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PRE-DOMINANCE OF DELTA VARIANT AMONG UNVACCINATED AND VACCINATED INDIVIDUALS IN MULTAN REGION

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ABSTRACT

The outbreak of pneumonia occurred in Wuhan, China, in December 2019, and a new virus entitled COVID-19, causing a severe pandemic worldwide, was found as the root cause. There was no effective treatment to control the infection so the epidemic was spreading day by day. To eradicate this global pandemic the SARS-CoV-2 vaccine is the only solution. Globally on 17 July 2022, 567,348,657 cases were reported to WHO of COVID-19, including 6,387,224 deaths. In Pakistan on 17 July 2022, there were 1,543,741 confirmed cases with 30,424 deaths. The goal of this research was to find out, in retrospect, the viral load difference in vaccinated and unvaccinated individuals, and to determine the predominant variant in COVID-19-positive individuals in the Multan region. Our study was conducted in the molecular section of the Pathology department of Nishtar Medical University Multan in the time period from July 2021 to February 2022. Nasopharyngeal and oropharyngeal swabs were collected in viral transport media and immediately transported to the molecular laboratory, Pathology Department Nishtar Hospital Multan. Multan has 2.205 million mio. inhabitants and is located in Punjab, Pakistan. A qualitative Real-Time PCR was performed to detect the positivity and viral load by cycle threshold value. The variant was detected by whole genome sequencing. Results of our study showed that 69.80% of individuals were vaccinated and 30.20% were unvaccinated in 500 study subjects. Symptomatic individuals were 83.8% and 16.2% were asymptomatic. This study found no significant difference between vaccinated and unvaccinated COVID-19-positive patients. Of 86 sequenced study subjects, 94.19% showed the delta variant and 5.82% individuals were of non-delta variant. In both populations, the delta variation was the more common one. Irrespective of immunization eminence, age, sex and mean virus load, determined by cycle threshold value were different in substantial numbers of asymptomatic and symptomatic persons infested with SARS-Cov-2 throughout the delta peak in the current investigation. When compared to asymptomatic COVID-19-positive people, symptomatic COVID-19-positive individuals had a higher viral load.

INTRODUCTION

A cluster of unexplainable pneumonia infections, was discovered in the Chinese province of Wuhan in December 2019. A new coronavirus was found that was the source of this enigmatic pneumonia a few days later. Currently, the COVID-19 epidemic is propagating across China and the rest of the world. This novel coronavirus human-to-human transmission extended a foothold worldwide[1]. On 11 March 2020, the World Health Organization announced that disease is a global contagion, citing a thirteen-fold increase in the number of cases outside of China and a three-fold increase in the number of countries affected[2]. Due to the exponential and quick spread of disease, government directives placed in lockdown. To control sickness, nearly all governments closed their borders and closed marketplaces, schools, and organizations. The virus quickly spread to Iran, Italy, Europe, the United Kingdom, and the United States[3]. Internationally on 30th June 2022, there have been 557,749,987 cases of COVID-19, including 6,367,213 deaths by WHO (Rubi et al., 2020). In Pakistan, on 30th June 2022, there have been 1,539,275 cases of COVID-19, including 30,403 deaths as reported by WHO. Coronavirus was given its name because of the crown-like spikes on the outer outer surfaceof the virus. Coronavirus is classified into four subcategories Alpha, Beta, Gamma[3] and Delta.

A variant of SARS-CoV-2 with greater viral loads and transmissibility is linked to B.1.617.2 delta, as well as partial resistance to polyclonal and monoclonal antibodies when compared to other variations. The expansion of the delta-variation has been associated with increased case counts and indicating rapid communal spread. Cases of SARS-CoV-2 have been on the rise in the United States by early July 2021, corresponding with delta SARS-CoV-2 becoming the most common lineage across the country. Understanding how and why the virus spreads in regions with high vaccination reportage has major public health insinuations. It's critical to discover that SARS-CoV-2 can spread from infected vaccinated people to others. The disease spreads more swiftly through families, friends, and the community due to close contact between individuals, asymptomatic incubation carriers, and air droplets[3]. COVID-19 has a number of 'hallmark' laboratory and radiographic parameters that can be used to track illness development. COVID-19, when taken together, 'Flu-like' symptoms can lead to multi-organ failure and systemic inflammation, which can be fatal.[27]

As quickly, as the first case was recorded, a competition for vaccine development began and the first vaccination became accessible to the public on January 12, 2021. The vaccine's main target was spike protein S. Many medications were utilized in the beginning, including ganciclovir, chloroquine, and hydroxychloroquine, but they were not approved by the FDA for the treatment of SARS-COV-2. These were not allowed since the use of drugs can increase the virus's resistance power, thus vaccine development and implementation to cure sickness is a better choice. It was difficult to recognize COVID-19 at the start of the pandemic and develop the vaccine. Within months SARS-CoV-2 genome sequence was studied which matched 80 percent with the genome sequence of SARS-COV and it made it possible to make a variety of vaccines[4].

Vaccines have archaeologically played a critical role in saving many lives from life-threatening diseases and in preventing the spread of infectious diseases in the community[5]. Biologists have created a variety of vaccine techniques to combat the disease, including inactivated, live-attenuated protein subunits, viral vector vaccine platforms (first, second, and third-generation vaccination), and nanomaterial-based vaccines[6].

