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Research Article

SARS-CoV-2 Catastrophe and aftermath in 3800 Indian patients with comorbidities: A retrospective study

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Abstract

Introduction: The severity of SARS-CoV-2 infections on the basis of hospitalization, ventilation support, oxygen therapy, and morbidity has been a major challenge witnessed by the medical fraternity during 2020 due to the global pandemic. The correlation and effects of the severity caused by the disease outcome due to SARS-CoV-2 infections along with co-morbid factors and lifestyle habits in the diverse Indian population are of greater interest to evaluate.

Objective: In this study, we aimed to investigate the association between various co-morbid factors, addiction habits, and their contribution towards disease severity, and the need for hospitalisation in a retrospective manner in a cohort of SARS-CoV-2 positive patients from the eastern part of India.

Methodology: Swab samples from a total of 75000 individuals were screened by RT-PCR technology between the months of July-November, 2020 at the inDNA Life Sciences laboratory as per ICMR guidelines. Out of 75000 individuals, 3800 SARS-CoV-2 positive individuals were included in this study. Retrospectively, the positive cases were considered for follow-up investigations. Clinical correlation between the severity of the disease and co-morbid factors along with addiction habits were performed.

Results: We observed that the young age group of 20-30 had the highest incidence of SARS-CoV-2 and the majority of infected individuals were males (66%). Disease transmission was high with the majority of asymptomatic patients presenting with low-grade fever. It was also observed that disease severity, mortality, and average recovery time (>14 days) were higher in patients with co-morbid conditions as compared to patients without co-morbidity. In addition, patients with a history of addiction exhibited higher severity of the disease and longer hospitalization with comparatively high mortality.

Conclusion: In this retrospective study we found, that co-morbid conditions such as diabetes, hypertension, COPD, asthma, liver disorder, and kidney disorder contributed to the severity or had detrimental outcomes of SARS-CoV-2 infections. Addiction to alcohol and smoking/chewing tobacco also results in poor clinical outcomes of the disease. Therefore, understanding the relationship between these risk factors and their association with disease outcomes can further assist the medical fraternity in managing the current patients in the clinic/ hospitals with a prior history of SARS-CoV-2 infection. Years after the outbreak, COVID-19 still remains a gray area with probably a lot of answers to be given to mankind.

Introduction

The outbreak of COVID-19 which later was declared as pandemic by WHO was caused by SARS-CoV-2, which belongs to the Coronaviridae family, in the order of Nidovirales and sub family of Orthocoronavirinae, four genera of coronaviruses have been identified so far namely Alpha-coronavirus (α-CoV), beta-coronavirus (α-CoV), gamma-coronavirus (α-CoV), and delta-coronavirus (α-CoV) [1]. Among these four genera, SARS-CoV-2 falls in the genera of α-CoV [1]. SARS-CoV-2 primarily affects the lower respiratory systems causing viral pneumonia, moreover, it has been also reported to infect the Gastrointestinal

(GI) system, liver, central nervous system, and kidney [2]. The latent period of incubation of SARS-CoV-2 varies from 5 to 25 days and the symptoms of the disease include high fever, loss of smell and taste, and severe respiratory distress, such as cough, sore throat, chest congestion, and muscle fatigue. Also, cases of severe headache and diarrhea have been reported to be involved with positive cases of SARS-CoV-2 [3,4]. Earlier studies have reported that patients predisposed with previous comorbid factors have very poor disease outcomes [5]. Amongst the prevalent comorbid factors, classifying the most significant risk groups is crucial for anti-2019-nCoV therapy decision-making [6]. It has been previously reported that, during a period of poor glycemic control in the body, the risk of infection is much higher in the case of patients having diabetes mellitus (DM) [7]. DM has been recently reported to be one of the most common comorbid factors in SARS-CoV-2 infections [8,9]. Also, few reports suggest that people with SARS-CoV-2 infection along with DM have increased mortality risk [10,11]. Among other comorbid factors, hypertension has been reported to be involved in COVID-19 infections with fetal outcomes [12,13], also Chronic Obstructive Pulmonary Disease (COPD), Asthma, and Liver and Renal disorders have been found to be lethal when associated with COVID-19 infections in patients [14,15]. Additionally, a study performed by Guan et al, showed that patients admitted to hospitals with severe COVID-19 and those with primary end-points (admission in ICU, the use of mechanical ventilation, or death) had a significantly higher percentage of diabetes, hypertension, coronary artery disease, cerebrovascular disease, COPD, chronic renal disease, and cancer [16]. Other than predisposition towards various co-morbid conditions, individuals with addiction towards alcohol and tobacco also has poorer diseases outcome [17-20]. Till date very limited number of studies has been conducted with a long-term follow-up of the patients, thus the findings should be interpreted carefully. In this study was performed in order to find a possible clinical correlation between COVID-19 infections with prevalent co-morbid conditions (diabetes, hypertension, COPD, asthma, kidney and liver disorders) and addiction through a retrospective three months follow-up study. This study will help in strengthening the available reports and also help in managing the disease outcome in case of COVID-19 cases. In this study we aim to evaluate the disease outcome of COVID-19 in patients when presented with other co-morbid conditions and addiction history in eastern part of India.

