Assessment of SARS-Cov-2 Antigen-Based Rapid Diagnostic Kit and Forecasting Using ARIMA Model Case Study of Pakistan

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Abstract

The pandemic of corona virus disease 2019 (Covid-19) has infected millions of individuals around the globe. The descriptive retrospective study was conduct From March 2020 to May 2021 utilizing publicly available data on COVID-19 total and active cases, fatalities, and recoveries from three key health centers in Shangla district (DHQ Alpurai, THQ Puran, and THQ Besham). A total 17734 suspects, 1257 (7.0%) cases were Covid-19 positive, in which 1250 (99.44%) recovered and 7 (0.56%) were dead. The most effected age groups were 16-30 years old 35% and 46-72 years old 27%. The most highly effected tehsil was Alpurai (57.5 %). The most highly effected month was April (2021) with 436 positive cases followed by May (2021) 168 and June (2020) with 213 positive cases. Mortality rate was high in the month of April (2020) with 3 deaths followed by May and June (2020) with single deaths, and April and May (2021) with single deaths. We concluded that Covid-19 spread was observed in a spike in the month of May (2021), prompting harsh adoption of the specified measures by the local authority of Shangla district at the commencement of the COVID-19 pandemic.

Key words: COVID-19, Pandemic, RT-PCR, KP, Infected, ARIMA

Introduction

The corona virus disease 2019 (COVID-19) outbreak was first described in Wuhan city, Since December 2019, the whole world is confronted with the problem of corona-virus pandemics and its effects on the people and their social life has been incredible. Every part of the world is virtually hit by COVID-19 infection. Most of the COVID-19 fatalities were aged people followed by the result of high death ratios as shown in data. [1,2]. Corona viruses (CoV) are members of the Corona viridae family, and they infect the pulmonary and enteric systems, causing respiratory syndrome and gastrointestinal sickness. Originally, they only infected animals, but since 1965, they have been able to infect humans [3-4]. Corona virus is a (+) strand enclosed RNA virus with the biggest genome of all RNA viruses. The coronavirus's RNA is polyadenylated and capped, and the genomic RNA is packed and enclosed by a nucleocapsid and an extra layer of envelop. Furthermore, the corona virus envelop contains unique glycolproteins such as hem agglutinin-acetyl esterase (HE) glycoprotein, various membrane glycoproteins, and spike glycoprotein. Which emerge from the envelop forming a crown-like shape[5]. Corona virus genome contains replicase genes with overlapping open reading frames (ORF1a and ORF1b) that can encode various non-structural proteins, as well as structural genes for Envelop, Spikes, membrane proteins, and nucleocapsid[6]. On January 30, 2020, the World Health Organization (WHO) designated the COVID 19 outbreak as the sixth public health emergency services (SPHEC) [7]. The COVID-19 is believed to be the third corona virus outbreak, affecting more than 209 nations, including Pakistan a total of 1,093,349 confirmed cases with 58,620 deaths, according to the World Health Organization (WHO). Till now, the United States has had the most positive cases, followed by Italy and Spain [8]. Pakistan's neighboring nations, especially China, were particularly hard hit, with the COVID-19 epidemic occurring for the first time. Italy has the largest number of COVID-19 fatalities in the west, whereas Iran has the largest number of COVID-19 fatalities in the north [9]. Pakistan is the second worst-affected nation in South Asia, with the third-highest case count in the WHO's EMRO area, behind Iran, and the 18th-highest case count worldwide [10]. The number of confirmed cases has risen to 66,457 across the country, with Punjab and Sindh being the worst-affected provinces [11]. On February 26, 2020, the Ministry of Health, Government of Pakistan, verified the first case of COVID-19 in Karachi, Sindh province. On the same day, the Pakistan Federal Ministry of Health in Islamabad verified another case [12]. Within fifteen days, there were twenty (20) confirmed cases (COVID-19 Positive) out of 471 suspected cases, with the Sindh province having the greatest number, followed by Gilgit Baltistan. All the confirmed patients had been to Iran, Syria, or London recently. And these cases are presently increasing at a rapid rate, making the situation much worse [13]. With the continuing rise in the number of CVOID-19 positive cases, Pakistan's geographical position necessitates a high degree of action, planning, and management. The Ministry of National Health Services, Regulation & Coordination Pakistan released a strategy titled "National Action Plan for Preparedness and Response to Corona Virus Disease (Covid-19) Pakistan" on February 12th, with the goal of controlling the disease. Local, regional, and national epidemics that might have a major influence on Pakistan's people and society [14]. According to the Ministry of National Health Services Regulation and Coordination, Sind province had 315,410 cases, KPK province had 131,775, Punjab province had 338,377, Baluchistan province had 25001, and the capital Islamabad had 81,007 cases. Gilgit Baltistan / Azad Jammu and Kashmir had 19108/5561 cases [15]. To far, the Pakistani government has adopted a variety of measures to combat the COVID-19 epidemic. We emphasized the many actions taken by the Pakistani government to combat CoVID-19, including designated hospitals,

