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Prevalence of Symptomatic and Asymptomatic Trends of COVID-19 and their Aggressiveness among Various Gender and Age Groups Due to Smoking

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ABSTRACT

COVID-19 remains a global threat and many developed countries are still troubled by the COVID-19 pandemic. During first wave of COVID-19, about 23 million people tested positive for the virus, worldwide. Hence, the factors that contribute towards enhancing the COVID-19 burden must be assessed. In the current study, RT-PCR testing method was used for the detection of COVID-19. Specimens used to detect the infection comprised nasopharyngeal swabs. After their collection, the specimens were processed further for the extraction of RNA and PCR. About 60% of sampled patients tested positive and 40% tested negative for COVID-19 infection. COVID-19 prevalence was found to be higher in male patients than female patients. Among COVID-19 positive individuals, 70% were smokers and 30% were non-smokers. Positive cases were more frequent in the age group of 40-60 years. Moreover, only 30% of the patients showed symptoms of COVID-19. Keeping in view the above statistics, it is safe to assume that smokers are more prone to infection by COVID-19 which was found to be more prevalent in men, mainly targeting their elderly population. A major portion of COVID-19 positive patients didn't show any symptoms. It was concluded that asymptomatic cases of COVID-19 are a silent threat to the world.

Keywords: asymptomatic, age groups, COVID-19, RT-PCR, patients, smokers, symptomatic

1. INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is caused by a new type of corona-virus. Its outbreak began in Wuhan, Hubei Province, China in December 2019. Due to it's rapid spread worldwide, the World Health Organization (WHO) declared (COVID-19) outbreak a pandemic [1]. The causative agent of COVID-19 is a new type of pathogenic coronavirus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). It is phylogenetically similar to SARS-CoV with approximately 80% similarity between the genomes. Coronaviruses are spherical virions. They have spike (S) proteins on their surface which give them a crown-like appearance. They also have three other viral structural proteins [envelope (E), membrane (M), and nucleocapsid (N)], along with a lipid membrane and ~25-31 kilobase viral genome [2]. The target of SARS viruses in the human body is the respiratory tract. They enter the cell through cell-entry receptor, namely ACE2 (angiotensin-converting enzyme 2) and cause an acute respiratory response. ACE2 is the only

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experimentally confirmed receptor for SARS-CoV-2 which also requires the activation of spike proteins found on its surface for cellular entry. The best targets for the activation of spike proteins are two host cell enzymes known as *Furin* and *TMPRSS2* [3]. The majority of human infections occur after exposure to the respiratory droplet [4]. The incubation period of SARS-CoV-2 is approximately 14 days. Signs and symptoms of COVID-19 include fever, cough, malaise, headache, sore throat, runny nose, diarrhea, dyspnoea, tachypnoea, and SpO2 < 93% [5].

The elderly, immunocompromised, and those with comorbid metabolic, pulmonary, and cardiac conditions are at greater risk of death from COVID-19 [6]. The role of comorbidities and other associated factors such as smoking cannot be neglected since it is presumably associated with the adverse prognosis of the disease. In this regard, extensive evidence has highlighted the negative impact of tobacco use on lung health and its causal association with a plethora of respiratory diseases [7]. Smoking is also detrimental to the immune system and its responsiveness to infections, making smokers more vulnerable to infectious diseases [8]. Previous studies showed that smokers are twice more likely than non-smokers to contract influenza and have more severe symptoms, while smokers were also noted to have higher mortality in the previous MERS-COV outbreak [9]. A study was conducted in March 2020 on COVID-19 that included information on patients' smoking status to evaluate the association between smoking and COVID-19 outcomes including the severity of the disease, the need for mechanical ventilation, the need for hospitalization in intensive care unit (ICU), and death. The total number of patients included in the study were 1099, out of which 173 showed severe symptoms and 926 showed non-severe symptoms. Among the patients with severe symptoms, 16.9% were current smokers and 5.2% were former smokers. On the contrary, among the patients with non-severe symptoms, 11.8% were current smokers and 1.3% were former smokers. Smoking is most likely associated with the negative progression and adverse outcomes of COVID-19 [10]. Another study explicates that smoking is the primary etiological factor behind chronic obstructive pulmonary disease (COPD) in the developed world, although environmental pollution and degraded air quality are also responsible for pulmonary diseases in developing countries. It is now the fourth leading cause of death in the world [11]. A study conducted on mice showed that exposure to smoking alters immune responses that promote viral infections and also cause alveolar damage [12].