COVID-19 must be detected accurately and quickly to control epidemics in hospitals by RT-PCR[7]. All countries aimed to discontinue transmission and avert the spread of the coronavirus to save lives. Our study will estimate the strength of the association between vaccination efficacy and COVID-19 prevalence. This study will give awareness to a population that vaccination is obliging to diminish the pandemic.

Material and methods

The design of the study was a Descriptive Cross-sectional study. The sample size was 500 COVID-19-positive patients. Our study was directed in the Molecular section of the Pathology department of Nishtar Medical University Multan. Nasopharyngeal/Oropharyngeal swabs were collected in viral transport media and immediately transported to the molecular laboratory, BSL-II, Pathology Department Nishtar Hospital Multan.

Sampling

Samples were collected from outdoor patients and patients admitted in isolation wards. The sampling type selected for patients will either he/she wants to give a nasopharyngeal or oropharyngeal sample.

Nasopharyngeal sampling

The nasopharyngeal swab is the most common technique for diagnosing COVID-19. The basic idea was to collect virus-infected respiratory early diagnosis of symptomatic patients and isolation of these all over the world [8]. The patient's head must be upright. It's easy to take samples from the nasal floor. Advise the patient to rest their head on the chair's head. Young patients should wear a surgical mask, which must be placed just beneath the nose. To minimize the exposure to droplet projections, the caregiver should be on the patient's side. Next, raise the tip of the nose to reveal the place. Roll the swab softly. Allow the swab to absorb secretions for some seconds. With the swab still rotating, slowly remove it [8]. The same swab can be used to get a sample from the nasal fossa, if testing is difficult due to a deviated septum or blockage, put the swab in the sample tube and the shaft retorted off to shut the tube when the sample is made [9].

Oropharyngeal sampling

Oropharyngeal swab sampling is usually used to detect viral nucleic acid [10]. A selected sample of the oropharyngeal mucosa of the posterior wall and base of the tongue is required for proper oropharyngeal swab execution. Because the target area was more reachable, an oropharyngeal swab is easier to do than a nasopharyngeal swab. In some circumstances, when the oropharynx is difficult to see with mouth open, a lingual retractor should be utilized. To reduce vagal activation, the patient was instructed to breathe gently and concentrate on breathing [11]. Put the swab on the posterior wall, parallel to the lingual retractor. Swabs are taken from one lateral side of the oropharynx, then the contralateral side, and finally the base of the tongue. The tip of the swab can swab the hard or soft palate or the palatine pillars as an alternative to the oropharynx if it is tilted cranially or too far laterally [11].

RNA extraction

The TAN-Bead nucleic acid extraction Kit was used to extract nucleic acids from samples that had been processed with proteinase K before being automated or a semi-automated process. The nucleic acids extracted can then be utilized in diverse applications like high-sensitivity real-time PCR. The idea behind this kit was that a silicon dioxide layer atop magnetic beads may absorb negatively charged molecules, allowing nucleic acid to be purified from materials [12].

Amplification of extracted RNA

The GB and Zybio Real-Time RT-PCR kits for COVID-19 were used in amplification test in vitro nucleic acid kits, that identify novel-coronavirus in respiratory tract specimens using reverse transcriptase polymerase chain reaction and Taq-Man probe technology. The World Health

Organization's technical guidance was used to build this kit, which was based on the conserved regions of the E gene and the ORF1ab region. One-step real-time RT-PCR, in one tube, was the test principle. The primers and probes for the kit were designed based on the conserved sections of the E gene, N gene, and ORF1ab of the SARS-CoV-2 strains, and covered other 369 SARS-CoV-2 strains sequences, as per the technical advice of the World Health Organization announcement. The GB and Zybio are kits that are used in vitro nucleic-acid amplification test kits that use reverse transcription for finding novel coronavirus (SARS-CoV-2) in respiratory tract samples. The enzyme mixture for RT-PCR was included in the GB SR. The master reagents specific to viral RNA were included in the RT-Master mix. The primers and TaqMan probes were found in the ORF1ab Mix, E Mix, and N Mix. Each positive control, negative control (H2O), and specimen passing through the entire procedure, including RNA isolation, amplification, and detection, must have the GB RNA IC, which is a specifically constructed non-infectious RNA molecule. During the testing, the internal control serves as a performance indication for the RT-PCR. The internal control was identified in the VIC channel, while the SARS-CoV-2 RNA was recognized in the FAM channel[13]. Bio- rad C1000 thermal cycler CFX-96 and ABI-7500 instruments were used for amplification.,

Whole genome sequencing

Study subjects N = 86 oropharyngeal and nasopharyngeal samples collected from COVID-19-positive patients at the pathology department in Nishtar sent to the virology department of the National Institute of Islamabad, positive samples with cycle threshold values of less than 27 were sent with proper triple packaging. Qiagen Viral RNA Mini Kit was used to extract RNA from nasopharyngeal swab samples. SuperScript-IV reverse transcriptase was used for the reverse transcription of ThermoFisher Scientific manufacturer. The National Institute of Islamabad used the Illumina DNA Prep Kit to sequence the whole genome. A Qiagen Viral RNA Mini Kit was used for the isolation of viral RNA from patient samples according to the usual technique (Germany, Qiagen). The ARTIC nCoV-2019 Panel was used to achieve the cDNA synthesis and amplification process [14]. The Illumina DNA Prep Kit was used to prepare the paired-end sequencing as per standard procedure. The produced libraries were sequenced and pooled on the Illumina platform at the National Institute of Health in Islamabad, Pakistan, in the Department of Virology. The fastest and most multipurpose sequencing solutions for DNA in the Illumina library prep portfolio were powered by Illumina DNA Prep technology. Whole

genome sequencing takes less time with on-bead tagmentation chemistry. The libraries were subsequently purified for use on an Illumina sequencing machine using Illumina Purification Beads [15].