Material and method

Study population

A total of 75,000 individuals were tested for COVID-19 infection from the month of July to November 2020 at inDNA Life Sciences Pvt. Ltd, Bhubaneswar, Odisha, India. 3800 COVID-19-positive patients from the months of August and September who willingly participated in the study were included in the current study. Detailed consent was taken from the patients or their nominees prior to participation in this study and complete confidentiality was maintained related to medical records of the patients.

Collection of samples

The samples were collected as both nasopharyngeal and oropharyngeal swabs and were suspended in 15 ml conical tubes containing 3 ml of Viral Transport Medium (VTM) primarily constituting of Hanks Balanced Salt Solution, protein antibiotics, buffers to control the pH and phenol red as pH indicator with range pH 7.0-7.6 (HiViral™ Transport Medium, HiMedia Laboratories, Pvt. Ltd., Nashik, MH).

Isolation of viral RNA and RT-PCR

The swabs were then further processed for RNA extraction with the help of a Viral RNA extraction kit [TRUPCR® viral RNA extraction kit, v1.0]. Following RNA extraction, Real Time-Polymerase Chain Reaction (RT-PCR) reaction was set up with the SARS-CoV-19 Real Time-Polymerase Chain Reaction (RT-PCR) test-kit [TRUPCR® SARS-CoV-19 RT qPCR kit, v2.0, manufactured by Kilpest India Ltd.] utilizing TaqMan chemistry. SARS CoV-2 infection was confirmed upon detection of fluorescent peaks in both the envelope-gene(E-gene) and nucleocapsid-gene(N-gene) below the cut-off of 35 cycles. The Human RNase-P gene was used as the housekeeping gene (internal control) for RNA extraction. Primer sequences for the specified target regions are, E-gene (Forward-ACAGGTACGTTAATAGTTAATAGCGT, Reverse-ATATTGCAGCAGTACGCACACA, Probe 5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-3'), N-gene (Forward-CACATTGGCACCCGCAATC, Reverse-GAGGAACGAGAAGAGGCTTG, Probe 5'-FAM-ACTTCCTCAAGGAACAACATTGCCA-3'), Human RNase-P gene (Forward-AGATTTGGACCTGCGAGCG, Reverse-GATAGCAACAACTGAATAGCCAAGGT, Probe 5'-VIC-TTCTGACCTGAAGGCTCTGCGCG-3').

A 25 µL reaction contained 10 µL of RNA, 10 µL of master mix(Hot-start DNA polymerase, reaction buffer, dNTPs, MgCl2, and stabilizers), 0.35 µL of enzyme mix(Reverse transcriptase), 4.65 µL of primer-probe mix. Primer and probe sequences, as stated above. All oligonucleotides were synthesised and provided as ready-to-use mixes (TRUPCR® SARS-CoV-19 RT qPCR kit, v2.0). Thermal cycling was performed at 50 °C for 15 min for reverse transcription, followed by 95 °C for 5 min and then 40 cycles of 95 °C for 5 s, 60 °C for 40 s (dye acquisition) and 72 °C for 15 s on a QuantStudio 3 & 5 Real-Time PCR Systems (ThermoFisher Scientific, Applied Biosystems, CA, USA).

Data collection

A detailed questionnaire was prepared and filled up based on different clinical parameters such as co-morbidity, disease symptoms, hospitalization, ventilation support, oxygen therapy, mortality, and lifestyle disorders such as addiction towards smoking/chewing tobacco and alcohol consumption from the patients who participated in this study (Supplementary Figure 1).