China has a high smoking rate among its male population. It is approximately 50% in rural areas and 44.8% overall [13]. Most of the deaths identified from the epicentre of the COVID-19 outbreak were of men from older age groups and of those with underlying conditions, such as chronic respiratory disease, cancer, hypertension, diabetes, and cardiovascular disease. The initial age distribution of COVID-19 cases was skewed towards older age groups with a median age of 45 years for patients who were alive or who had an unknown outcome at the time of reporting. The median age of patients who were dead at the time of reporting was 70 years; this data is also supported by an early epidemiological study of 99 COVID-19 cases from Wuhan, China [14]. In a meta-analysis, five (5) studies regarding the effect of smoking along with COVID-19 were included, out

of which only one study found active smoking to be a significant predictor of COVID-19 severity. While, in the other four studies, the association was not found to be statistically significant. Despite the fact that a trend towards higher risk was appreciable, no significant association could be found between active smoking and the severity of COVID-19 when the data of individual studies were pooled. Hence, the results of this preliminary meta-analysis based on Chinese patients suggested that active smoking is not significantly associated with an enhanced risk of progression towards disease severity in COVID-19 [15].

2. METHODOLOGY

The current study was planned to cover a period of two months. The knowledge of diagnostic tests for SARS-CoV-2 is still evolving and a clear understanding of the nature of tests and the interpretation of their findings is important [16]. The most commonly used and reliable test for the diagnosis of COVID-19 is the RT-PCR test performed by using nasopharyngeal swabs or other upper respiratory tract specimens. The thermocycler of BioRad CFX maestro was used for PCR. The COVID-19 RT-PCR test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 [17]. Moreover, data was collected from 1000 patients through the designed questionnaire. These patients were categorized in terms of their age, status of smoking, and the associated symptoms of COVID-19 including fever, cough, and flu [17].

2.1. Specimen Type

A total of 1000 samples were collected from patients using the simple random sampling technique. Nasopharyngeal swabs were collected as specimens for the detection of nucleic acid from SARS-CoV-2.

2.2. Viral Gene Targets

ORF genes were the main target in this method.

2.3. Specimen/Sample Processing

The patients' nasopharyngeal swabs were collected and directly processed for RNA extraction.

2.4. RNA Extraction

RNA was extracted from clinical samples using the MagNA Pure 96 system (Roche, Penzberg, Germany) which uses the magnetic glass particle technology or from cell culture supernatants using the viral RNA kit.

2.5. Primers and Probes for Real-Time RT-PCR for SARS-CoV-2

Primers and probes were used in RT-PCR for the detection of COVID-19.

2.6. Real-Time Reverse-Transcription PCR

A 25 µL reaction contained 5 µL of RNA, 12.5 µL of 2 × reaction buffer (containing 0.4 mM of each deoxyribont triphosphates [dNTP] and 3.2 mM magnesium sulphate), 1 µL of reverse transcriptase / Taq mixture from the kit, 0.4 µL of a 50 mM magnesium sulphate solution (invitrogen), 1 µg of nonacetylated bovine serum albumin, primer sequences, and probe sequences.

Thermal cycling was performed at 55°C for 10 mins for reverse transcription, followed by 95°C for 3 mins, 45 cycles of 95°C for 15s, and of 58°C for 30s.

2.7. Detection of Viral RNA

Viral RNA was measured in terms of the cycle threshold (Ct). It becomes

detectable as early as Day 1 of symptoms and peaks within the first week of symptoms' onset [18].