The double-stranded DNA library denatured earlier. Bio-Rad C-1000 Touch thermal cycler and BioRad DNA Engine Tetrad 2 thermal cyclers were employed in this work [16]. The read quality of sequenced data was assessed using the FastQC program [17].

Data analysis

For statistical analysis and data entry, the SPSS computer software statistics package for Social Science version 26 was utilized. The predominance and prevalence of the delta variant were estimated in positive samples. Threshold cycle detection of genes demonstrated with the help of charts and graphs.

Results

More than 500 questionnaires were filled out by patients willingly. All of them were literate and they could easily understand the questions and interpret them. All the patients in our study were above 18 years old. Patients with the first COVID-19 positive test will be considered for collection of samples. The swabs were transported in the PCR section for processing of the sample. In the PCR section, RNA extraction and master mix preparation were performed in Biosafety cabinet class II. We run the amplification process on ABI-7500 and CFX-96 thermal cycler. In our study of 500 study subjects, 241 were women and 259 were male. The minimum age was 18 and the maximum was 80 years, the mean and standard deviation were 39.22 ± 17.285. In this study, 140(51.85%) males got nasopharyngeal sampling, and 130(48.14%) females got nasopharyngeal sampling as it has more sensitivity than oropharyngeal sampling. 119(51.73%) males and 111(48.26%) female give oropharyngeal sampling (Figure 1).

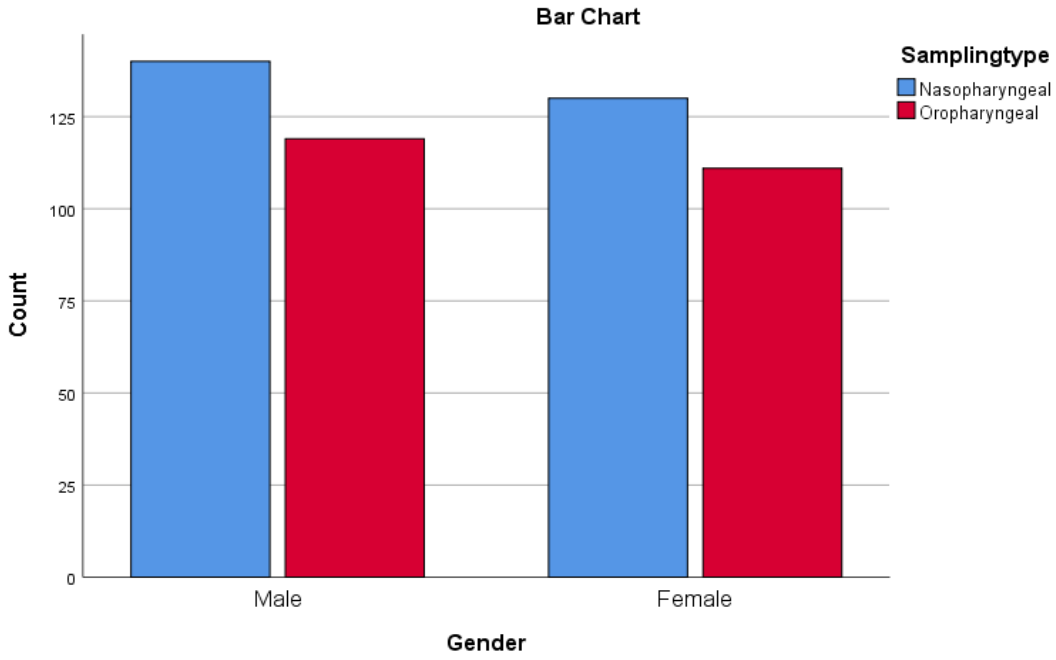

Figure 1: indicates that 140(51.85%) males got nasopharyngeal sampling and 130(48.14%) females got nasopharyngeal sampling as it has more sensitivity than oropharyngeal sampling. 119(51.73%) males and 111(48.26%) females gave oropharyngeal sampling as it was convenient and less painful than nasopharyngeal sampling.

In 500 study subjects, 349 individuals were vaccinated and 151 individuals were unvaccinated which describes that people were familiar with the vaccination importance in the 3rd wave and 4th waves. Nasopharyngeal Sampling status was 271 and individuals with oropharyngeal sampling was 229 shown in (figure 2 & figure 3).

