Detection of Covid-19 Variants and Antibodies Profiling in patients from Malakand Khyber Pakhtunkhwa.

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Abstract- Coronavirus is an enveloped RNA virus belongs to *Coronaviridae* family. It has four genotypes alpha, beta, gamma, delta and size ranges from 80 to 140 nm with incubation period of 1 to 14 days. The main purpose of the study was to find out the overall prevalence, antibodies level and Covid-19 variants detection in hospitalized patients of Malakand division as part of the 4th wave. This study was conducted at Saidu Teaching Hospital Swat. This project was completed in six months, from August 2021 to January 2022. In this descriptive cross sectional study, 1500 swab samples were evaluated for Covid-19 using Genrui RT-PCR amplification kit. Among these cases, 200 positive samples having PCR CT value less than 33, were examined for antibodies titer by CLIA (Chemiluminescence immunoassay) method and 100 positive samples with PCR CT value less than 27 done for variant detection by using GSD Nova III amplification kit. According to our findings the overall prevalence in Malakand division was 31.5% (473), with highest prevalence was recorded in district Swat 22.4% (336). According to gender wise distribution the highest frequency was recorded in males 20.7% (311) and old age people 10.3% (155) respectively. The highest antibody levels were recorded in Upper Lower (Mean=12.82), Males (Mean=12.15) and people with age of 21-40 years (Mean=14.57) respectively. According to our findings all obtained samples were positive for (Delta) variant of Coronavirus during 4th wave. This study concludes that the PCR based detection of positivity ratio is very high during the fourth wave of Covid-19. The antibodies level in patients was very low and vaccination is needed for high antibodies. The delta variants of Coronavirus is found in overall districts of Malakand division.

Index Terms- Antibodies, Covid-19, RT-PCR, Variants

1. INTRODUCTION

Coronavirus belongs to the *Coronaviridae* family and disease caused by this virus term as Covid-19.[1] Letovirinae and Orthocoronavirinae are the two subfamilies that make up the *Coronaviridae*. The four genera of the *Orthocoronavirinae* subfamily are the alpha, beta, gamma and delta Coronaviruses.[2] SARS-CoV-2 like all Corona viruses, is an enclosed RNA virus that produces virions that range in size from 80 to 140 nm.[3] This virus appearance is indicated by the word "Corona" even (Latin: crown or halo).[4]

The Coronavirus incubation period lasts between 1 and 14 days, but the median is 4-6 days.[5] Previous studies have demonstrated that viral excretion starts several days before the onset of symptoms and peaks at symptom onset +/- 2 days later.[6,7] Therefore, a significant fraction of the transmissions already take place during the incubation stage.[8] Patients with COVID-19 experience non-specific symptoms that are comparable to those of other respiratory illnesses. In comparison to MERS-CoV and SARS-CoV the symptoms are more severe and vary between individuals. The most common sign and symptoms of Covid-19 are; flu, fever, chills, tiredness, cough, sneezing, dry throat and nasal congestion, joint and muscle pain, Eye and mucous membrane inflammation, pneumonia and shortness of breath, diarrhea, nausea, Loss of smell and taste.[9]

The ongoing COVID-19 pandemic has had a profound impact on global health, with numerous cases reported across the globe.[10] As the virus continues to spread, it undergoes genetic changes, leading to the emergence of different variants. These variants often possess distinct characteristics, including increased transmissibility or potential resistance to certain treatments or vaccines. It is imperative to closely monitor these variants and understand their prevalence to effectively control and manage the pandemic.[11]

The primary method for demonstrating the presence of an infection is antibody detection. There are several techniques for detecting SARS-CoV-2 antibodies, primarily ELISA (Enzyme-linked Immunosorbent Assay), CLIA (Chemiluminescence-Immunoassay) and fast assays (lateral flow tests). They are transformed into spike protein or the nucleocapsid protein, which are used to identify the presence of IgA, IgM, IgG or total antibodies as virus antigens.[12] Rapid antigen tests (point-of-care tests) have drawn a lot of attention during the pandemic because of resource-saving and can rapidly determine whether a SARS-CoV-2 infection is present. The quality of testing utilizing an antigen test also depends on how the swab is conducted correctly and how precisely the results are read.[13]

