REVIEW ARTICLE

Comparison of 2nd and 3rd wave of SARS-C0V-2 based on gender and age.

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ABSTRACT... SARS-COV-2 is a deadly virus which emerged in China in late 2019. In this article, the difference in European variant (2nd wave) and UK variant (3rd wave) of COVID-19 in Pakistan was investigated. Polymerase chain reaction (PCR) technique was applied to evaluate the difference in gene sequence of both the variants. The UK variant (3rd wave) was more terrible due to its resistance and genomic mutation. When performed PCR in both the waves, there was nucleocapsid protein (N gene) in UK variant while envelop protein (E) was more prominent in European variant after ORF 1ab in both the strains. People of all age groups were tested and was reported that aged individuals were at more risk of infection as compared to young ones. Similarly females were having more immunity in defence against COVID-19 infection as compared to males that increased more risk of infection to them.

Key words: SARS COVID-19,

INTRODUCTION

The word corona virus is not a new term in the scientific world. A family of corona viruses was discovered in 1960s[1] which was sub divided into four basic categories as alpha, beta, gamma and delta which was grouped on the basis of certain attributes including the type of nucleic acid, on the basis of specific morphology and either the presence of lipid envelop or not.[2] All the viruses of this family are zoonotic as they are transferred at some point from animals (domestic as well as wild) to humans by an event named spill over event. Only sub-group alpha and beta are harmful for human beings. Some members of this family cause respiratory diseases including simple cold as well as certain rare respiratory infections. These infections include Severe Acute Respiratory Syndrome (SARS), believed to be arisen in bat but presence was reported in civet cats and latterly caused disease in humans[3] in 2002 and Middle East Respiratory Syndrome which was reported initially in dromedary camel through bats and was confirmed in humans[4] in 2012.[5]

A new virus of this category was reported in December 2019 in Wuhan city of China. Initially reported cases of this virus showed their link with the Huanan SeaFood Market of Wuhan in which wild as well as domestic animals were being sold. Initially it was resembled with influenza virus because of its symptoms were almost similar with influenza. But later it was revealed that the cycle of infection of influenza was quite different from this newly emerged disease.[6] Also the infection rate of influenza was more in children (20-30%) as compared to adults (5-10%).[7] As it was at some angle associated with animals and was attacking the respiratory system it was added in the family of corona viruses. It was named as Severe Acute Respiratory Syndrome Corona Virus Disease (SARS COVID-19). Soon it was clear that this can now be transmitted from human to human that means the carriers of disease include not only animals but humans as well on 20th of January 2020.[8-9] So it raised a new challenge for the world as thousands of people got infected by this in China and all the physicians were unable to cure the patients and to find out the protective

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measures against the infection. Keeping in view that worst scenario the Chinese government decided to stop all sort of transport to and from Wuhan just to keep Wuhan isolated from China and whole world[10], as infection was reported only in Wuhan. But condition was out of control and its area of infection was spread in Europe (mainly Italy and Spain) and Iran as well. By considering the alarming situation, on 30th January 2020 WHO confirmed it a 6th Public Health Emergency of International Concern.[11,12] As there was no cure from that deadly virus, mostly all countries mainly European and Iran decided to stop all trade and transport as well and closed the national boundaries just to minimize the risk of viral infection.[13]

First case of COVID was reported in Pakistan on 26th of February 2020 in Karachi[14-15] and it was associated with the history of travelling of person to Iran.[16] But Pakistan was not so much attacked with it as compared to European countries because of humid and hot weather condition, strong immune system of population, the more young population of the country as well.[17] In the same time, virus attacked more than 80 countries of the world. While having a serious observation over the entire situation, the Center of Disease control and prevention (CDC) Centers of United States expressed a view that the ongoing situation of whole world, virus will more likely cause pandemic.[18] And within three months of outbreak of COVID-19 in China (Wuhan) WHO declared it a pandemic.[19-20] Virus was spreading at a nonstop speed in no time in the whole world. In Pakistan too, the government decided to go for a complete lockdown which was initially of two weeks and then prolonged to months. But although there was complete lockdown, the ratio of corona positive cases was going to increase on daily basis and it was probably due to weak immune system of old ones who were at a risk of infection and its high rate of spread[21] and it took months to take protective measures against it. In Pakistan condition was quite normal in summer due to humid conditions in Pakistan and all the social restrictions were uplifted by the government in August.[22] But it was not the end of it, a new wave was arising in Europe[23] and within no time it