Statistical analysis

All the statistical analysis was performed by using GraphPad Prism(v.8.2.0) software. A chi-square test was performed

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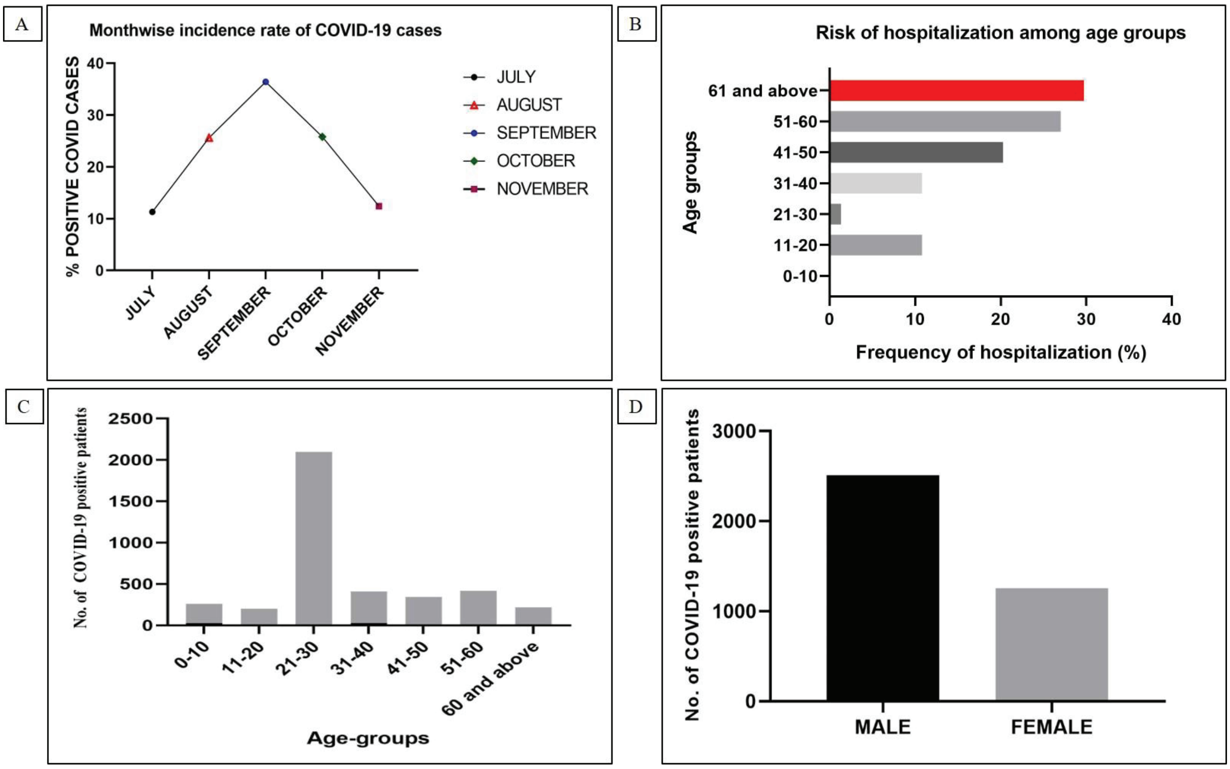

Figure 1: Prevalence of COVID-19 in our cohort A) Month-wise occurrence of positive COVID-19 cases tested at inDNA life sciences B) Risk of hospitalization among different age groups. C) Histogram representing the rate occurrence of COVID-19 cases across different age groups. D) Histogram representing the fraction of males and females affected with COVID-19.

to evaluate the association between different co-morbid factors and the severity of the disease based on parameters like hospitalization, ventilation support, oxygen therapy, and mortality. A *p* - value < 0.05 was considered statistically significant.

Results

We have tabulated the month-wise distribution of parentage of COVID-19-positive patients detected by RT-PCR referred to inDNA Life Sciences for COVID-19 tests. A total of 75,000 individuals suspected of COVID-19 infection from the month of July to November 2020 were screened by RT-PCR assay. During the COVID-19 pandemic scenario, we observed the highest peak in infection rate in the months of August and September as compared to other months (Figure 1A). A retrospective study was designed to further analyze the implication of the disease outcome based on various clinical parameters, co-morbid factors, and addiction habits. Detailed consent was obtained from 3800 COVID-19-positive patients with complete medical records, and were included in the study.

