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Identification of SARS-CoV-2 Variants in different regions of Punjab and Health Concern

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Abstract

The SARS-CoV-2 pandemic has been occurring in several phases across the globe since the emergence of the virus in China. As part of the pandemic, from February 2021to April 2022, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) began to spread in different regions of Punjab with variable impacts on population with a total of 81,517 confirmed cases of COVID-19. During this period, we were able to gather critical information on the transmission and evolution of pathogens. through genomic surveillance. Particularly, the objective of our study was to track putative variants of SARS-CoV-2 (novel and/or previous) circulating in different regions of the state and to identify the impact of different variants on the infection rate. Patients who showed symptoms of COVID-19 were routinely tested for SARS-CoV-2 infections *via* RT-PCR at Viral Research and Diagnostic Laboratory, Government Medical College, Amritsar. Whole-genome sequencing was performed on 403 clinical samples collected from different regions of Punjab at National Genome Sequencing Sites authorised by Indian Council of Medical Research. As a result, we identified a local population is infected with different variants of SARS-CoV-2 viz., Alpha (23.82%); Delta (36.23%). In addition we identified 26 different lineages of delta strain along with 6 of omicron and 2 of alpha. Such high variations in Covid-19 is due to uncontrolled mutation rates because of poor proofreading mechanism during replication; co-infection within two viral lineages in same host and RNA-editing mechanism. Climate based variations was also been reported in the study as we found 10 different variants of delta stains infected the population which was quite high in comparison to other seasons. Gender biasness have been seen in the study as 68.18% male were found to be infected with Covid-19 was vaccinated and hospitalized (70.58%) in comparison to female 31.82% and 29.42% respectively. Since, the appearance

of several variants of SARAS-CoV-2 in such a sample geographical region of country and its highly versatile impact on population with respect to climatic changes and its gender biasness scientist need to be more concerned about the consistent monitoring of the virus epidemiology, mutation rate and its causes and surveillance of the different variant and related disease severity.

Keywords: SARS-CoV-2 Variants, Covid-19, Gender Biasness, RT-PCR, Genome Sequencing

Introduction

The current SARS-CoV-2, unlike all viruses is an graciously engineered viral structure. It's a positive-sense, single-stranded RNA virus with a genome size of ~30kb comprising of 14 open reading frames (ORF) encoding 27 proteins [(1)]. Genome sequencing of SARS-CoV-2 has been taken place all around the globe since the beginning of the pandemic, which contributes in understanding the viral evolution and its potency to cause COVID-19 disease. The epidemiology of SARS-CoV-2 has been often described by interchangeably the terms *mutation*, *variant*, and *strain* but at the same time there distinctions are important. *Mutation* refers to the actual change in sequence, for instance D614G is an aspartic acid-to-glycine substitution at position 614 of the spike glycoprotein. *Variants* are defined when the genomes sequence are often different from each other because of either one or many mutations. Precisely speaking, a variant is a *strain* when it has a demonstrably different phenotype which further results in difference in antigenicity, transmissibility, or virulence than previous strains. SARS-CoV-2 accumulates mutations i.e. changes in its genetic code over time as it replicates. The virus has its unique RNA repair mechanisms, as a result it accumulates mutations at a relatively slower rate in-comparison to other RNA viruses. The virus undergoes mutation at a rate of $\sim 1.1 \times 10^{-3}$ substitutions per site per year which corresponds to one substitution every ~11 days [(2)].This compares to a rate of $\sim 4 \times 10^{-3}$ substitutions per site per year for the HIV virus [(3)].