3. RESULTS

The results determined that 60% of the patients tested positive for COVID-19 and 40% tested negative, as shown in Figure 1. Among positive cases, the percentage of COVID-19 infection was higher in male patients than in female patients, as shown in Figure 2. It could be due to more frequent outdoor activities and public gathering attended by men as compared to women. The findings showed that 70% of the patients were smokers (of both genders), as shown in Figure 3. This suggests that smoking could be the major risk factor that contributes in the spread of COVID-19 because smoking upregulates the expression of ACE-2 receptors on epithelium receptors, especially in lungs. These receptors are vital for SARS-COV-2. Significantly, a major portion of COVID-19 patients were in the age group 40-60 years, as shown in Figure 4. On the contrary, the infection rate in the age group 20-40 years was 20% and in the age group 60-80 years, it was 10%. It is interesting that only 30% of the patients presented the symptoms of COVID-19, while the remaining 70% were asymptomatic, as shown in Figure 5. The positive results showed a CT of below 30, as shown by sigmoid curves. Bold lines are also indicative of positive results and NA means negative, as shown in Figure 6
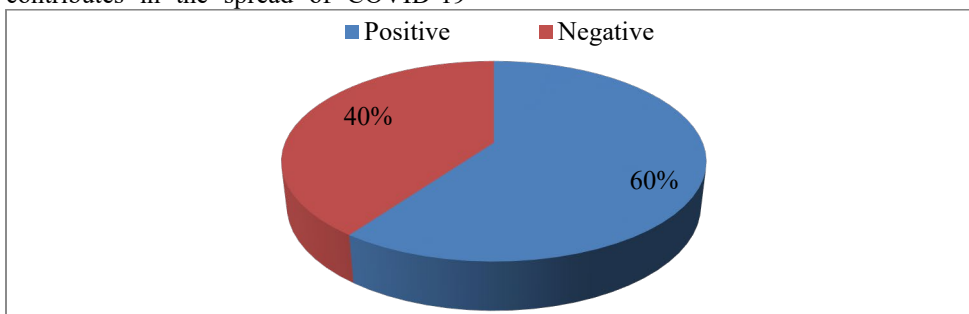

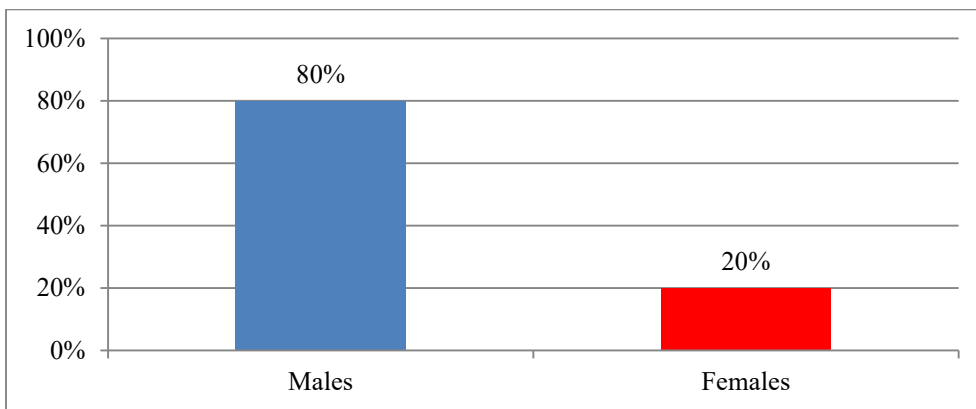**Figure 1.** Prevalence of Positive and Negative Cases of COVID-19

Figure 2. Gender-Wise Distribution of COVID-19 Positive Patients
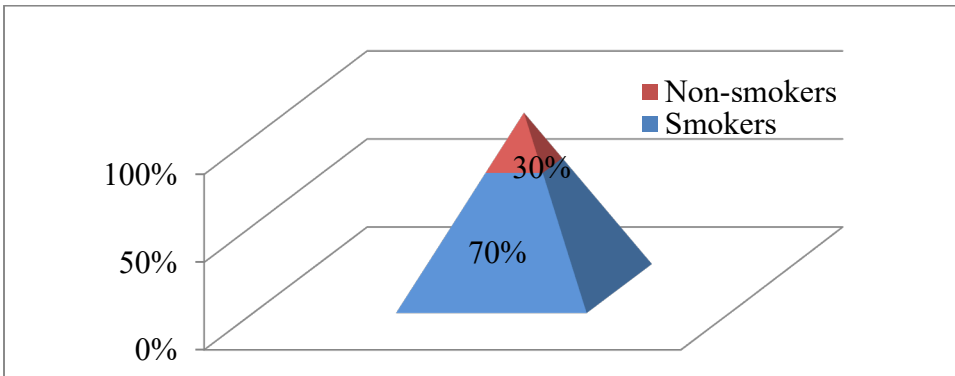
Figure 3. Percentage of Smokers and Non-smokers among COVID-19 Positive Patients
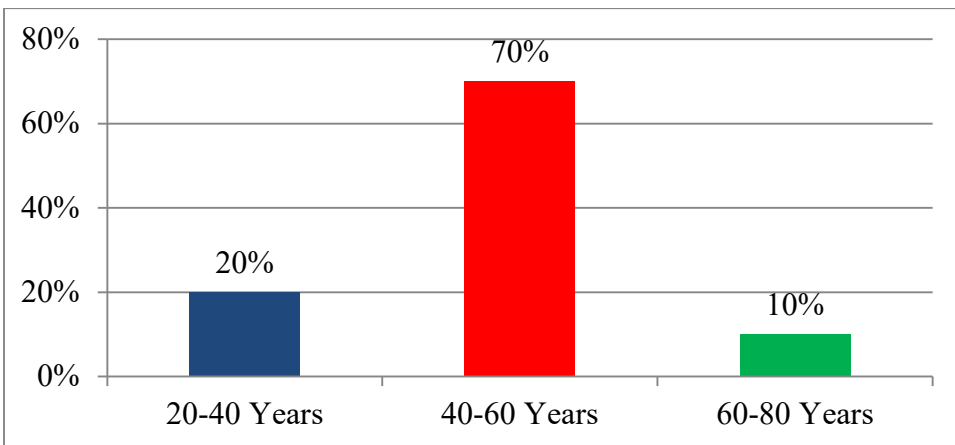