There are many vaccines available for Covid-19 are Pfizer (BioNtech), Moderna (NIAID), AstraZeneca (ChAdOx1 nCoV-19), Covishield (AZD1222), Johnson & Johnson's (Ad26.COV2.S or JNJ-78436735), Sputnik V (Gam-COVID-

Vac), Sinopharm (BBIBP-CorV) and CoronaVac made in China by Sinovac Biotech.[14,15] All of these vaccine have different efficiency rate to control the Coronavirus.

This study aims to investigate the detection of COVID variants and antibody profiling in patients from the Malakand region of Khyber Pakhtunkhwa. The Malakand region in Khyber Pakhtunkhwa, Pakistan, has experienced a significant burden of COVID-19 cases. However, limited research has been conducted to investigate the prevalence and characteristics of COVID variants in this specific geographical area. Understanding the distribution of different variants and their potential implications for disease severity and transmission dynamics is essential for implementing targeted public health measures and optimizing treatment strategies.

Moreover, investigating the antibody profiles of COVID-19 patients in the Malakand region will provide valuable insights into the immune responses elicited by the virus in this population. Antibodies play a crucial role in neutralizing the virus and preventing reinfection. Analyzing the antibody profiles will help identify any variations in immune responses among individuals and potentially guide vaccination strategies and therapeutic interventions.

To achieve these objectives, this research will employ robust genomic sequencing techniques to detect and identify the prevalent COVID variants in patients from Malakand. Nasopharyngeal swabs will be collected from individuals diagnosed with COVID-19, and the samples will undergo genetic sequencing and variant analysis. Furthermore, blood samples will be obtained to analyze the antibody responses mounted against the virus and investigate the presence of specific antibodies against different viral components.

By investigating the detection of COVID variants and antibody profiling in patients from the Malakand region of Khyber Pakhtunkhwa, this study aims to contribute valuable data to the growing body of knowledge regarding the evolving nature of the COVID-19 virus. The findings will provide insights into the prevalence and characteristics of different variants in this specific population, as well as the immune responses elicited by the virus. Ultimately, this research will inform public health strategies, vaccination efforts, and therapeutic interventions, enabling more effective control and management of COVID-19 in the Malakand region and beyond.

Rationale: Effective control of the COVID-19 pandemic requires continuous monitoring of viral variants and understanding the immune responses in specific populations. The Malakand region in Khyber Pakhtunkhwa, Pakistan, has experienced a significant number of COVID-19 cases, yet little research has been conducted on variant prevalence and antibody profiles in this area. This study aims to fill this knowledge gap by detecting COVID variants and analyzing antibody responses in patients from Malakand. The findings will provide valuable insights for implementing targeted public health measures, optimizing vaccination strategies, and developing effective therapeutic interventions. By improving disease management and outcomes in the local population, this research will contribute to controlling the spread of COVID-19 in the Malakand region and beyond.

2. METHODOLOGY:

Study design: This study was conducted at Saidu Teaching Hospitals Swat, to find out the prevalence, antibodies level and Covid-19 variants in hospitalized patients of Malakand division as part of the 4th wave.

Study Settings: Information such as Signs and Symptoms, vaccination status, contact with known Covid positive case, attended a social gathering and travel history in past 14 days were obtained. Patients admitted in the hospitals included in the study were residents of Malakand division of Districts; (Buner, Lower Dir, Upper Dir, Malakand, Shangla and Swat).

Study Duration: This project was completed in six months, from August 2021 to January 2022.

Sample Size: To find out the prevalence 1500 swab samples collected, for antibodies level 200 blood samples were collected and for variants detection 100 swabs samples were examined.