Among all the viruses sequenced till date, thousands of mutations have been emerged, which in turn have given rise to thousands of different variants. The majority have no perceivable impact on the virus or disease biology and can act as a useful genetic 'barcode' for tracking viral spread and evolution. Such strains of SARS-CoV-2 have genotypic and/or phenotypic changes compared to the reference genome and are categorized as variants of interest (VOI) [(4)]. However, several variants have been identified that appear to increase transmissibility and potentially have an impact on disease severity. They have been labelled as variants of concern (VOC). Initially, there were four internationally confirmed VOCs − B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and Cluster 5 (Delta) [(5)]. While, the most notable case that has caused alarm around the globe is the recently emerged Omicron (B.1.1.529), which was first reported in November 2021. This variant has over 50 mutations compared to the original Wuhan strain, with over 30 of them located at the Spike ORF. Alongside this variant has doubling time of 2 days, and has a very high reinfection rate i.e., 5.4-fold increase than Delta [(6)]. As the pandemic continues to unleash, it is quite possible to have many more VOCs, preferably in the presence of new selection pressures, such as vaccination. So, it is of paramount importance to understand the complete evolution of all the COVs as an entire group (subfamily).

Keeping in view, the current study was carried out for the period of 15 months to identify the variants of concern (VOCs) of current SARS CoV-2 among the different regions of Punjab and to corelate there impact upon the

viral trans missibility and spread, antigenicity, or virulence.

Materials and Methodology

Due to significant rise in number of SARS-CoV-2 patients, a rapid and accurate detection of virus and/or its mutagens is vital for diagnose the source of infection and help patients to prevent the progression of illness. Among many approaches a nucleic acid detection-based technology is considered as the 'gold standard' for the rapid and reliable detection of viruses. As a result, real-time reverse transcriptase-PCR (RT-PCR) a qualitative assay played a vital role in detection of SARS-CoV-2 virus because of its high sensitivity, specificity and simplicity. The study includes approximately 20,08,687 SARS-CoV-2 suspected samples received from different regions of Punjab (Amritsar; Pathankot; Tarn Taran; Gurdaspur; Hoshiarpur; and Kapurthala) at Viral Research and Diagnostic Laboratory (VRDL), Government Medical College (GMC), Amritsar for the period of 15 months i.e., between February 2021 to April 2022. The samples received were properly sorted and processed as per the guidelines of ICMR, government of India. Afterwards, the nucleic acid viz., RNA was extracted by using automated magnetic particle-based extraction system for high yield throughput and eluted in 50 µl elution buffer (Kingfisher Flex, Thermo Fisher Scientific, Waltham, MA, USA). The extracted RNA was amplified for different genes viz., ORF1ab gene (open reading frame 1a and b), N gene (nucleocapsid protein), RNase P gene (Ribonuclease P) gene using ICMR recommended RT-PCR multiplex detection kit (CoviPath™ COVID-19 RT-PCR Kit, Thermo Fisher Scientific, Waltham, MA, USA). Quant Studio™ 5 Real-Time Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) was used to amplify the RNA using standard cycling conditions. The interpretation of the test sample result along with one positive and negative control was performed by corelating the amplification curve for each molecular target along with its respective Ct value (cycle threshold). The Ct values of RT-PCR were taken as indicators of the copy number of SARS-CoV-2 in samples, the ones with lower threshold value corresponding to higher viral copy numbers. The known 403 positive samples of SARS-CoV-2 with CT value less then 25 were sent to National genome sequencing centres authorised by ICMR for the detection of mutagenic strains of Covid-19.

Results and Discussion

Since, viruses manifest the unique property of undergoing innate mutations on the basis of various physiographic conditions which constantly leads to many variants across the globe. Out of which few emerges and few disappear while some persists. WHO has traced 11 new variants of SARS-CoV-2 to date, out of which Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) variants are enlisted as "Variants of Concern" (VOC), and Eta (B.1.525), lota (B.1.526), Kappa (B.1.617.1) and Lambda (C.37) as "Variants of Interest (VOI)" [(7)]. The emerging variants not only exhibit a novel attribute of increased transmissibility, morbidity and mortality in comparison to its precursor but also have the ability to evade the detection by various previous diagnostic tests. This further results in decreased susceptibility to treatment including antivirals, monoclonal antibodies and convalescent plasma and also possess the ability to cause reinfection in previously recovered and vaccinated individuals. Keeping in mind the present study was planned to investigate the different variants of SARS CoV-2 and their impact on different regions of Punjab state of India during second and third wave.