was in Pakistan when the government of Pakistan confirmed a new wave (probably 2nd wave of COVID-19) on October 28, 2020 when daily positive cases increased from 630 (on average of last month) to more than 750 confirmed new cases per day and again hospitals were under stress of corona patients.[24] But it was not so much tough time for nation as people were familiar with the precautionary measures to prevent the spread of disease. Pakistan was soon out of that worse condition due to proper strategies adopted by the management. As we were not completely out of it, a new chapter of SARS COVID-19 started when after the European variant (2nd wave) a new variant of UK (3rd wave) hit Pakistan on December 29, 2020[25] in Islamabad. Within a year of outbreak of SARS COVID-19, Pakistan has suffered from three strikes of it and this third episode of it is much alarming as ratio of positive cases has been increased from 10% with more than 4000 new cases being reported on daily basis and more than 100 patients of SARS COVID-19 are being dead daily. As SARS COVID-19 is a problem worldwide, a lot of scientific articles are published on various aspects of it in every minute.[26]

This research was conducted to have some nearly authentic figures of viral spread in of all age groups and have the genomic difference in different viral strains in Pakistan.

OBJECTIVES
The main objectives include.
To check the infection rate of 3rd wave of SARS-CoV-2 in Pakistan.
To find out main reason of infection in different age factors.
To evaluate that which gene is more prominent in this UK variant of SARS-CoV-2.

MATERIAL & METHODS
After transport of viral sample inside the laboratory, it was loaded in the extraction kit (as shown in Figure-1). 300 μl of viral sample was added in extraction kit from viral transport medium with the help of pipette.

Auto-Pure 32 A
After sample being loaded in extraction kit,

Auto-Pure 32 A was used. This extraction robot (as shown in Figure-2) was used to extract the viral RNA from 2nd and 8th well to 6th and 12th well respectively.

Figure-1. (Maccura extraction kit) The wells of kit consists of magnetic beads in 1st and 7th wells, lysis buffer in 2nd and 8th well, wash buffer 1 in 3rd and 9th, wash buffer 2 in 4th and 10th well remaining wells have elution buffers.
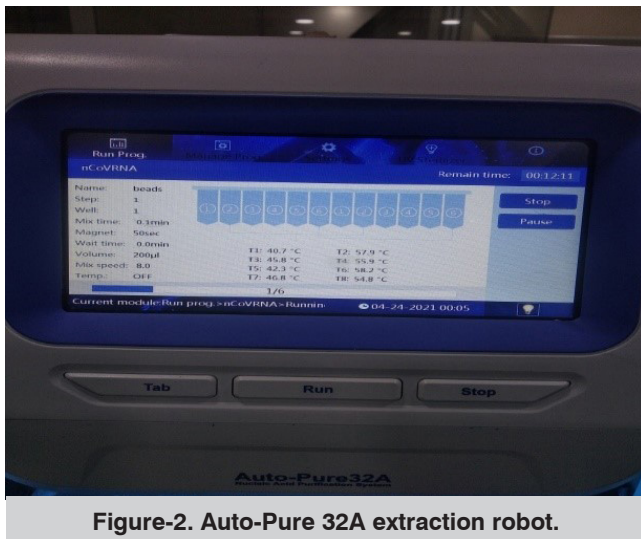

Figure-2. Auto-Pure 32A extraction robot.

Biosafety Cabinet

The process of sample loading and loading of viral RNA in master mix was performed in biosafety cabinet 2 (as shown in Figure-3). Whole process of pipetting was performed inside the cabinet.