The risk of hospitalization was highest in the old age affected by COVID-19.

From our cohort, it was observed that there was an increased likelihood of hospitalization in the age group of 61 and above (29.72%) followed by the previous age group of 51-60 (27.02%). COVID-19 patients belonging to the age group of 0-10 and 21-30 possess a very low risk of hospitalization (Figure 1B).

The maximum no. of COVID-19-positive cases was found to be in the age group of 20-30 as shown in (Figure 1C). Reported COVID-19-positive cases were majorly males i.e. 66% as compared to females 33%. (Figure 1D)

Disease transmission was high with the majority of asymptomatic patients presenting a low-grade fever.

The transmission of the SARS-CoV-2 virus has been previously reported to be very high [21]. Upon investigating its rate of transmission in Odisha patients, the disease was transmitted in 53% of the cases in spite of strict lockdowns and social distancing indicating the virus is highly infectious (Figure 2A). The symptoms of the COVID-19-positive patients were investigated. An increase in the number of asymptomatic patients to symptomatic patients was observed in our cohort (Figure 2B) and a maximum number of symptomatic positive cases demonstrated low-degree fever (Figure 2C).

Disease severity was higher in patients with co-morbid conditions.

It has been previously reported that COVID-19-positive patients with co-morbidities demonstrated poorer prognosis and therapy response [6,22-25]. Upon further analysis of the comorbid condition of our patient cohort, we observed that COVID-19-positive cases with pre-existing co-morbid factors had poor disease outcomes (Table 1). Furthermore, the patients with diabetes were segregated and its correlation with the severity of the disease and their response to routine treatment was studied. We observed patients with diabetes had a greater prevalence of 4%. Diabetic patients experienced the

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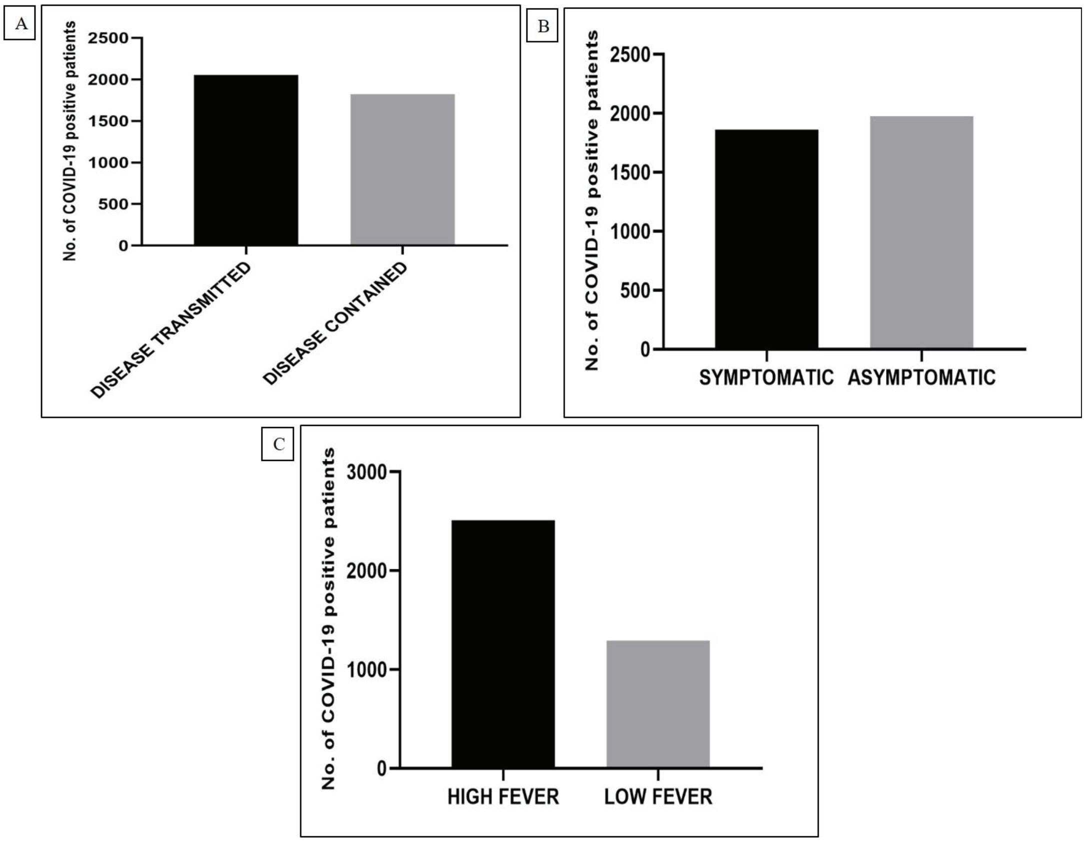