In the present study, the total of 20,08,687 SARS CoV-2 samples were examined at VRDL, GMC, Amritsar by RT-PCR technique from February 2021 to April 2022 to detect the presence of Covid-19 virus. A total of 81,517 were found to be positive whereas 19,27,170 were tested negative for the virus. Out of positive cases (4.06 %) a total of 403 samples were sent for whole genome sequencing at ICMR recommended sequencing institutes. Sequencing data reveled that 23.82% were Alpha (B.1.1.7); 36.23% were Delta (B.1.617.2) while 39.95% were Omicron (B.1.1529) (Figure 1).

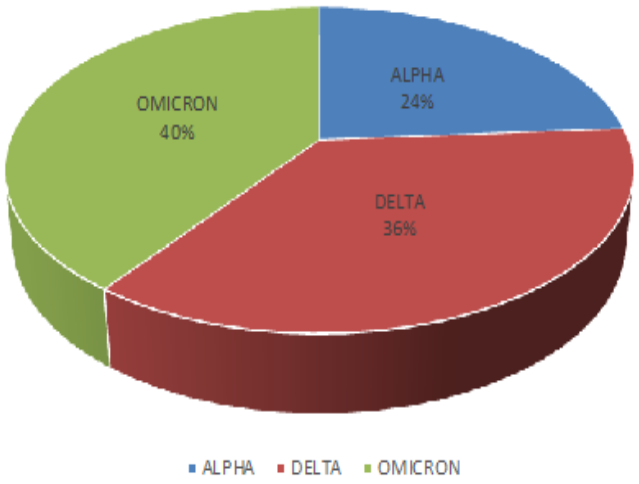

Figure 1: Pie chart depicting the different strains of SARS-CoV-2

The study addressed that the patients in initial period viz., February 2021 to November 2021 was mostly infected with Delta strain (B.1.617.2) while during the latter half i.e., from December 2021 onwards till April 2022 infected with Omicron strain (B.1.1.529). However, reinfection of previous strain viz., Alpha (B.1.1.7) was also been observed in the month of March and September 2021 which was found to be highly infectious during the first waves of infection. Alongside infectious of all the strains and there lineages have been observed throughout the study like omicron showed its presence in the month of April 2021 while dominate in later half i.e. after December 2021 similarly few cases of delta strain was also found in the month of march 2022. Moreover we also found one patient infected with original strain of SARS CoV-2 i.e. A which was one of the two original haplotypes of the pandemic (A and B) originating from China and other one with B.1. Which is one of the largest European lineages originated roughly from Northern Italian outbreak early in 2020 [8]. Even more threatening impact of mutagenic ability of SARS-CoV-2 was investigated in our study mainly during the monsoon region as with the identification of many novel lineages originated at different geographical regions around the globe. Such variations were the impact of inter-strain mutations and we observed as many as 26 different lineages of delta strain along with 6 of omicron and 2 of alpha (Figure 2).

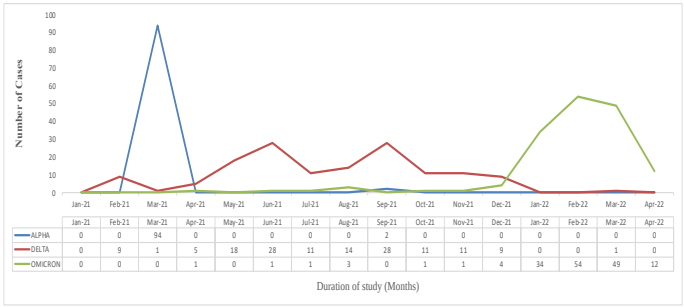

Figure 2: Line graph depicting the impact of mutation in SARS CoV-2 during the study

Our finding addressed that the ongoing coronavirus disease 2019 is the male bias with respect to various factors. We analysed 68.18 % male were infected with different mutagenic strains of covid-19 virus in comparison to female patients (31.82%) among different age groups (Figure 3).