PCR Detection: A total of (1500) nasopharyngeal and oropharyngeal swab samples were obtained in VTM (UTM) tubes for the detection of Coronavirus in order to determine the prevalence and CT value among the hospitalized patients. For automated nucleic acid extraction, the Ascend Hero 32 Instruments and plates were used. The automatic DNA/RNA extraction technology used in this methodology was based on magnetic beads.[16]

The master mix was prepared in laminar flow hood under strict aseptic condition. One-step Genrui amplification reagent was used to amplify SARS-COV-2 RNA in specimens. Probes specific for the gene of interest are labeled with fluorophore FAM and VIC, allowing the N and S genes to be distinguished in parallel. Internal standard gene probes and primer sets serve as internal positive controls for monitoring sample quality, RNA extraction and detecting inhibitors of PCR reactions.[17]

The amplification device (BIORAD CFX-96) and device configuration ABI Prism 7500 were used in study. First, open the ABI Prism 7500 Setup window and set the positive control to (PC), the negative control to (NC) and the other samples to (unknown) in the correct order. The instrument window configures the cycle as follows: The first phase was set at 37 C^0 for 2 minutes, the second phase at 50 C^0 for 15 minutes, the third phase at 95 C^0 for 2 minutes, and 40 cycles. The data acquisition camera was ON in this step to detect viral load. This kit has a detection limit of 200 copies/mL and a CT value of less than 38.[18]

Antibodies Detection: Among 1500 patients those individuals having CT values under 33 were screened for serum antibodies detection. Covid-19 antibodies were tested using a fully automated Chemiluminescence immunoassay analyzer (COBAS e-411) and Elecsys Anti SARS-CoV-2 reagent kits. Following

calibration, external Positive and Negative controls were performed.[19] For Coronavirus Antibodies, the cut off value is 1.0 U/mL. If the antibody level is above 250 U/mL, the antibody is diluted (100X) according to the manufacturer's protocol.[20]

Detection of Variants: Among (473) positive swab samples those with CT value of less than 27 were screened for variant detection. Ascend Hero 32 Automated Extraction for nucleic acid extraction Instruments and plates were utilized for variant detection.[16]

The ABI Prism 7500 instrument configuration and the (BIORAD CFX-96) amplification instrument were utilized for the determination of variants. A GSD Nova Type III amplification reagent was used for the master mix. The kit contains the spike (S) gene as an amplification target region and also detects mutations E484K, E484Q, and E484R. An additional E484 wild-type probe allows E484K/Q mutations to be distinguished from wild-type sequences. The detection kit also includes an internal standard detection system (internal standard "RNAseP") used to monitor sample collection, nucleic acid extraction, and PCR amplification processes. This system helps reduce the possibility of false negatives. The CT value for RT-PCR GSD Nova III detection is less than 38 and detects four types of variants such as Epsilon, Kappa, Beta/Gamma, and Delta Coronavirus.[21]

Data Analysis: The statistical analysis was performed using Microsoft Excel for the tables and figures. The Mean and Standard deviation was performed between different variables of the data.

3. RESULTS:

PCR Detection: The overall prevalence of Covid-19 in Malakand division was 31.5% (473). Five major groups were created out of these samples based on District, Gender, Age, Vaccination and Signs and symptoms wise.

Samples from each district was sorted into six subgroups, including Districts: (Buner, Lower Dir, Upper Dir, Malakand, Shangla and Swat). In terms of gender distribution, two major grouping were carried out (males and females). In terms of age wise distribution, four major grouping were carried out, 1st was age under 20 years, 2nd was age between 21-40 years, 3rd was age between 41-60 years and 4th age above 60 years. In the vaccination-wise distribution, three major grouping were carried out 1st was partially vaccinated patients, 2nd was fully vaccinated patients and 3rd from non-vaccinated patients samples. The results for prevalence of Covid-19 according to District, Gender, Age and Vaccination wise are below (see table 1).