Figure 2: Disease transmission and symptoms of COVID-19-positive cases of our cohort. A) Graph representing over 53% of the cases were transmitted. B) Histograms representing a higher incidence of asymptomatic cases as compared to the symptomatic ones. C) Graph indicating the fraction of low-grade fever and high-grade fever (<99%) amongst individuals with COVID-19 infection.

Table 1: Frequency distribution of various risk factors associated with the severity of COVID-19 along with pre-existing comorbidities and average recovery time.

Comorbid conditions	FREQUENCY DISTRIBUTION OF Individuals affected (%)	Hospitalization (%)	Ventilation support (%)	Oxygen therapy administered (%)	Morbidity (%)	Average time taken to recover
Diabetes	4.00	2.89	0.11	0.34	0.34	7 days
Hypertension	3.13	2.11	0.11	0.21	0.21	7 days
COPD	1.45	0.87	0.29	0.29	0.18	13 days
Asthma	1.68	1.13	0.34	0.24	0.34	13 days
Kidney disorders	1.34	1.13	00	00	2.37	15 days
Liver disorders	0.66	0.11	0.11	00	0.11	12 days

severe impact of the COVID-19 disease where hospitalization was required for a maximum no. of patients (2.89%) for proper clinical management as shown in Figure 3A. The correlations between the diabetic patients and the severity of the disease in terms of hospitalization, requirement of oxygen support, and morbidity showed a strong correlation with p-values <0.0001, <0.0001, and 0.0010 respectively (Table 2).

We further segregated patients with COPD and then studied the correlation with the disease response. We observed patients with COPD had a poorer clinical response where oxygen therapy and ventilators were used in 1.45% of the patients for their proper care after hospitalization as observed in (Figure 3B). The correlations between patients with COPD and the severity of COVID-19 disease in terms of hospitalization, the requirement of ventilation, the requirement of oxygen support, and morbidity showed a strong correlation with p-values <0.0001, <0.0001, <0.0001, and 0.0009 respectively (Table 2)

COVID-19 has been implicated in severe pulmonary responses. Therefore, to investigate the implication of the infection on the patients, we segregated patients with a previous history of asthma and correlated it with the recovery rate and the disease response. We observed patients having asthma also had poorer disease outcomes where oxygen therapy and ventilators were used in more than 1.68% of the asthmatic patients for their proper care after hospitalization. (Figure 3C). The correlation between patients with asthma and the severity of COVID-19 disease in terms of hospitalization, the requirement of ventilation, oxygen support, and morbidity showed a strong correlation with p-values <0.0001, <0.0001, <0.0001, and <0.0001 respectively (Table 2). Further, we also checked the correlation between patients with hypertension, kidney disorders, and liver disorders and the severity of COVID-19 disease in terms of hospitalization, the requirement of ventilation, oxygen support, and morbidity. It was observed