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quarantine centers, testing facilities, therapies, public awareness, and the local community's response to the epidemic. Despite the country being under partial lockdown for the ninth week in a row, the number of cases has continued to rise. The situation is still quite fluid, with statistics changing at a rapid rate. The goal of this study was to look at the COVID-19 cases as well as the general epidemic scenario in Shangla district.

Methodology

Study population and Sample collection

The current study was conducted among 17734 suspected COVID-19 subject during the period of June 2020 to March 2021 from District Shangla. After pre-test counseling by trained counselor, the specimens were obtained from SARS-CoV-2 suspected patients with respiratory symptoms and/or fever and international travel history or close contact with SARS-CoV-2 confirmed patients, by trained personnel at, the department of Pathology District head Quarter hospital shangla Kp, Pakistan. Ethical approval was obtained from all 17734 participants included in this study. About 3 ml blood sample was obtained from everyone in a gel vacutainer tube. The relevant demographic information like age and gender were also recorded

Inclusion criteria

All those individuals who were tested positive for the SARS-CoV2 according to the WHO guidelines for the detection and diagnosis of COVID-19 were included in this study.

Sample Processing

The extracted RNA was further processed with PCR reagents as per guidelines. For 1 sample 26µl PCR mix (Primers 4.62%, Probes 1.15%, dNTPs 3.85%, MgCl2 0.77%, RNase 0.48%, PCR buffer 89.13%) and 4 µl enzyme mix (RT enzyme 62.5%, Taq enzyme 37.5%).

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Real Time PCR

All the collected samples were then analyzed with Real-Time PCR (Rotor-Gene Q) for the diagnosis of COVID-19/ detection of SARS-CoV-2 RNA/genome.

- The PCR tubes were placed in the specimen wells of thermal cycler.
- The 2019-nCoV-PCR-Positive control and negative control were set up and named in the system.
- The PCR test channel was selected: a) FAM (ORF-1ab region) and ROX (N gene) to test nCoV nucleic acid. b) CY5 channel to test the internal control.

Statistical Analysis:

On the bases of the available data, we used to predict the number of COVID-19 cases and mortalities in the people of district Shangla for upcoming 7 months period. Therefore, we used the simple series

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methods of Auto-Regressive Integrated Moving Average (ARIMA) Model [16]. In other words, the ARIMA model is known more accurate in predicting than exponential smoothing [17]. Due to the scarcity of data, we used non-seasonal models to define the pattern through time using SPSS 20 version. While using Microsoft Excel 10, the data was analyzed as descriptive statistics such as percentages and frequencies.

Results

In overall 17734 suspects, 1257 (7.0%) cases were found Covid-19 positive, in which 1250 (99.44%) recovered from disease and 7 (0.56%) were lost their lives. While the COVID-19 positive male individuals were 1074 (85.5%) and female individuals were 183 (14.5%) reported as shown in table. No1.

Table.No.1. Demographic presentation of Covid-19 Pandemic in male and female:

Gender	Covid Positive	Percentage %
Male	1074	85.5%
Female	183	14.5%
Total	1257	N/A

Age wise presentation of Covid-19

The age group 16-30 years old was the most affected (35 percent), followed by 46-72 years old (27 percent), 31-45 years old (26.6 percent), and 1-15 years old (11.3 percent) shown in table.No.2.