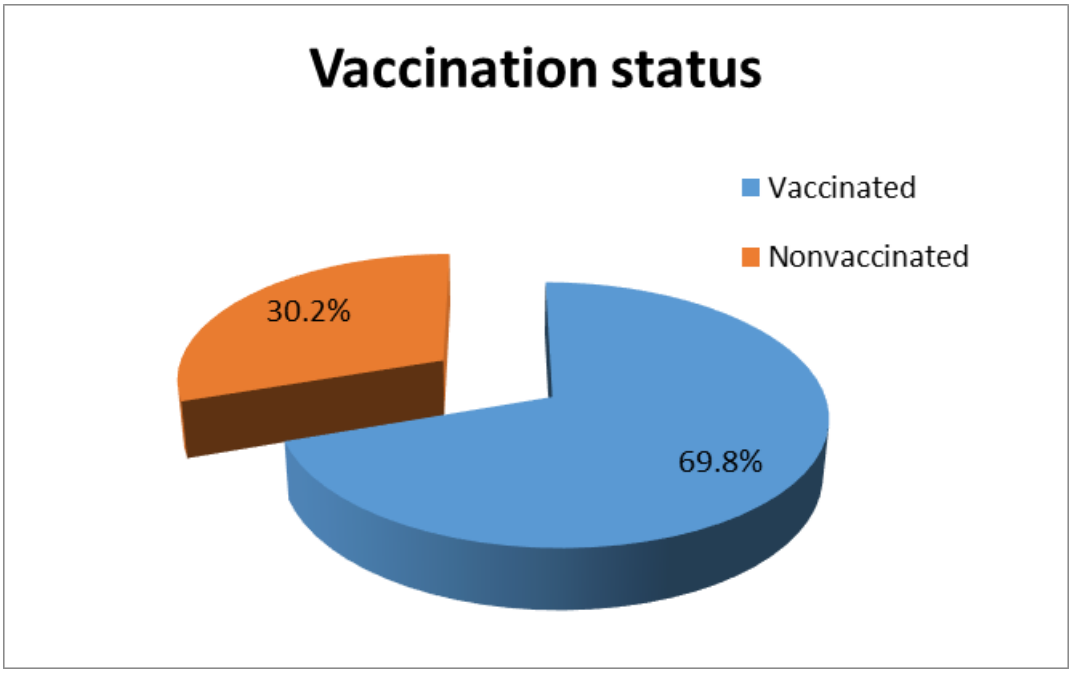

Figure 2: shows the Vaccinated and Unvaccinated individual ratio in 500 COVID-19-positive patients there were 349(69.80%) were vaccinated and 151(30.20%) were unvaccinated. This data was collected from July 2021 to February 2022.

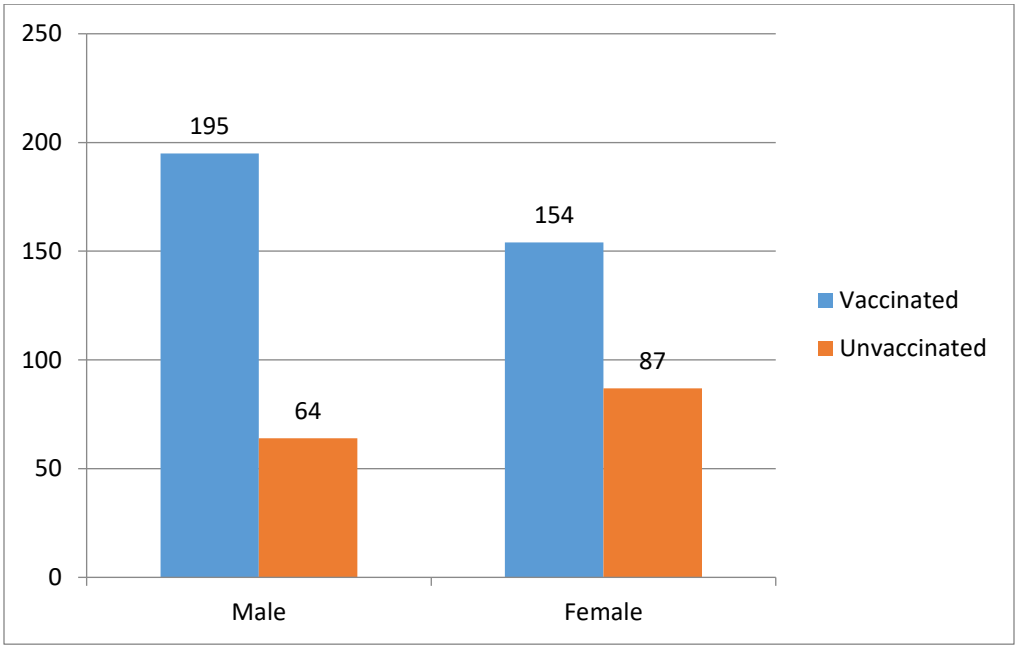

Figure 3: shows that out of 241 females, there were 154(63.90%) were vaccinated and 87(36.09%) were unvaccinated. Out of 259 males there were 195(75.28%) were vaccinated

and 64(24.71%) were unvaccinated. This data was collected from July 2021 to February 2022.