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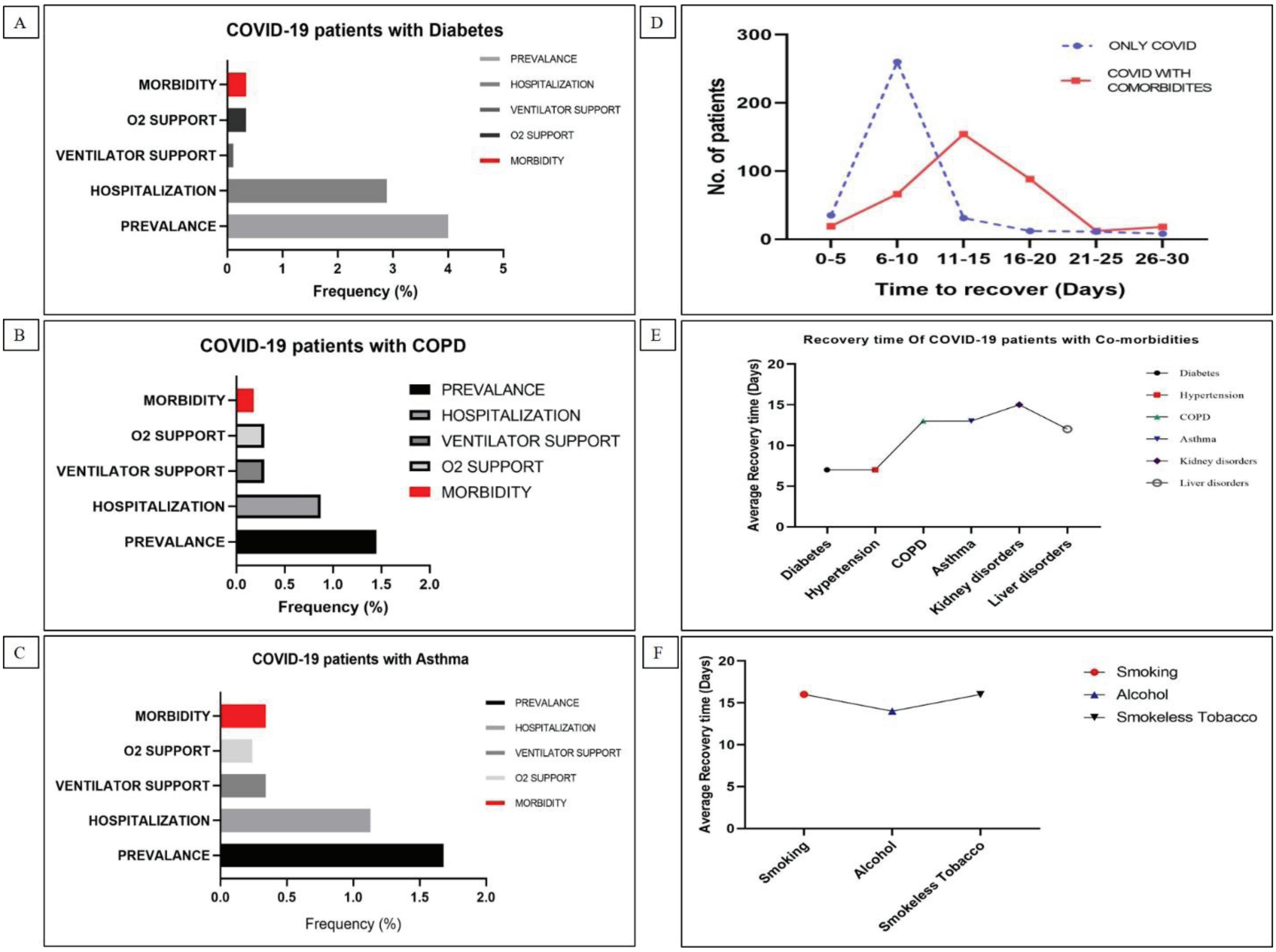

Figure 3: Disease severity and average recovery time in COVID-19-positive cases with co-morbid factors or smoking history. A) Graph representing patients with diabetes had a higher frequency of mortality and required hospitalization. B) Graph representing patients with COPD had a higher frequency of hospitalization and mortality. C) Graph representing patients with asthma required ventilator support and the likelihood of mortality is highest in these cases. D) Graph representing longer recovery time for COVID-19 patients with pre-existing co-morbid factors. E) Graph representing the average recovery time of COVID-19-positive patients with co-morbidities. F) Graph representing the average recovery time of COVID-19-positive patients with various addiction habits.

that patients with hypertension were strongly correlated with the risk of hospitalization, the requirement of ventilation, oxygen support, and morbidity with p-values <0.0001, 0.0380, 0.0002, and <0.0001 respectively (Table 2). Patients with kidney disorders strongly correlated with risk factors of hospitalization and morbidity with p-values of <0.0001 and <0.0001 respectively (Table 2). Furthermore, patients with liver disorders strongly correlated with risk of ventilation and morbidity with p-values of 0.3597 and 0.0053 respectively (Table 2).