Table.No.2. Age-wise distribution of Covid-19 patients:

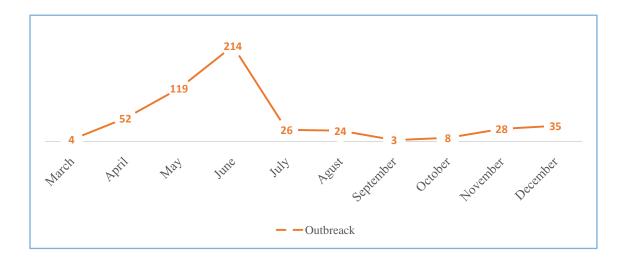
Age Group	confirmed cases	percentage
1-15	142	11.3%
16-30	440	35%
31-54	335	26.6%
46-72	340	27%

Tehsil wise presentation of Covid-19

The most highly effected tehsil was Alpurai (57.5 percent), followed by Tehsil Puran (28.4 percent), and Tehsil Besham (14.0 percent). Shown in table.No.3.

Table.No.3. Tehsil wise Distribution of Covid-19 Infected patients from Shangla:

Tehsil-wise	Positive case	Percentage%
Alpuria Tehsil	724	57.5%
PuranTehsil	357	28.4%
Besham Tehsil	176	14%
Total	1257	N/A



Year wise prevalence of Covid-19 pandemic

Figure.No.1. Covid-19 outbreak 2020 in District Shangla:

In the year 2020, the initial spike of the Covid-19 pandemic was most effective in the months of June, May, and April, respectively. In addition, a spike in cases was seen in the months of April, May, and March in the year 2021. Shown in Figures.No.2 & 3.

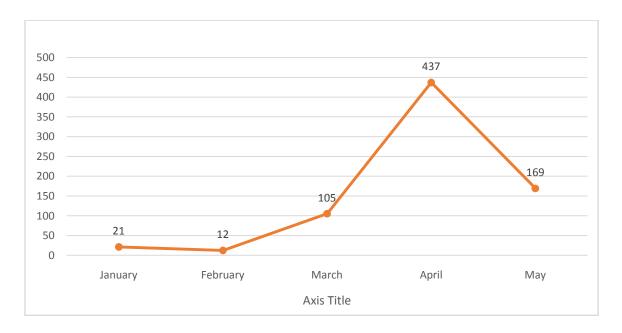


Figure.No.2. Covid-19 outbreak 2021 in District Shangla:

Month wise presentation of Covid-19

The most highly effected month with 436 positive cases was April (2021) followed by June (2020) with 213 positive cases, May (2021) with 168 positive cases, May (2020) with 118 positive cases and March (2021) with 105 positive cases. In other words, mortality rate was high in the month of April

(2020) with 3 deaths followed by May and June (2020) with single deaths, and April and May (2021) with single deaths. Shown in Figure.1.

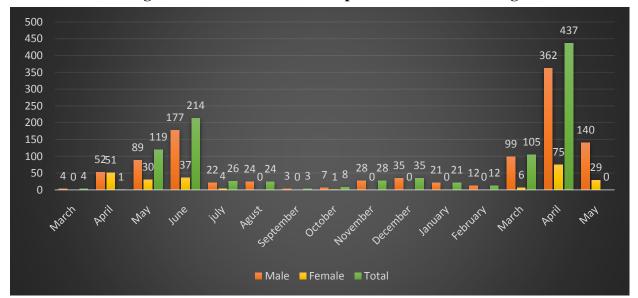


Figure.3. Month wise Covid-19 patients in District Shangla:

Data forecasted with ARIMA model

Using 15 months data from March 2020 – May 2021 and ARIMA model, we forecasted the data up to December 2021using SPSS 20 version. As, we dealt with time series and non-stationary data, the mean and variance of data was variable in nature. ARIMA (0, 2, 1), was applied to produced plots for the number of positive cases and deaths over time (Months/Years) as shown 1, 2, models in Fig 4. Results with the 95% prediction interval, Positive cases from the model show a little increase, although mortalities show a line below of the 0 percent. As a result, if the restrictions will strictly be followed in the region, the expected pattern of cases will be adapted in the coming months.