Table 1: Classification of Covid-19 Patients base on District, Gender, Age and Vaccination wise.

Distribution		Obtained Samples	Positive Samples	Percentage %
District Wise	Buner	38 (2.5%)	12	0.8%
	Dir Lower	56 (3.7%)	19	1.3%
	Dir Upper	79 (5.3%)	23	1.5%
	Malakand	33 (2.2%)	17	1.1%
	Shangla	191 (12.7%)	66	4.4%
	Swat	1103 (73.6%)	336	22.4%
Gender Wise	Male	827 (55.1%)	311	20.7%
	Female	673 (44.9%)	162	10.8%
Age wise	Below 20 Years	151 (10.1%)	67	4.5%
	21-40 Years	417 (27.8%)	120	8.0%
	41-60 Years	473 (31.5%)	131	8.7%
	Above 60 Years	459 (30.6%)	155	10.3%
Vaccination Wise	Partial	162 (10.8%)	61	4.1%
	Fully	201 (13.4%)	79	5.3%
	Non Vaccinated	1137 (75.8%)	333	22.2%

History obtained from patients was distributed according to signs and symptoms (see Table No:2), (Flu, Fever, Cough, Sore throat, Diarrhea and Shortness of breath). Out of 1500 patients, cases of fever 1182 (70.8%), flu 420 (28%), cough 1065 (71%) cases, sore throat 450 (30%), diarrhoea 69 (4.6%), and shortness of breath 849 (56.6 percent). While 138 (9.2%) of the 1500 suspected patients had no symptoms.

Table 2: Different complications of Covid-19 Patients.

Signs & Symptoms	Total Cases	Obtained Cases	Percentage(%)
Fever	1500	1182	78.8%
Flu		420	28.0%
Cough		1065	71.0%
Sore Throat		450	30.0%
Diarrhea		69	4.6%
Shortness of Breath		849	56.6%
Asymptomatic		138	9.2%

Antibodies Detection: In this study, blood samples (200/473) were obtained from PCR positive patients who had CT values below 33 performed for antibody detection. The results showed that the overall antibodies level was (Mean=10.66) and 6 samples were negative for Covid Antibodies.

Three major groups were created from these samples as area, gender and age wise. Samples from each district were sorted into six subgroups including; Buner, Lower Dir, Upper Dir, Malakand, Shangla and Swat. Samples by gender were distributed into two subgroups namely; (Male and Female). In terms of age distribution, four major grouping were carried out, 1st was age below 20 years, 2nd were between the ages of 21 and 40, 3rd were between the ages of 41 and 60 and 4th was age above 60 years. The results for antibodies level according to District, Gender and Age wise are below (Table No:3).

Table 3: Distribution of Covid-19 Antibodies base on (Gender and Age Wise).

Distribution		Samples (%)	Mean (M)	Negative Samples
District Wise	Buner	4 (2.0%)	9.49	00
	Dir Lower	11 (5.5%)	12.82	00
	Dir Upper	5 (2.5%)	7.60	00
	Malakand	7 (3.5%)	10.75	00
	Shangla	19 (9.5%)	10.25	00
	Swat	154 (77%)	10.68	06
Gender Wise	Male	125 (62.5%)	12.15	06
	Female	75 (37.5%)	10.11	00
Age wise	Below 20 Years	19 (9.5%)	8.71	00
	21-40 Years	45 (22.5%)	14.57	00
	41-60 Years	69 (34.5%)	9.83	05
	Above 60 Years	67 (33.5%)	9.24	01

Variants Detection: Samples of (100/473) positive patients samples were used for detection of variants which has CT value less than 27. Three major groupings were created from these samples; (District wise, Gender wise and Age wise).

Samples from each district were distributed into six subgroups including; (Buner, Lower Dir, Upper Dir, Malakand, Shangla and Swat). In terms of gender distribution, there were two major groupings (males and Females). Age-based distribution includes age from 21-40 years, from 41-60 years and from age above 60 years.