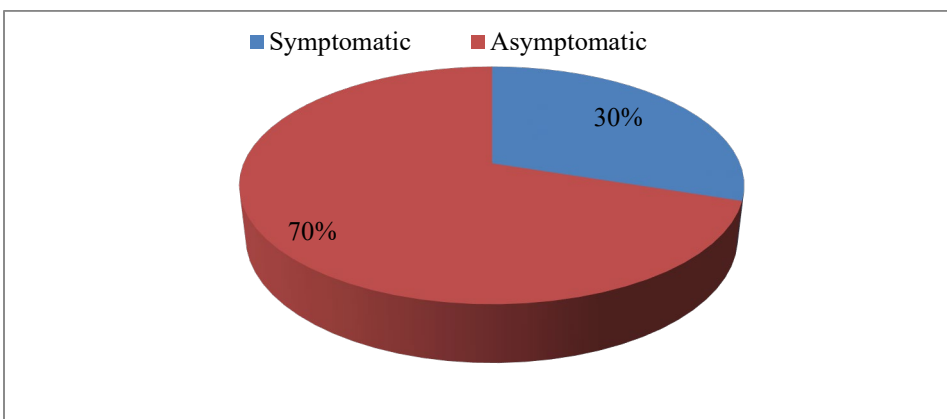**Figure 4.** Age-wise Distribution of COVID-19 Positive Patients

Figure 5: Percentage of Symptomatic and non-symptomatic cases among COVID-19 positive patients
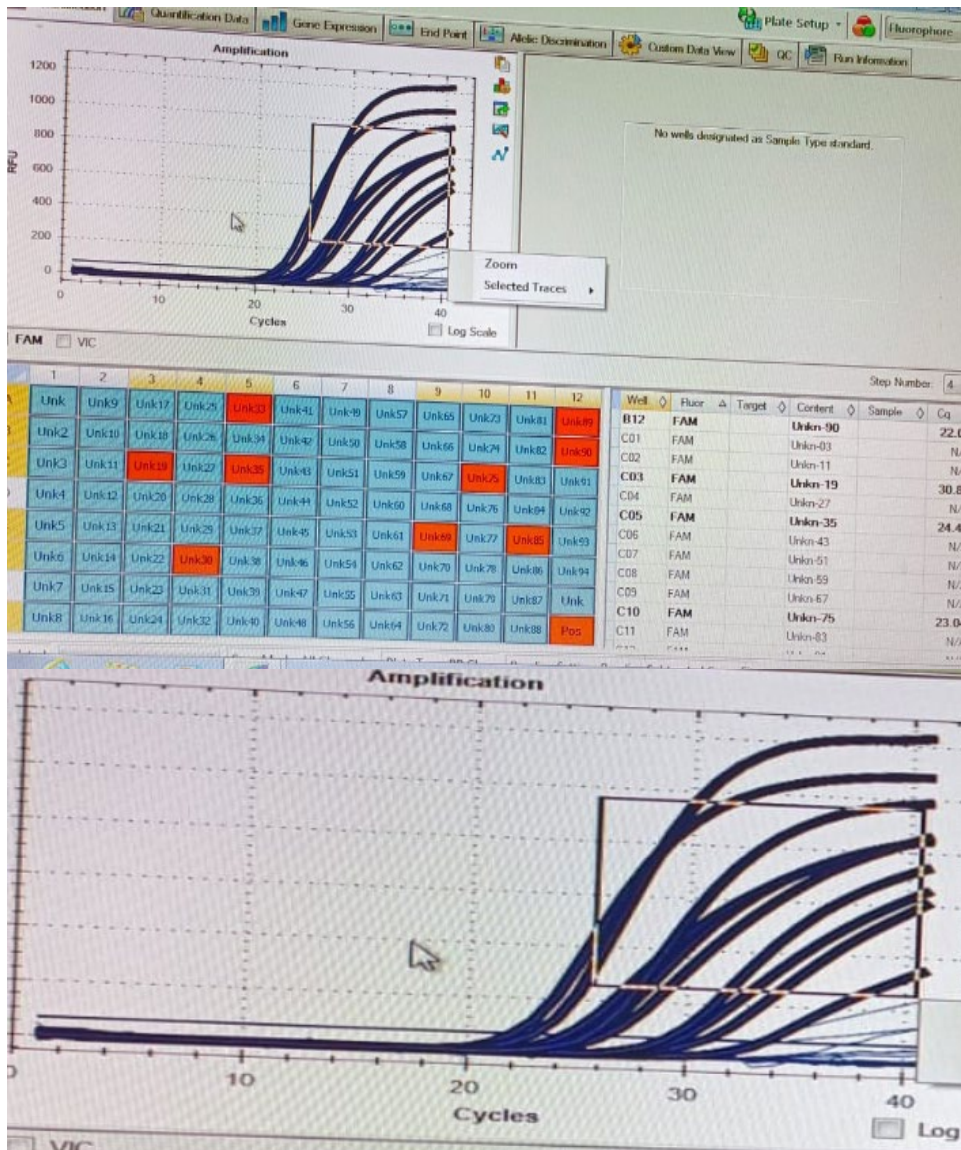
Figure 6. CT (Cyclic Threshold) of Below 30 as Shown by Sigmoid Curves