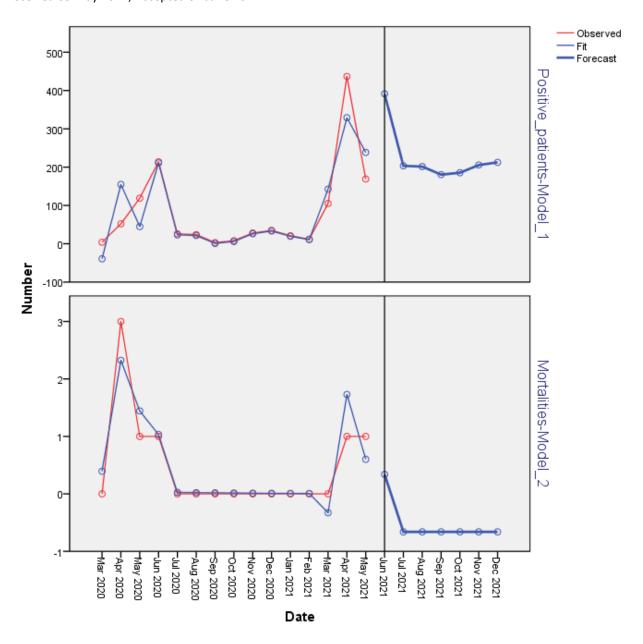


Figure. 4. Shows Seasonal Forecasting for COVID-19 pandemic in next 7 months.

Discussion

The initial stage of Covid-19 pandemic was highly affected on people and threaten every individual about the seriousness of this deadly disease in all over the world, as first China then Europe, USA, Brazil, Russia, India, and Pakistan. The first incidence of COVID-19 in Karachi was confirmed by Pakistan's Ministry of Health on February 26, 2020. Pakistan has been designated as the second worst-affected country in South Asia, with the third-highest case total in the WHO's EMRO area, after Iran, and the 18th-highest case count globally. Then the country's testing capacity was expanded, as well as reporting of positive cases among groupings of pilgrims, Pakistan saw a rise in cases beginning in the first week of April 2020. Pakistan has performed over 508,086 tests by the end of May, with a positive rate of 24%. The number of tests performed per million individuals in KPK is much lower (1486 tests per million people) than in other provinces. In KPK province, there

were 132170 confirmed cases as of 30 May 2021, with 122795 recovered and 4060 fatalities due to the corona (Covid-19) pandemic [18].

The acquired data in this study disclosed that, the initial stages of the COVID-19 pandemic, which was hit hot most of the regions in the world as well as Pakistan, many other areas, also effected the Shangla district. The highly effected was male group 85.5% than female group 14.5%. Which suggested that male individuals were mostly visited to the COVID-19 pandemic hit regions and effected. Likewise, the age group 16-30 years age individuals effected more, followed by 46-72 years age, in other words the death cases were noted in between the age group of 46-72 years age individuals. Moreover, the effected tehsil was Alpurai, due to district headquarter (DHQ Hospital) more infected individuals were visited and referred by Doctors of other local health centers. While tehsil Puran (THQ Hospital) reported with 28.4% followed by tehsil Besham (THQ Hospital)with 14%. In other words, the residence of tehsil Puran and tehsil Besham reported mostly visited individuals to the pandemic regions. In the year of 2020 most, effected month was April with 3 fatalities due to COVID-19 pandemic. While the month of June was noted with surge in pandemic, followed by the months of May and July. Adding to that in the month of March (2021) once again COVID-19 was noted with surges in people, while the month of April conformed to high positive cases.