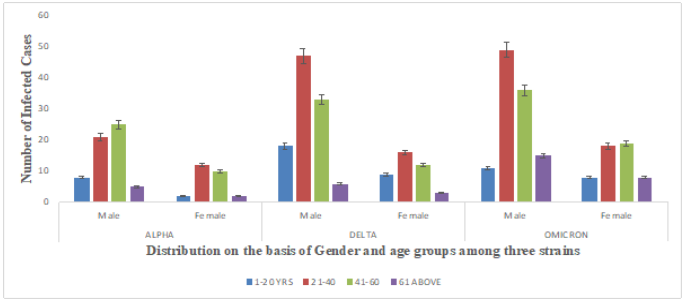

Figure 3: Distribution of Patients infected with different

strains of SARS CoV-2 on the basis of Gender among different age groups

Moreover, it was observed that even vaccinated male were more prone to infection in comparison to female patients. Alongside, hospitalised admission data clearly represent the drift toward the male patients of different age groups with all the possible symptoms of infection in comparison to female patients (Figure 4).

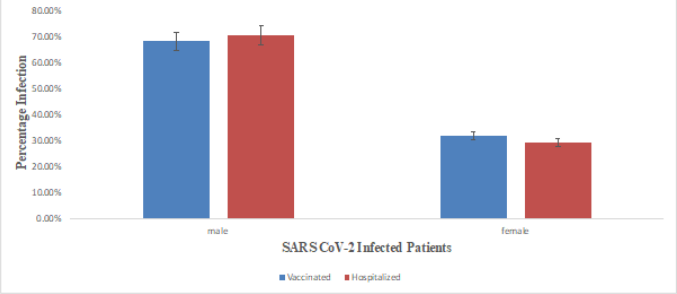

Figure 4: Impact of Infection by different mutagenic strains of SARAS CoV-2 on Hospitalization and Vaccination and its gender biasness

The study also analysed the impact of climatic changes on the infection rate of different strains of SARS CoV-2. During the rainy season (mid of July to end of September) population was found to be infected more by different types of variants of SARS CoV-2 strains hence the rate of mutation was found to be more in virus in comparison to summer or winter seasons [9]. Such drastic variations in single stranded RNA virus reported in the study clearly represent it's most pressing and challenging threat to the present global public health and economy.

Keeping in mind, all such kind of variations which we observed throughout the study it was very hard to commit the specific strain or its impact was diminished rather we observed that many new lineages with in the strains along with the old ones due to high rate of mutations in this single stranded RNA virus. COVID-19 is highly contagious and indiscriminate regarding any parameters viz., age, sex, nationality, and ethnicity. Chilamakuri et all., (2021) explained the impact of rapid mutations acquired by the SARS CoV-2 single stranded RNA virus and its assorted transmission routes have significantly contributed to the pandemic [10]. Nonetheless, the vaccination is a key strategy to prevent its widespread transmission and reduce morbidity and mortality but the rapid rate of mutations is a big challenge. Higher mutation rate in SARS-CoV-2 outcomes to quickly adopt the new mode of transmission, virulence, and immune eluding [11-12]. Current epidemiological evidence shows that since the end of 2020 and the mid of 2021, the continuous and uncontrolled evolution due to high rate of mutations in SARS-CoV-2 has resulted in the emergence of novel lineages in viral strains around the globe [13-15]. Scientists reported in their respective studies that at present around the globe, Delta variants has become the most dominant strain with as many as 10 mutations in spike protein (16). Corresponds to such variations studies showed the increased replication adaptability of the Delta variant of SARS- CoV-2 and the decreased in sensitivity to neutralizing antibodies have led to the recent rapid and large-scale spread of the virus [16-18].