Further, the effect of co-morbidities on the overall recovery rate of the patients suffering from COVID-19 was evaluated. In order to check that, we segregated COVID-19 patients with comorbid factors and checked their average recovery time in days. We observed that patients with no co-morbidities recovered faster on an average of 7 days on the other hand patients suffering from COVID-19 with multiple co-morbidities took longer to recover on an average of 14 days of time as shown in (Figure 3D). Also, the average recovery time of COVID-19 patients with various co-morbidities was checked (Figure 3E) and it was observed that COVID-19 patients with kidney disorders took the longest time (16 days) to recover followed by patients with COPD, asthma, and liver disorders (14 days) and patients with diabetes and hypertension took least time to recover (7 days).

Disease severity was higher in patients with a history of addiction.

In order to observe the effect of lifestyle on the severity of the disease, we segregated patients with habits of smoking/ chewing tobacco and alcohol intake. We observed the majority of the patients with COVID-19 infection had a habit of smoking (66%) and maximum patients required hospitalization support (83%) for proper management of the disease. Smoking also showed a higher hospitalization risk (71%) among patients infected with COVID-19. The highest mortality rate of 9.09% was observed in patients with a habit of alcohol consumption (Table 3). We also evaluated the average recovery of COVID-19-positive patients with a history of addiction (Figure 3F), it was observed that, patients with addiction to consumption of tobacco (smoked or smokeless) in any form required a longer time to recover as compared to patients addicted towards alcohol.

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Table 2: Correlation between individual co-morbidities and various severity parameters of COVID-19 disease.

Co-morbidities	No. of patients	Clinical parameters for severity of COVID-19							
		Hospitalization		Ventilator support		Oxygen therapy		Morbidity	
		Yes	No	Yes	No	Yes	No	Yes	No
Diabetes									
Yes	152	110	42	04	148	13	139	13	139
No	3648	1907	1741	49	3599	49	3599	109	3539
p - value		<0.0001		0.1604		<0.0001		0.0010	
Hypertension									
Yes	119	80	39	04	115	08	111	08	111
No	3681	628	3053	37	3644	44	3637	1017	2664
p - value		<0.0001		0.0380		0.0002		<0.0001	
COPD									
Yes	55	33	22	11	44	11	44	07	48
No	3745	637	3108	34	3711	42	3703	102	3643
p - value		<0.0001		<0.0001		<0.0001		0.0009	
Asthma									
Yes	64	43	21	13	51	09	55	13	51
No	3736	1395	2405	34	3702	47	3689	98	3736
p - value		<0.0001		<0.0001		<0.0001		<0.0001	
Kidney Disorders									
Yes	51	43	08	00	51	00	51	09	42
No	3749	751	2998	47	3702	55	3694	102	3692
p - value		<0.0001		>0.9999		>0.9999		<0.0001	
Liver disorders									
Yes	25	04	21	04	21	00	25	04	21
No	3775	785	2290	47	3728	55	3720	106	3669
p - value		0.3597		0.0003		>0.9999		0.0053	

Table 3: Frequency distribution of the severity of COVID-19 individuals with a history of addiction.

Addiction towards	FREQUENCY DISTRIBUTION OF					Average time taken to recover
	Individuals affected (%)	Hospitalization (%)	Risk of ventilation (%)	Risk of oxygen therapy (%)	Morbidity (%)	
Smoking	66.66	83.33	00	00	5.55	16 days
Alcohol	40.74	100	00	00	9.09	14 days
Chewing Tobacco	51.85	71.42	00	14.28	NIL	16 days
Multiple addiction	37.03	70	00	20	NIL	14 days