Following a sharp increase in cases, the federal and provincial governments implemented a variety of strategies to limit the spread of COVID-19, which were vigorously enforced by local administrative bodies, resulting in the district's COVID-19 pandemic being halted. In the early phases of the COVID-19 pandemic, a smart lockdown in hotspot locations, which enabled in contain corona spread more effectively than in other nations throughout the world. In a televised speech to the nation on June 13, 2020, Pakistani Prime Minister Imran Khan declared that a "smart lockdown" plan will be implemented in specific hot locations around the country "since people's apathy to the epidemic was threatening the lives of the elderly and chronically sick [19]. Similarly, district administrations across Pakistan implemented the same techniques devised by the National Command and Operation Center (NCOC), which contained the COVID-19 outbreak that was raging at the same time and saved people's lives. While similarly, Shangla district's District Police Officer (DPO) advised residents on (26th March 2020) to avoid leaving their houses needlessly. People should stay at home and be safe. While police officers and 'jawans,' along with volunteers, were executing their responsibilities, only medical stores were open in several marketplaces throughout the district, advising the public to avoid unwanted gatherings [20]. Furthermore, the Corona pandemic was in remission in the following months, causing many to lose faith in government-imposed strategies, but the pandemic resurfaced in some locations. Due to a COVID-19 pandemic increase, again in (2021) the National Command and Operation Center (NCOC) has recommended a smart lockdown in districts such as Peshawar, Swat, Mardan, Swabi, Charsadda, Nowshera, Kohat, Abbottabad, Shangla, and Bajaur in Khyber Pakhtunkhwa province [21]. Add to that, we made a short-term forecast about COVID-19, Positive cases, as well as the number of related deaths. The results of this study revealed a little bit raise in the positive cases, which suggests the COVID-19 outbreak not fully controlled in the district, but the mortalities could be controlled at the end of this year (2021) in the district Shangla if the current tendency with smart lockdowns (restrictions) continues. In other words, to keep the COVID-19 pandemic from spreading throughout the Shangla district, residents must strictly adhere to the NCOC's laws and safeguards (strategies) as well as those enforced by the local government.

Conclusion

The strategies recommended by the Nation Command and Operation Center were closely implemented by the local administration of Shangla district at the start of the COVID-19 pandemic, which helped to contain the epidemic's further surges. However, the corona spread was noticed in a spike in the month of May (2021), necessitating stringent application of the proposed procedures.

Conflict of interest:

The Authors declare that there is no conflict of interests.

Ethical approval

Ethical approval was taken from the Research Committee in the Department of Biotechnology and Genetic Engineering at Hazara University Mansehra and the medical superintendent of District Head Quarter hospital Shangla

Informed Consent

Consent form will be filled with the mutual agreement of the family which will be about to keep the data personal and confidential.

References

- [1] Chellapandi, P., & Saranya, S. (2020). Genomics insights of SARS-CoV-2 (COVID-19) into target-based drug discovery. *Medicinal Chemistry Research*, 1-15.
- [2] Anwar, F., Tayyab, M., Khan, J., & Haq, I. (2020). COVID-19 and taking care and protection of patients with intellectual disabilities, need special care and equity.
- [3]Fulton, R. W., Herd, H. R., Sorensen, N. J., Confer, A. W., Ritchey, J. W., Ridpath, J. F., & Burge, L. J. (2015). Enteric disease in postweaned beef calves associated with Bovine coronavirus clade 2. *Journal of Veterinary Diagnostic Investigation*, 27(1), 97-101.
- [4] Le Coupanec, A., Desforges, M., Meessen-Pinard, M., Dubé, M., Day, R., Seidah, N. G., & Talbot, P. J. (2015). Cleavage of a neuroinvasive human respiratory virus spike glycoprotein by proprotein convertases modulates neurovirulence and virus spread within the central nervous system. *PLoS pathogens*, 11(11), e1005261.
- [5]Qamar, Z., Anwar, F., Ahmad, R., Haq, I., Khan, A.M.K., Hussain, R., Shahzad, Z., Ahmad, I., Malik, M.S.M. and Khan, J., 2021. Prevalence of Hepatitis C virus and determination of its genotypes in subjects of Tehsil Daggar District Buner, KP, Pakistan. *Clinical Epidemiology and Global Health*, 12, p.100809.
- [6] Sawicki, S. G., Sawicki, D. L., & Siddell, S. G. (2007). A contemporary view of coronavirus transcription. *Journal of virology*, 81(1), 20-29.
- [7]Bilgin, S., Kurtkulagi, O., Kahveci, G. B., Duman, T. T., Meryem, B., & Tel, A. (2020). Millennium pandemic: A review of coronavirus disease (COVID-19). *Experimental Biomedical Research*, *3*(2), 117-126.
- [8] Anwar, F., Khan, M., Salman, M., Ahmad, S., Ullah, F., Khan, J., ... & Abbas, M. (2021). Seroprevalence of hepatitis B virus in human population of district Buner Khyber Pakhtunkhwa Pakistan. *Clinical Epidemiology and Global Health*, 10, 100688.

