated Pulmonary Aspergillosis (CAPA) was between 1.6% and 38%[14].So our study results corroborate with this study. In another study by *Alenazi T et al*, 28% of patients with severe COVID19 pneumonia patient had bacterial coinfection with *Acinetobacter baumanii*[15]. So a discrepancy has been noted in this study with respect to our study. In another study by *Said BK et al*, screening was done to detect different types of post COVID patients and there were – 37% patients with *Klebsiellapneumoniae*, 18.6% were *Escherichia coli*, 8.5% were *Pseudomonas aeruginosa*[16]. These data does not corroborate with our study which may be due to smaller sample size in our study and also due to shorter duration of study in our case. Another study by Yang *Set al*, they found *Burkholderiacepacia*infection in 18.8% and *Staphylococcus aureus*infection in 7.3% post COVID patients[17].In another study by *Toc DA et al*, 2.6% post COVID patients suffered from *Enterococcus species* infection[18].This data corroborates with our study. In another study by *Alemu A et al*, the proportion of acute pulmonary tuberculosis among COVID19 patients was 1.07%[19]. This data almost corroborates with our study results. In another study by *YanX et al*, the prevalence of influenza co-infection among patients with confirmed COVID19 disease was found to be 2.45%[20]. This data almost corroborates with our study.Though many patients developing post COVID infection had history of Corticosteroids intake, some of the patients had no such history, in such patients the risk of acquiring different infections in post COVID period can be defined by the fact that COVID infection itself may weaken the immune system otherwise known as immune amnesia[1]. In present study, overall most common Mucorales found was *Rhizopusarrhizus*, most common Candida species was *Candida albicans*and the only isolate causing pulmonary aspergillosis was *Aspergillusfumigatus.* Infact most common cause of mucormycosis worldwide is due to *Rhizopusarrhizus*, most common candida spp. is *C.albicans*though there is an increasing trend noted in percentages of non-albicans Candida species these days and most common Aspergillusspecies isolated in different clinical samples is *Aspergillus fumigatus*[21]. In our study, 14.2% patients died due to different bacteriological and mycological post COVID infections. In an ICMR study, it was shown that 6.5% COVID19 patients died after discharge or during recovery period due to development of few other infections or different hospital acquired infections[22]. So, this data does not corroborate with our study results and this discrepancy may be due to difference in antibiogram amongst different bacterial and mycological isolates resulting in few highly resistant isolates in our setting. In our study the percentages of different drug susceptibilities among gram negative bacilli were-PolymixinB (95%), Meropenem (80%), Tigecycline (75%), Levofloxacin (70%), Ampicillin-Sulbactam (65%), Cotrimoxazole(50%), Ceftriaxone (45%); among gram positive cocci- Vancomycin (80%) and Linezolid (85%).In another study by *Khalid N et al*, PolymixinB were found to be 100% sensitive among gram negative bacteria and gram positive bacteria were highly sensitive to Vancomycin (98.2%) and Linezolid (99.4%)[23].So, our study does not corroborate which may be due to difference in antibiograms. In our study, the percentage of different drug susceptibilities among fungal isolates especially Candida species were- Voriconazole (80%), Liposomal Amphotericin B (70%), Fluconazole (65%) and Itraconazole (50%). In another study by *Gupta S et al*, the resistances of Candida species were- Voriconazole (87%), Fluconazole (65%), Itraconazole (19%) and AmphotericinB (37%) among *Candida albicans* urinary isolates[24]. This discrepancy may be due to difference in sample types studied in our set up and difference in antibiograms. In our study, different acute phase reactants showed an increasing trend from COVID19 disease patient at admission to these patients while developing post COVID infections. In another study by *Ali N*, it was noted that there was an increase of severe events by 5% for every one unit increase in CRP concentration in COVID19 disease patients[25]. In another study by *Marhaeni W et al*, there was an increased level of ferritin in patients with COVID19 than in healthy people[26]. In another study by *Copur S et a*l, elevated creatinine level was seen seen in COVID patients and more so when they developed different post COVID infections probably due to acute kidney injury[27]. In a study by *Sharma N et al*, in few COVID patients anemia was noted[28]. Total leukocyte count was raised in patients developing post COVID infections as compared to patients during admission[1]. In another study by *Nemec HM et al*, there was an elevation of D-dimer level in COVID19 disease and this plasma D-dimer levels was shown to be a good prognostic factor for COVID19 disease outcomes[29]. Increased levels of D-dimer may be due to injury to vascular endothelial cells by the virus leading to coagulopathy[1].So, all these studies supports the increasing trend of CRP, ferritin, creatinine, haemoglobin, total leukocyte count and D-dimer between patients at the time of admission