Thermo Cycler

When whole procedure of extraction was performed Applied Biosystems (ABI) thermo cycler (as shown in Figure-4) was used to perform reverse transcription of viral RNA.
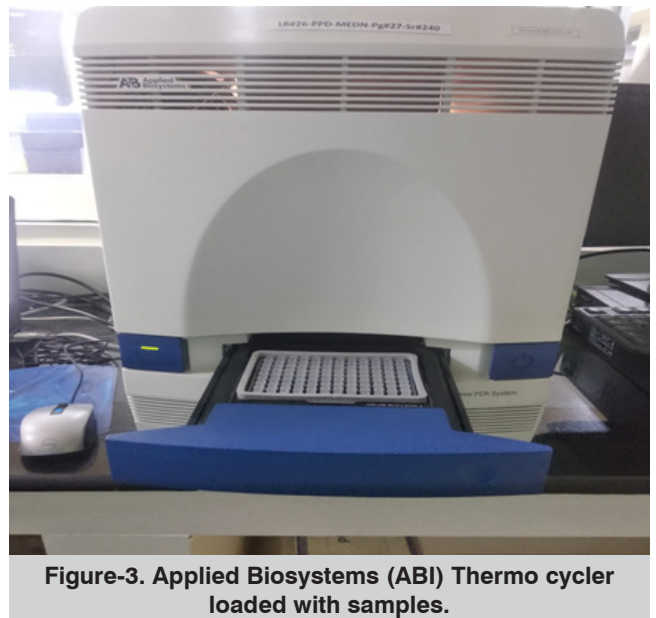

Figure-3. Biosafety Cabinet 2.

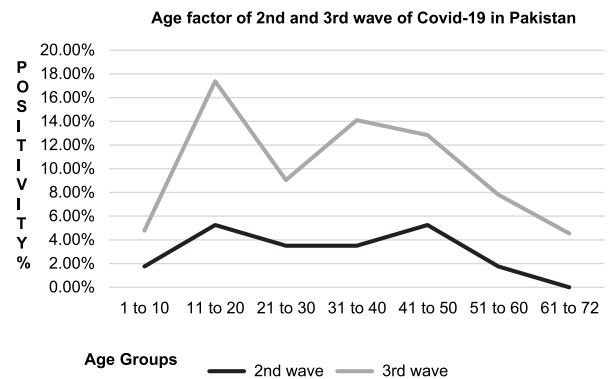**Figure-3. Applied Biosystems (ABI) Thermo cycler loaded with samples.**

RESULTS

After passing the viral RNA from all the practices from sample medium, it run finally on the real time RT-PCR to detect the genes of viral RNA including Open Reading Frame (ORF1ab) making 2/3rd of total COVID genome with that encode sixteen proteins (non-structural) while the remaining 1/3rd portion encode four structural proteins including spike (S glycoprotein), nucleocapsid protein (N),

envelop protein (E) and matrix protein (M) and at least six accessory proteins[27-29] following all of its 5 steps including reverse transcription, initial denaturation, denaturation, annealing and at last extension step with different temperature at every step gave us the final expression of genes of virus present by following the guidelines provided by WHO to test the suspected case of COVID-19.[30] Taking in account the 2nd wave of COVID-19 in Pakistan, three main genes were detected by PCR including ORF, E Gene and S Gene in the mid of October 2020 when National Command and Operation Center (NCOC) accounted a rapid increase in the infection curve.[31] While in mid of January, when again samples were tested then genome sequencing in National Institute of Health (NIH) confirmed that the UK variant of SARS COVID-19 is spreading in Pakistan showing the nucleocapsid protein (N gene) was more prominent as compared to E gene of 2nd wave and was later confirmed by National Command and Operation Center (NCOC).[32-33] The age factor and the gender specification of both the 2nd and 3rd layer of virus as discussed.

Age Factor
The positivity ratio in different age groups of both the waves was remarkably different because of the geographical atmosphere in both of waves (as shown in Graph-1). The samples of 2nd wave were tested in October when the temperature was humid as compared to January when the samples of 3rd wave were tested and genome sequence was observed. More cases were reported in winter while 3rd wave was at its peak because the survival rate of virus on various things was remarkably more in winter as low temperature can increase the survival duration of SARS COVID-19 up to 28 days while temperature (30 ☐ C) can limit the survival duration to few days.[34] The 2nd wave was more common among adults of older age[35] as compared to young people. While in 3rd layer along with older people, severity of infection was also reported in the younger and healthy people as well.[36] In 3rd wave some patients showed their interaction with COVID recovered patients which proved that infection recovered patients were also a cause of infection to the healthy ones.[37-38]
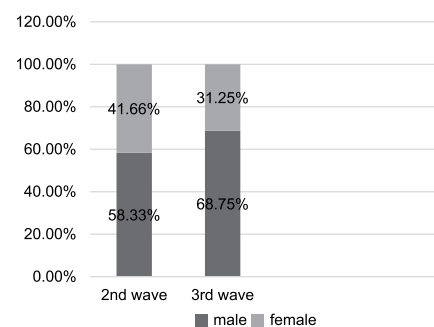

Graph-1. Age factor of 2nd and 3rd wave of Covid-19 in Pakistan.