Symptomatic status in study subjects (n=500) shows that in 500 patients 419 were symptomatic and 81 were asymptomatic. Asymptomatic individuals showed less viral load and symptomatic individuals showed high viral load in qualitative RT-PCR. Of 349 vaccinated individuals 289 were symptomatic and 60 were asymptomatic. Mostly aged individuals were symptomatic and young individuals showed no symptoms as observed in our study. Symptomatic status in study subjects (n=500) showed that in 500 patients 419 were symptomatic and 81 were asymptomatic. Asymptomatic individuals show less viral load and symptomatic individuals show high viral load in qualitative RT-PCR in our study (Figure 4 & Figure 5)

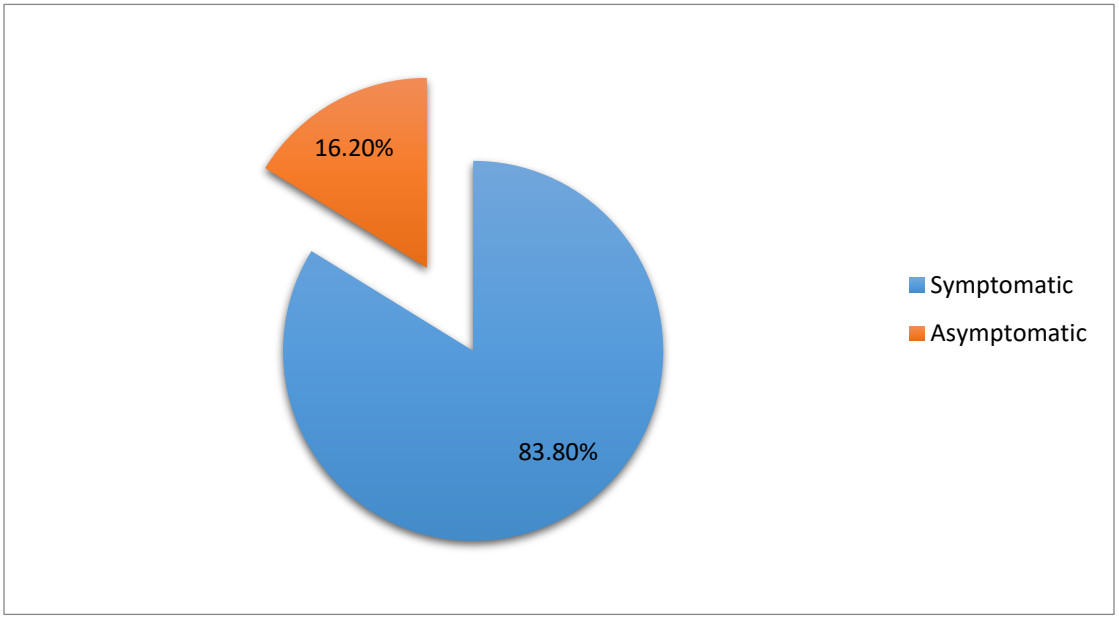

Figure 4: Symptomatic status in study subjects (n=500) shows that in 500 patients 419(83.80%) were symptomatic and 81(16.20%) were asymptomatic. Asymptomatic individuals were mostly young. This data was collected from July 2021 to February 2022.

Smoking status in N=500 individuals showed that there 47 smokers in 259 males. All smokers were male, our study describe there was correlation between smoking status and viral load status shown in (Figure 6).

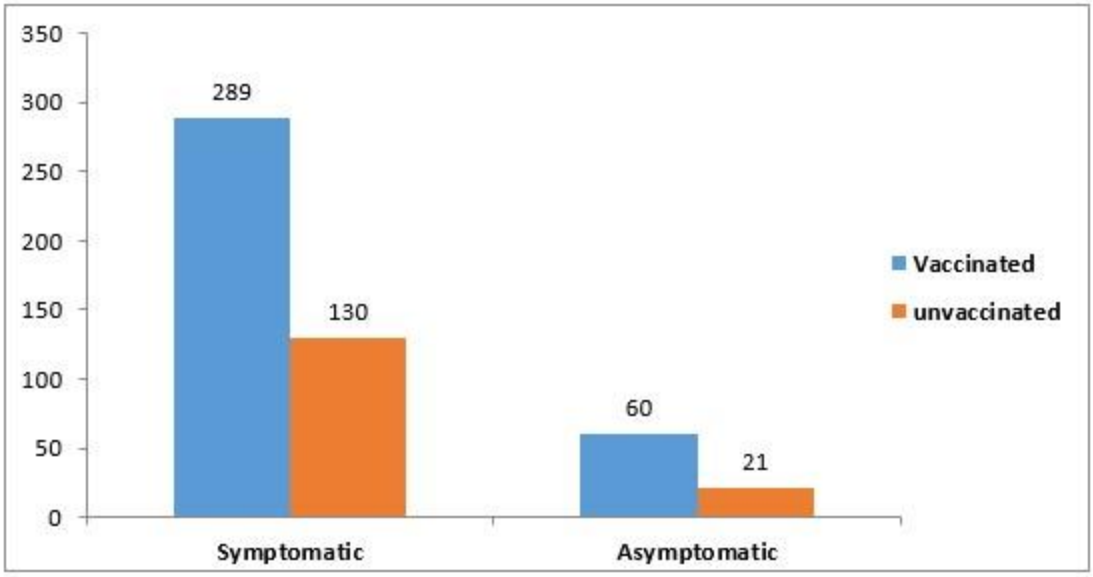

Figure 5: shows that of 419 symptomatic individuals, there were 289(68.97%) vaccinated and 130(31.02%) were unvaccinated. In 81 asymptomatic individuals 60(74.07%) were vaccinated and 21(25.92%) were unvaccinated. This data was collected from July 2021 to February 2022.