- [9] M. Saqlain, M.M. Munir, A. Ahmed, A.H. Tahir, S. Kamran.Is Pakistan prepared to tackle the coronavirus epidemic? Drugs TherPersp (2020), pp. 1-2.
- [10] Anwar, F., Tayyab, M., Salman, M., Abdullah, Din, M., Khan, J., & Haq, I. (2020). Dengue outbreak 2018 in district Shangla KPK; clinical features and laboratory markers of dengue virus infection. *Future Virology*, *15*(10), 693-699.
- [11] Government of Pakistan (2020) COVID-19 Health Advisory Platform by Ministry of National Health Services Regulations and Coordination.
- [12] Anwar, F., Ahmad, S., Haroon, M., Haq, I. U., Khan, H. U., Khan, J., ... & Shah, I. A. (2019). Dengue virus epidemics: A recent report of 2017 from district Mardan, Khyber Pakhtunkhwa province, Pakistan. *International Journal of Mosquito Research*, 6(1), 46-49.
- [13] Anwar, F., Zubair, M., Shah, M., Ahmad, S., Mehmood, M., Bakht, S., Zahir, F., Din, Z.U., Ullah, S., Bibi, T. and Nasar, Z., 2021. Molecular epidemiology of SARS-COV-2 in Mardan, Khyber Pakhtunkhwa Pakistan: A real world clinical experience. *Bioscience Research*, pp.1608-1613.
- [14]Khan, J., Anwar, F., Shah, S. S., Qamar, Z., Ullah, W., Ali, A., ... & Ullah, F. (2021). Dengue virus epidemics: A recent report of 2018 from district Swat, Khyber-Pakhtunkhwa Pakistan. *International Journal of Mosquito Research*, 8(1, Part B), 105-108.
- [15] Anwarl, F., Khan, M., Rehman, N., Akbar, F., Ahmadl, S., Yousaf, M., Haq, I., Zahir, F., Shahzad, Z., Khan, F. and Bakht, S., 2021. Review and forecasting on COVID-19 outbreak; An insight to in silico candidate drug discovery. *Bioscience Research*, pp.1198-1210.
- [16] Yousaf, M., Zahir, S., Riaz, M., Hussain, S. M., & Shah, K. (2020). Statistical analysis of forecasting COVID-19 for upcoming month in Pakistan. *Chaos, Solitons & Fractals*, *138*, 109926.
- [17] Chen, P., Yuan, H., & Shu, X. (2008, October). Forecasting crime using the arima model. In 2008 Fifth International Conference on Fuzzy Systems and Knowledge Discovery (Vol. 5, pp. 627-630). IEEE.
- [18] Bashir, Z., Ahmad, S. U., Kiani, B. H., Jan, Z., Khan, N., Khan, U., ... & Mahmood, T. (2021). Immunoinformatics approaches to explore B and T cell epitope-based vaccine designing for SARS-CoV-2 Virus. *Pak. J. Pharm. Sci*, *34*(1), 345-352.
- [19] Anwar, F., Tayyab, M., Haq, I., & Shah, O. U. (2021). Viral overload of COVID-19 pandemics: Overweight people a soft target to get an infection.
- [20] Shah, I. A., Anwar, F., Haq, I. U., Anwar, Y., Aizaz, M., & Ullah, N. (2018). HBV burden on population, a comparative study between two districts Mardan and Charsadda of KPK, Pakistan. *International Journal of Contemporary Research and Review*, 9(09), 20269-20274.
- [21] Ahmad, S., Anwar, F., Ullah, I., Alam, M., Khan, J., Abid-ur-Rehman, F. A., & Ullah, R. (2020). Epidemiological and clinical manifestation of dengue virus infection: A Recent Report of 2018 from District Battagram Khyber Pakhtunkhwa. *International Journal of Mosquito Research*, 7(6, Part A), 5-8.