Anecdotal evidence suggests that Coronavirus disease 2019 (COVID-19), caused by the coronavirus SARS-CoV-2, exhibits biasness with respect to genders. The prevalence and outcomes of infectious diseases caused by various microbes viz., bacteria, virus, fungal and parasite among all the age groups was dominated by human males [19-21]. The immune response (innate and adaptive) have been previously reported with sex difference may account for the female advantage in COVID-19. In the past, coronavirus outbreaks have demonstrated the similar trend of sex bias. SARS-CoV-1 epidemic in Hong Kong showed an age- adjusted relative mortality risk ratio of 1.62 (95% CI = 1.21, 2.16) for males while little in female [22]. During the same outbreak in Singapore, male sex was associated with an odds ratio of 3.10 (95%

CI = 1.64, 5.87; p≤0.001) for ITU admission or death [23]. Alghamdi et al., (2014) reported in their study that MERS outbreak in 2013-14 in Saudi Arabia exhibited higher fatality rate in men (52%) in comparison to female (23%) [24]. These data suggest that pandemic may be influenced by various socio-economic factors. Beside theses the difference in immune response between males and females are likely to be a driving factor behind the significant sex-bias observed in the COVID-19 pandemic. The adaptive immune system of females have higher numbers of CD4+ T cells [25-26], more robust CD8+T cell cytotoxic activity [27] and increased B cell production of immunoglobulin compared to males [25,28]. Mori et al., (2015) reported in their study that women were more susceptible to severe local and systemic side effects. Hence, antibody titres was reported in response to the various trivalent inactivated seasonal influenza vaccination (TIV), as well as against other pathogen vaccines was found to be higher [29]. Moreover, female B cells also produce more antigen- specific IgG in response to TIV. Females produce more Type 1 interferon (IFN), a potent antiviral cytokine, upon toll-like receptor 7 sensing of viral RNA was reported to be produced more in female than males, which is important for the early response against COVID-19 infection [30]. These findings imply that females have an increased capacity to mount humoral immune responses compared to males which could be the major reason for sex-bias response to SARS-CoV-2 infection between sexes.

Variations due to climatic changes possibly determines the effects of monsoon weather which results in increase in the frequency and intensity of mutations in the virus and thus become more transmissible and contagious. Dimitrova et all., (2020) in their study shed a light on the possible detrimental effects of monsoon weather on Indian population and revealed the similar impact of weather responsible for irresistible infection rate among the different age group individuals [31]. Hijioka et all., (2014) also revealed in their study that increase in the transmission of communicable diseases like SARS-CoV-2 during the monsoon season, along with other risks [32].

Gender-based socio-cultural and behavioral differences could contribute to the sex difference seen in disease severity caused by various strains of COVID-19. Men are more likely to smoke, although smoking has not emerged as a clear risk factor for severe disease. Men are less likely to wash their hands with soap after entering a restroom, and in many cultures, men may be more likely to leave the house and enter crowded areas [33-34].

Conclusion

The present study revealed the endemic variations of SARS-CoV-2 due to mutations in different regions of Punjab, India for the first time with respect to sex-bias and climatic changes which could be related to the milder, local COVID-19 epidemic. Expanding the ongoing sequencing efforts to monitor SARS-CoV-2 subtype will be critical in identifying future variants of concern and understanding the mechanisms of innate immune evasion by which SARS-CoV-2 adapts to a new host environment. The study demonstrates that although there is not much sex difference in the proportion of test people infected with different mutagenic strains of SARS- CoV-2, males were significantly at higher risk than females. As many reports described the differences between sexes with respect to immune response to infection, which include a more robust antiviral innate interferon response and increased adaptive immunity towards viral antigens in females. In people infected with SARS-CoV-2 these differences were likely to be more effective viral control in females, which may contribute to the relatively lower risk of developing severe disease. Although further studies are needed, these data have

implications for the clinical management of COVID-19 and highlight the importance of considering sex as a variable in fundamental and clinical research.

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