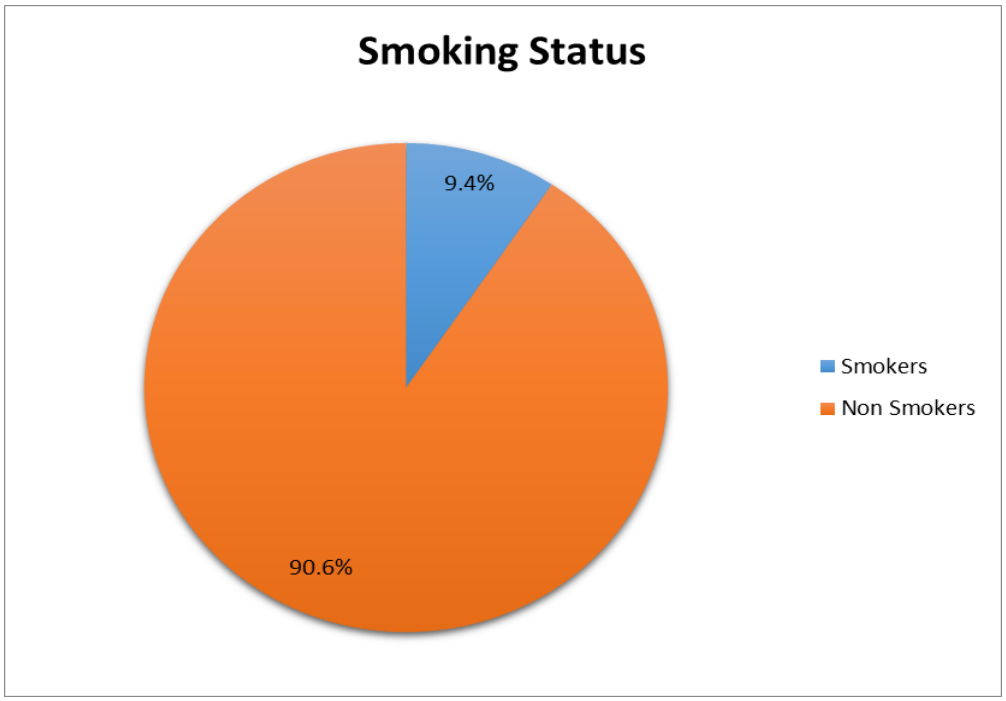

Figure 6: Smoking status in N=500 individuals shows that there are 47 smokers in 259 males. Our study shows there is a weak positive correlation between smoking status and viral load of coronavirus. This data was collected from July 2021 to February 2022.

Frequency of study subjects according to their location:

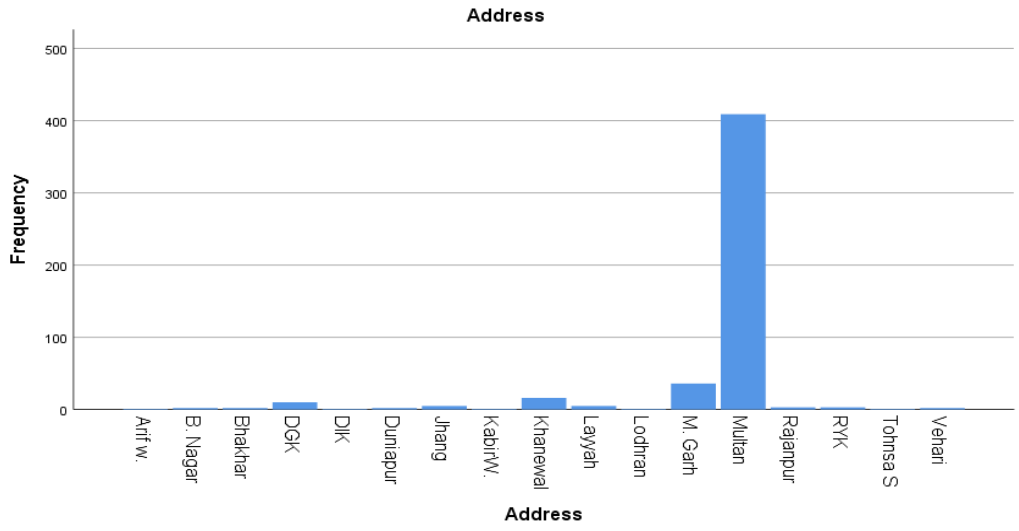

Figure 7: Frequency according to cities shows that there is a high count of Multan. In 500 study subjects there are 409(81.8%) of Multan city. It may be due to awareness of covid pandemic in big cities while in backward cities people don't bother or it may be due to a lack of corona center facilities. This data was collected from July 2021 to February 2022.

Of the 500 study subjects, there were 409(81.8%) belonged to the Multan city. Muzaffargarh city, near Multan, had 36 no of individuals. It may be due to awareness of covid pandemic in big cities while in backward cities people don't bother or it may be due to a lack of corona center facilities. Our study revealed that COVID-19 spread or attack in individuals with comorbidities and healthy ones has no significant correlation (Figure 7).

Our study illustrates that the delta variant was the more common variant seen in the Multan region and its territories. Delta variant was spreading rapidly. The delta variant was significantly prominent variant delta variant was 94.82% and the omicron was 5.18% in n=86 study subjects (Figure 8).