to hospital and at the time of development of several post COVID infections. In our study, the different risk factors were associated. There were 20% patients who required mechanical ventilation and subsequently developed different post COVID infections. In another study by *Hannah W et al*, the rate was 2.3% to 33.1%[30]. So, our study results corroborates with this study. In our study, pre-existing uncontrolled diabetes mellitus was found to be a risk factor for post COVID infection present in 25%. In another study by *Lim S et al*, underlying diabetes mellitus was found to be a risk factor for severe COVID19 with worse outcomes like post COVID infections and hence high mortality and a tight glycemic control is crucial to prevent severe courses of COVID19 disease[31]. In our study, immunosuppressive drugs including Coticosteroids therapy was associated risk factor for post COVID infections in 72% patients. It has been found that Corticosteroids when given during first 7 days of hospital admission that reduces mortality. However, use of inappropriate and high dose systemic steroids during COVID19 disease may increase risk of mucormycosis, aspergillosis infections and may also lead to reactivation of latent tuberculosis[32]. So, our study results corroborate. In our study we have seen out of all COVID19 patients developing post COVID infections, 70% of them had prolonged hospital stay (>3 weeks). Prolonged hospital stay in COVID19 patients may increase the risk of various bacterial and mycological infections due to contaminated hospital environment, patient to patient spread and due to use of broad spectrum antimicrobial agents[1].In our study it was seen that obese patients with body mass index (BMI) of more than or equal to 35 kg/m^2 was present as risk factor for post COVID infections in 30.9% patients. In another study by *Alqahtani FY et al*, showed that obesity with BMI of more than or equal to $40kg/m^2$ was associated with higher risk of severe COVID and influences disease presentation and due to insulin resistance in such patients, may lead to occurrence of deranged glycemic control increasing risk for opportunistic infections. Also obesity increased severity of influenza infection seen during Influenza 2009 H1N1 pandemic. So, post COVID influenza infection may be more severe in obese patients[33]. In our study, malignancy was a risk factor in 5% patients with post COVID infections. In another study by *Fillmore NR et al*, COVID19 patients with malignancy required higher frequency of hospitalization, ICU admissions, respiratory support, higher mortality and due to use of immunosuppressive chemotherapeutic agent they developed more post COVID opportunistic infections[34]. So, these studies corroborate with our study results. In our study, few common post COVID non-infectious complications were seen amongst patients like Diabetes mellitus in 15% (n=6), hypertension in 7% (n=3), myocardial infarction in 4.8% (n=2) and cerebrovascular accident in 4.8% (n=2). However, it is not fully known whether SARSCoV2 infection is associated with temporary hyperglycemia during active phase of COVID or if metabolic alterations persists which increases risk of subsequent diabetes among post COVID infection period; however use of high dose and long term corticosteroids may be responsible for development of post COVID diabetes mellitus[35].So, our study corroborates with this study. In another study by *Vyas AP et al*, new onset hypertension was found in 32.3% of patients at one year follow up post COVID19 disease recovery[36]. This data does not corroborate with our study. This discrepancy may be due to smaller sample size and shorter duration of study. In another study by *Zuin M et al*, the rate of post discharge COVID19 patients developing acute myocardial infarction was between 0.1-1.1%[37]. This discrepancy with our study may be due to smaller sample size. In another study by *Bass DI et al*, the rate of cerebrovascular accident was found in 5.7% patients with severe disease and 0.8% in patients with mild to moderate disease[38]. This study almost corroborates with our study.

Strength of study: 1) An extensive study, different types of post COVID infections were studied. 2) The demographic profile, risk factors and non-infectious post COVID complications were studied. 3) Also different laboratory markers determining severity of patient's condition while developing post COVID infections were enlightened.

Limitations of study: 1) Though antimicrobial susceptibility testing were performed for all bacteria and Candida species; Antifungal susceptibility was not performed for mucorales and Aspergillus, however they were treated as per current guidelines. 2) The study period was short and sample size was smaller.

CONCLUSION

COVID19 is altering the landscape of hospital acquired infections and several opportunistic infections which may be due to prolonged hospital stay and use of immunosuppressants like Corticosteroids, warranting further investigations. Though other risk factors are not statistically significant but may be contributory. We must be vigilant whenever a recovering COVID patient turn symptomatic as often healthcare associated infections and opportunistic infections increase the mortality manifold.

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