Discussion

According to published data from WHO and ICMR, India stood second only after the United States of America in terms of total number of individuals infected with COVID-19. Indian populations with higher comorbidities along with addiction possess a higher threat of infection and a higher rate of morbidity. Patients with diabetes followed by hypertension, COPD, and asthma were most affected in our study cohort, and possess an increased risk of hospitalization. It was observed that the highest rate of infection was in the age group of 20-30 years. This indicates that the younger population was mobile despite restrictions and probably contributed to the rapid transmission of the disease as well as increased the risk of the vulnerable group of elderly in the family. From the above results, we could comment that COVID-19-infected individuals belonging to the age of 51 and above are more likely to be worst affected and hence they are at a greater risk of hospitalization with oxygen and ventilator support. On the other hand, the age group of 20-30 was the most affected age group in our cohort with the highest number of individuals infected with COVID-19. Symptoms of the COVID-19 patients in our cohort were mainly low-grade fever with loss of smell and taste, and the majority of the patients were asymptomatic in nature. Out of 3800 COVID-19 patients, the majority were male individuals. Individuals with a positive COVID-19 report along with the presence of other co-morbid factors are at an increased risk of hospitalization with ventilation support and isolation. Amongst the six co-morbid factors studied in our cohort, individuals with a history of diabetes were most affected followed by individuals with a history of hypertension. Recent studies have shown that COVID-19-positive individuals with a history of diabetes are more likely to be hospitalized with a low recovery rate and poor prognosis [15,22,26,27]. Individuals affected with diabetes and hypertension are known to express greater amounts of angiotensin-converting enzyme 2 (ACE2) as compared to normal individuals [8,28,29]. Human coronaviruses SARS-CoV and SARS-CoV-2 are known to bind with ACE2 which is expressed in the epithelial lining of blood vessels, lungs, intestines, and kidneys [30-35]. Therefore, increased expression of ACE-2 in patients with diabetes and hypertension poses a greater risk of infection with COVID-19 [25]. Diseases like diabetes, hypertension, COPD, and asthma are associated with poor functioning of the respiratory and cardiovascular systems. Post-COVID-19 infection, inflammation in the cardiovascular system accompanied by expression of various cytokines and cardiac enzymes (troponin) may lead to arrhythmias, acute coronary

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syndrome, myocardial infractions, and death [13,23,36-39]. In our cohort of COVID-19-positive cases, individuals predisposed towards any co-morbid conditions represented a higher risk of hospitalization as compared to COVID-19-positive individuals without any known comorbidities. A significant correlation was observed between co-morbidities such as diabetes, hypertension, COPD, asthma, and the severity of the disease in terms of hospitalization, requirement of oxygen support, and morbidity. Additionally, a significant positive correlation was also observed in COVID-19 patients with kidney and liver disorders with the severity of the disease in terms of risk of hospitalization, ventilator support, and morbidity.

Amendable risk factors such as smoking have been previously reported to be involved in the severity of the disease outcome of COVID-19 and these habits also increase the likelihood of acquiring infection in individuals [17-20,40]. In concordance with the above-mentioned study, we also observed an increased frequency of hospitalization and mortality rate in individuals pre-exposed to cigarette smoke, alcohol consumption, and both.

The absence of a comprehensive, unified, and open-access national database for listing and sharing important parameters of COVID-19 patient data poses a major limitation in our study. All the data were collected individually from patients retrospectively over telephonic communication only. Hence, a detailed clinicophysiological correlation in the COVID-19-positive study group could not be established. Additionally, the ACE2 expression profile in patients belonging to the higher-risk group needs to be studied to validate the findings. Also, the correlation between other pathophysiological parameters and with severity of COVID-19 disease outcomes needs to be validated in a larger population.

Conclusion

A health policy towards better management of post-COVID-19 infection must provide priority to patients with other non-communicable diseases, addiction history, and especially old age population. The younger population was the most affected and were also the first ones to be vaccinated across the country.

The current study highlights the importance of, co-morbid conditions such as diabetes, hypertension, COPD, asthma, liver disorder, and kidney disorder that contributed to the severity or had detrimental outcomes of SARS-CoV-2 infections. Additionally, addiction to alcohol and smoking/ chewing tobacco were also found to be associated with poor clinical outcomes of the disease. Although the world has recovered from the global pandemic, the after and long-term effects of the infections are yet to be known and explored in the population. India being a heterogeneous population with a diverse genetic pool is an ideal population to understand the aftermaths of the devastating infection episodes. Years after the outbreak, COVID-19 still remains a gray area with probably a lot of answers to be given to mankind. Therefore, understanding the relationship between these risk factors and their association with disease outcomes can further assist the medical fraternity in managing the current patients in the clinic/ hospitals with a prior history of SARS-CoV-2 infection.

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Ethical declarations

The study was conducted abiding by the Helsinki Guidelines, 2013. The study was reviewed and approved by the Independent Ethics Committee-Institutional Review Board (IEC-IRB) of inDNA Life Sciences, Bhubaneswar, Odisha, India with approval number [IEC-ILS/IRB/MB/002/20].

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