4. DISCUSSION

A study conducted by Ivan Berlin et al. [19] suggested that smoking contributes in inducing complications due to COVID-19. and This finding supports the results of the current study. Hua cai conducted a study in

March 2020. They found that some smokers showed positive results for COVID-19, although the majority showed negative results. So, they concluded that there is no strong evidence for any correlation between smoking and COVID-

19 infection. Their finding contradicts the results of the current study [20]. A similar result was found by Liu et al. [15]. They found that more positive cases of COVID-19 were reported for a group of smoker patients as compared to a group of non-smoker patients. Zhou et al. [21] concluded that there was no significant difference in the death rate of smoker and non-smoker patients who died by COVID-19. Another research conducted by Guan et al. [22] supported the results of the current study. The study found that smokers were at a higher risk of COVID-19 and they developed severe complications as compared to non-smokers. Badawi and Ryoo found that male patients were more affected by COVID-19 infection as compared to female patients. The results were the same as the current study which explicates that male patients are more affected by COVID-19 [23]. Another study with the same effect was conducted by Channappanavar et al. [24]. According to the survey, the male population was more affected by COVID-19. In the same vein, it was found that the age group 40-60 was more affected by the disease [25]. Another study arrived at a similar conclusion [26]. Likewise, the study conducted by Irena Voinsky et al. [27] found that the age group 40-60 experienced more COVID-19 related complications. These results are the same as those of the current study. Another study suggested that the age group 20-40 years was more affected by COVID-19, which contradicts the results of this study [28]. A study in China suggested that only 1% cases were asymptomatic, which contradicts the results of the current study as well [29].

Furthermore, a study conducted in China showed the same results as this study, since it found that there were many asymptomatic carriers of COVID-19,

detected only by antibody testing [30]. Another research conducted in China showed that many COVID-19 patients were asymptomatic, which is the same as the results of the current study. Many other countries, particularly low- and middle-income countries, are at high risk of COVID-19 infection spread due to the silent mode of asymptomatic cases [31]. A study conducted in Japan suggested that most COVID-19 patients showed no symptoms, although they tested positive for COVID-19 [32].

4.1. Conclusion

The current study found that the prevalence rate of COVID-19 was 60% among the tested patients, which shows that only 40% of the sample tested negative for COVID-19. The high rate could be related to non-adherence with the SOPs for COVID-19 prevention and huge public gatherings. Furthermore, it was found that 70% of the COVID-19 positive cases had a history of smoking, indicating that smoking makes a person more prone to infection. Moreover, smokers were found to be at a greater risk of complications associated with COVID-19. This study also analyzed the prevalence of COVID-19 in patients of different age groups and found that 70% of patients belonged to the age group 40-60 years. The results showed that 70% of patients were asymptomatic, that is, they had no symptoms of cough, fever, flu, and sore throat. Hence, COVID-19 remains a silent threat to the whole world.

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