According to data that has been collected in 2nd wave of COVID-19 positivity rate in age group 1-10 were 1.75%, positivity rate in age group 11-20 were 5.26%, positivity rate in age group 21-30 were 3.50%, positivity rate in age group 31-40 were also 3.50%, positivity rate of age group 41-50 were 5.26%, positivity rate in age group 51-60 were 1.75%, positivity rate in age group 61-70 were 0%.

Comparatively in 3rd wave of COVID-19 positivity rate in age group 1-10 were 3.03%, positivity rate in age group 11-20 were 12.12%, positivity rate in age group 21-30 were 4.54%, positivity rate in age group 31-40 were also 10.60%, positivity rate of age group 41-50 were 7.57%, positivity rate in age group 51-60 were 6.06%, positivity rate in age group 61-70 were 4.54%.

Gender Specification

Graph-2. Positivity ratio b/w male and female in 2nd and 3rd wave of COVID-19.

According to data that was collected in 2nd wave of COVID-19 positivity percentage in males and females were 58.33% and 41.66% respectively. While on other hand, in 3rd wave of COVID-19 positivity percentage in males and females were 68.75% and 31.25% respectively. Initially it was not clear that which gender is at greater risk of COVID-19 infection[39] because not much information was known about the virus that how the immune system of individuals behave in response of infection. However some reports revealed the differences in the fatality rate between female and male patients exists.[40-42] The result indicated us that in both 2nd and 3rd wave, the males were at higher risk of severe infection as compared to females.[43-46]

The immune response of individuals is the main reason of development of infection or not. As females have more effective innate as well as adaptive response of immune system and develops quickly[47] as compared to males because more genes related to immune response are located on X chromosome.[48] Along with these genes ACE2 gene is also situated on the same chromosome, so females have more ACE2 genes as compared to males that help to protect females from more severity of disease.[49] 1468V and K26R are the two variants of ACE2 genes are very effective against the COVID-19 infection because these variants possibly lower affinity of S protein binding to host.[50] So ACE2 genes can be a protective barrier against the lung infections and older age causes a noticeable decrease in genes of ACE2.[51]

In addition to the beneficial role of ACE2 genes in females against viral infections, there exists harmful effect of these genes as well. It has been reported that receptor of Angiotensin Converting Enzyme 2 behaves like a barrier for viral entry into the tissues of host individual.[52] Just like ACE2, HLA allelic region in the genome of humans has also been proved to be involved in both the risk as well as resistance against infections.[53] Another thing observed that increases the resistance of females against the COVID infection was the higher IgG antibody level in them as compared to males which causes male to be a more likely to be infected with SARS COVID-19.[47]

From all the individuals whose test was performed in both the waves, 19 of them were with blood of A group type and 1 person with brain tumour. Out of 19 persons, 13 were positive and the infection was at severe stage. So A blood group was believed to be risky factor for severe infection.[54-55]

DISCUSSION

3rd wave of COVID-19 pandemic in Pakistan was more severe as compared to 2nd wave due to resistance and mutation of virus. The mutation rate of single stranded COVID-19 virus is more as compared to mutation rate of humans.[56] Another edge in its more critical stage was the temperature, as viruses survive more in cold temperature due to increase in persistent period of virus.[34] In Pakistan, COVID-19 infection was more severe for people of older age as compared to young people.[35] Similarly females were at safe end from virus as compared to males due to rapid and effective immune system response.

Also people who are suffering from chronic disease (s) are also at greater risk of viral infection and could prove deadly for them. So proper protective policies should be followed to minimize the risk of viral spread because virus can spread from a number of ways including direct contact, aerosol droplets and via oral-fecal route[57-59] from both the patients developing symptoms and without symptom developed patients.

CONCLUSION

The infection rate of UK strain of COVID-19 was double as compared to European variant due to more survival rate of that strain because of winter season. People with low immune response were at more risk of infection. Variation in Nucleocapsid (N Gene) was detected in UK variant in replace of envelope protein (E) gene in European variant. **Copyright© 20 Oct, 2021.**

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2	Noor ul Ain	Write paper.	
3	Abdul Rehman	Analysis of data.	
4	Sajjal Rashid	Analysis of Data.	