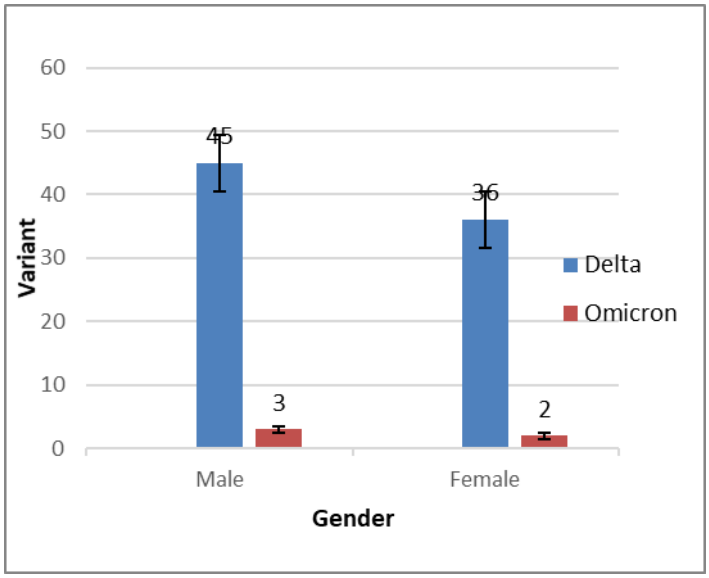

Figure 8: shows the variant data according to gender. It was seen that the delta variant was prominent in the fourth wave and fifth wave in the Multan region and its surrounding territories.

Discussion

The novel coronavirus pandemic has spread across the world, causing a worldwide health catastrophe that has been exacerbated by the virus's recent development of new strains. The pandemic had claimed over 6 million lives globally. In approximately 80% of cases, the course of SARS-CoV-2 infection is mild or asymptomatic. However, the virus can cause severe, progressive pneumonia, leading to respiratory failure and multiple organ dysfunction. The overall mortality rate is around 2%, though certain groups are at a higher risk of severe outcomes. These include older adults, males, individuals with obesity, chronic cardiovascular, respiratory, or kidney diseases, cancer, and those with weakened immune systems[18-21].

In Pakistan, there was relatively little information on the distribution of these variations. Our study gives information on the delta variant as a predominant variant in the Multan region in the fourth wave and fifth wave from July 2021 to February 2022.

Our discovery explains how delta variation is so effectively conveyed in populations with high vaccination coverage. Current COVID-19 vaccinations are still effective in averting serious sickness and death. Our data imply that in situations where exposure is close and protracted, immunization alone is insufficient to avoid the transmission of the delta variation [22.]

According to recent studies, patients over the age of 60 years are at a larger risk than youngsters, who are less likely to become infected or, if they do, may experience minor symptoms or even silent infection. People who have been vaccinated and those who have not been vaccinated are both at risk of catching the Delta strain. Vaccination appears to slow the progression of the disease. To stop the pandemic from spreading further, vaccination rates and coverage must be increased.

Our study showed that the Mean ± SD of Age (years) was 39.22 ± 17.285, and the median age was 59.5 Females were 241 (48.2%) and males were 259 (51. 8%). This also illustrated that 140(51.85%) males got nasopharyngeal sampling and 130(48.14%) females got nasopharyngeal sampling as it has more sensitivity than oropharyngeal sampling. Nasopharyngeal sampling is the gold standard and many recent studies declare it a more sensitive and accurate method (Charlotte B. et al., 2021). 119(51.73%) males and 111(48.26%) females give oropharyngeal sampling as it is convenient and less painful than nasopharyngeal sampling. In our 500 study subjects 271(54.20%) got nasopharyngeal sampling and 229(45.80%) had oropharyngeal sampling type and 349(69.80%) were vaccinated 151 (30.20%) were unvaccinated.

Our study shows that there is no difference seen in corona attacks either in comorbidities or in healthy ones. It spreads easily. Proper use of SOPs is the only way of protection. Smoking status in N=500 individuals shows that there are 47 smokers in 259 males. All smokers were male, our study shows there is a positive correlation between smoking status and viral load status.

Several studies have reported no significant difference in Ct-values between those who have been vaccinated and those who have not been vaccinated. On the other hand, other studies have found that breakthrough infections, have a reduced viral load and, as a result, are less prone to spread, particularly among asymptomatic people. In our study, we found similar results. In 500 study subjects 419 were symptomatic and 81 were symptomatic. By comparing their viral load, we find 327 individuals have CsT-value in 20-30 which depicts a higher titer as compared to asymptomatic which shows 64 individuals have CT-vale in the 20-30 range.

Our study showed that out of 241 females, there were 154(63.90%) vaccinated and 87(36.09%) were unvaccinated. Symptomatic status in study subjects (n=500) shows that in 500 patients 419(83.80%) were symptomatic and 81(16.20%) were asymptomatic. Asymptomatic individuals were mostly young.

Smoking status in N=500 individuals showed that there were 47 smokers in 259 males. Our study shows there is a weak positive correlation between smoking status and the viral load of corona.

About COVID-19, the studies presented here clearly demonstrate the complexity and multi-factorial etiology of smoking. The findings of epidemiological meta-analyses reveal that active smoking is significantly connected to a higher likelihood of COVID-19 severity [23].