Results for variants detection showed that all (100) swabs samples were tested positive for delta variant Coronavirus. The results showed that in 4th wave most of infection caused by Delta variant of Coronavirus in Malakand Division, Khyber Pakhtunkhwa Pakistan (Table No:4).

Table 4: Distribution of Covid-19 Variants based on (District, Gender and Age Wise).

Distribution		Obtained Samples	Detected Variant
District Wise	Buner	3 (3%)	Delta
	Lower Dir	4 (4%)	
	Upper Dir	5 (5%)	
	Malakand	2 (2%)	
	Shangla	7 (7%)	
	Swat	79 (79%)	
Gender Wise	Male	65 (65%)	Delta
	Female	35 (35%)	
Age wise	Below 20 Years	6 (6%)	Delta
	21-40 Years	15 (15%)	
	41-60 Years	34 (34%)	
	Above 60 Years	45 (45%)	

4. DISCUSSION:

The present study determined the overall prevalence of Covid-19 in Malakand division, along this the antibodies titer and variant detection of Covid-19 was evaluated in these individuals. According to our findings the overall prevalence of Covid-19 was (31.5%) in Malakand division.

According to our findings the overall antibodies level of Coronavirus was (97%) with (Mean=10.66) in Malakand division. According to present study 194 samples were positive for antibodies and 6 samples were negative for antibodies. Among 154 samples collected from Swat only six samples were negative for antibodies detection. In Gender wise distribution the Coronavirus antibodies level was higher in the males (Mean=12.15) and lower in female (Mean=10.11). This difference in antibodies among the males and females is due to difference in the sample sized processed for the study. A study conducted by Haq *et al.*, (2020) on the sero-prevalence of Covid-19 antibodies has similar findings with (68.1%) in males as compared to females (31.9%) which supports and endorsed our study findings.[22]

According to our findings the highest antibodies level was recorded in age group of 21 to 40 years (Mean=14.57) and less antibodies level found in age group of 1-20 years (Mean=8.71). The highest level was found in age group of 21 to 40 years because of their good immune status as compared to old people

and children. This showed that the vaccination is very important for antibodies against Coronavirus, because at that time vaccination is only recommended for age above 18 years of age. According to a study conducted by Haq *et al.,* (2020) highest antibodies level (45%) was reported in age group 20–29 years followed by (31%) in the age group 30-39 years and less antibodies level (3.4%) was in age group older than 60 years.[22]

To find out the variations in Coronavirus and to determine the actual number of different genotypes present in Malakand division. According to our study in fourth wave of corona all the cases were Delta, genotype no case of Alpha, and Beta variants was found, Hasan *et al.* (2021) conducted a study in Qatar during the second and third waves of the COVID-19 pandemic. They evaluated non-type able samples for genotype confirmation. Hasan *et al.* (2021) determined the genotype of Coronavirus in second and third wave between April and June 2021. According to their findings, out of 9792 samples, 5500 (56.2%) of the variants of the Coronavirus were Beta, 2970 (30.3%) were Alpha variants, 40 (0.4%) were Delta variants and 1282 (13.1%) were wildtype. The difference in the result was due to the number of samples processed and the wave of Coronavirus used to determine the Variant detection.[23]

5. CONCLUSION:

This study concludes that the PCR based detection and variants of positivity ratio is very high during the fourth wave of Covid-19 epidemic and with high number of cases are reported in District Swat. This study demonstrated that males are more affected than females with most common symptoms of fever and cough. This study revealed that vaccination does not provide full protection against Coronavirus. Antibodies base data demonstrated high ratio of antibodies among the people of District Dir Lower, males have high antibodies as compared to females and young age people were antibodies rich as compared to others. According to this study Delta variant was the only variant in the fourth wave in Malakand Khyber Pakhtunkhwa.

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