Our study compared to 20 shows the same results. Individuals in both the vaccinated and unvaccinated groups showed significant variance, with Ct-values ranging from >14 to < 32 in both. Likewise, no statistically significant variations in the mean threshold of asymptomatic and symptomatic samples, either overall or by vaccine status, were identified. Ct-values were found to be similar across age groups, vaccination kinds, and genders [24].

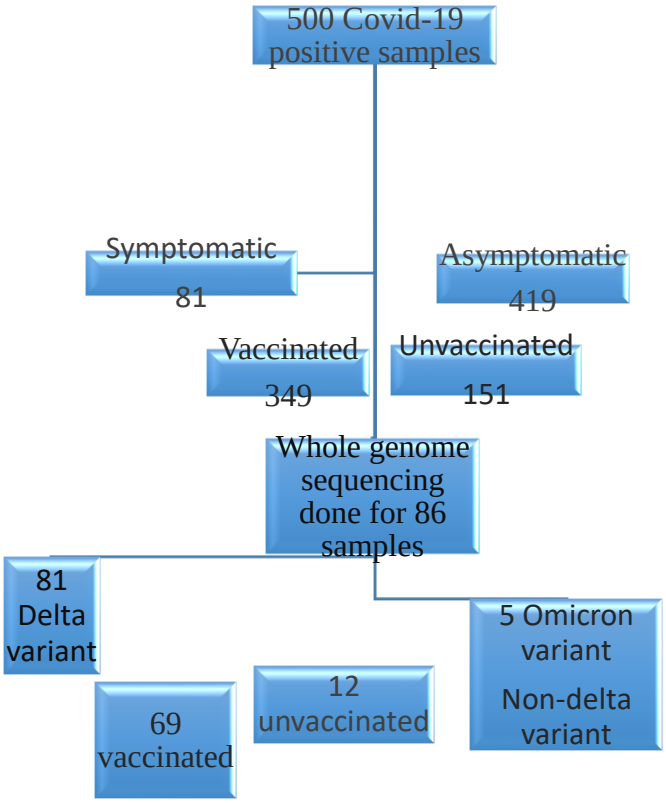

Figure 9: Workflow schematic diagram

There is no significant difference in Ct-value between vaccinated and non-vaccinated Delta-infected persons. In addition, tn fully vaccinated asymptomatic individuals had viral-load < 28

symptomatic and vaccinated persons, according to a study from San Francisco. Our findings were similar to those of the Matan research [25].

The findings of the Riemersna study are consistent with other recent publications that reveal similar virus levels in vaccinated and un-vaccinated individuals in environments where delta variant transmission is present. In a subset of studies comparing 24 unvaccinated cases and 11 vaccinated cases in a Wisconsin study cycle, threshold values were identical, and culture positive was not different [18].

In a recent study in Massachusetts and Singapore, in both studied, individuals with vaccination showed the same results. Several studies back up our conclusions, conducted in different cities in Pakistan and other countries [26].

In our study, we sent 86 samples for whole genome sequencing to NIH Islamabad. Samples had with lower CT-value, which shows 81 were delta variant and five were of omicron variant. These findings resemble those of a study conducted by the Lahore Institute of Molecular Biology and Biotechnology in Punjab, Pakistan [24].

The information recorded in this study during the expansion of the Delta variant strongly reinforces the notion that neither vaccine status nor the presence or absence of symptoms should influence the recommendation and implementation of good public health practices, such as mask-wearing, testing, social distancing, and other measures, aimed at preventing SARSCoV-2 spread. Figure 10).

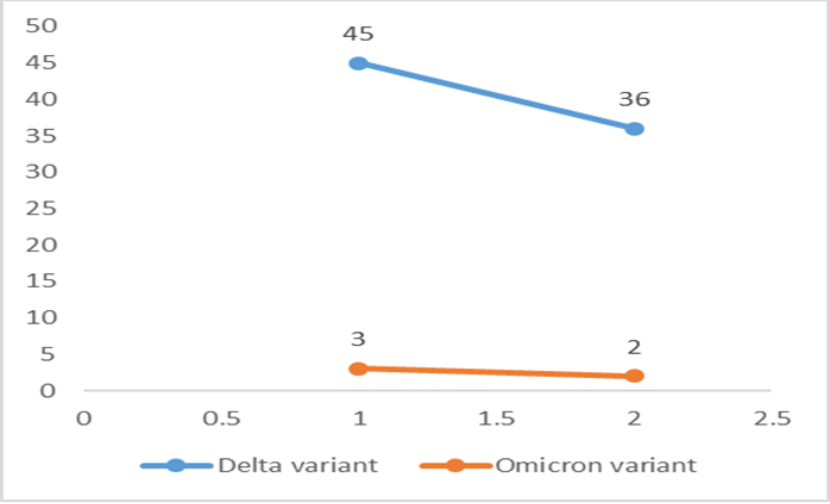

Figure 10: Delta variant and non-delta variant comparative occurrence

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Authorship & Contribution

1. Conception and design of or acquisition of data or analysis and interpretation of data.
2. Drafting the manuscript or revising it critically for important intellectual content.
3. Final approval of the version for publication. All authors agree to be responsible for all aspects of their research work.
4. Analysis of the study and proofreading of the final draft contributed to the data.

Data Availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

Authors declare no conflict of interest

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