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Molecular epidemiology of SARS-CoV-2: A tertiary care hospital experience from Pakistan

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ABSTRACT

Objectives: The current study was conducted to assess the molecular epidemiology of SARS-CoV-2 in the general population.

Methodology: This study was conducted from April to July 2020, at the Molecular Diagnostic Laboratory, Pakistan Atomic Energy Commission (PAEC) General Hospital Islamabad, Pakistan. A total of 28,274 nasopharyngeal swabs were collected in Viral Transport Medium (VTM) media from symptomatic and asymptomatic individuals at the sample collection centers of our hospital and other affiliated hospitals. RNA was extracted using both automated and manual extraction platforms as per the manufacturer's instructions. Multiple qualitative reverse transcription real-time PCR kits for the identification of SARS-CoV-2 were used.

Results: The results showed that 1,722 (6.09%) were positive for SARA-CoV-2 RNA. The males exhibited a prevalence of 2.76% while females showed a high prevalence of 13.44%. Among males, most patients 424 (31.47%) were in the age group of 31-40 years followed by the age group of 41-50 years 306 (22.71%). Similarly, among females, the majority of patients were from the age group 31-40 years with 91 (24.66%) followed by 41-50 years of age group 70 (18.66%) confirmed cases.

Conclusion: The molecular epidemiological data may support the national policy formulation, transmission tracking, and the execution of measures to control viral transmission.

Keywords: SARS-Cov-2, COVID-19, Pakistan, Molecular Epidemiology, Coronavirus, Pandemic.

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Introduction

The SARS-CoV-2 and other related viruses belong to the bigger family of viruses termed as "Coronaviridae" having the capacity to infect both animals and humans.[1] The phylogenetic analyses revealed that it is not the first incident of human infection with a member of this family of viruses.[2] In the past, we have seen infections in many regions across the globe such as Severe Acute Respiratory Syndrom-Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) in 2003 and 2015 respectively.[3]

The first case of novel coronavirus resulting in mortality was identified in Wuhan, China in December 2019.[4] The cause of this disease was instantly identified as a beta coronavirus.[5] The disease was not restricted to China and in January 2020, the World Health Organization (WHO) declared the outbreak as a public health emergency of international concern; on January 31, 2020, the United States declared a public health emergency.[6] Later, the WHO declared the disease a pandemic on 11th March, 20207. Pakistan is a neighbouring country of China, however, the country remained unscathed from the virus till February when

the first cases were reported. 8 Until now, 0.33 million confirmed cases of COVID-19 infection with a ratio of 2.03% mortality rate have been reported.[9] There are high risks of person to person transmission through aerosols. The virus may get transmitted from any surface which has been touched by the infected person after sneezing and coughing.[10] The asymptomatic and undiagnosed patients are a real concern not for the general public but health care workers as well if HCW (healthcare worker) are not provided with proper PPE (personal protective equipment), they may become an easy victim of the disease.[11,12]

There is a dire need to gather molecular data of the disease for a better understanding of disease progression and to formulate more effective policies based on available information. Scanty epidemiological data from Pakistan are available, particularly at the molecular level. The present study reports the epidemiological development of SARS-CoV-2 in Islamabad, the federal capital of Pakistan.

Methodology

This cross-sectional study was conducted from April to July 2020, at the Molecular Diagnostic Laboratory, PAEC General Hospital, Islamabad, Pakistan. All samples were received from the collection center of COVID-19 sample collection center of PAEC and reference collection centers of affiliated hospitals across the province Punjab and Islamabad Capital Territory, Pakistan.The study was approved by the ethics committee of the PAEC General Hospital, Islamabad.

A total of 28,274 nasopharyngeal swabs were collected from symptomatic and asymptomatic individuals. All samples were stored in VTM media, processed, and analyzed at the central Molecular Diagnostic Laboratory.

RNA was extracted using both automated and manual extraction platforms as per the manufacturer's instructions. Manually RNA was extracted using Quick RNATM Viral Kit (ZYMO Research Cop) and Viral Nucleic Acid Extraction Kit (Favrogen Biotech. Corp). The automated platform used for RNA extraction with recommended kits included Verscent®kPCR Molecular system (SIEMENS Corp), Lab Aid Zeesan (Xiamen Zeesan Biotech. Co Ltd), and Ascend Hero 32 (Luoyang Ascend Biotech. Co. Ltd).

Multiple qualitative reverse transcription real-time PCR kits for the identification of SARS-CoV2 were used. These kits included (Kit 1) Real-Time Fluorescent RT-PCR Kit for detecting 2019-nCoV by BGI China (IVD CE marked; Catalogue No. MFG030010), takes ORF 1ab gene as the target domain, (Kit 2) qRT128 PCR for Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing, 129 IVD marked) by Sansure Biotech (Sansure Biotech Inc China, Ref No. S3102E) 130 novel coronavirus (2019-nCoV) ORF-1 gene and a conserved coding nucleocapsid protein N131 gene as the target regions as per manufacturer protocol.

For statistical analysis, the chi-square test was applied using IBM SPSS version 22.0 (SPSS Inc., Chicago, IL), and a p-value of less than 0.05 was considered significant.

Results

Among 28, 274 screened individuals, males were 25,485 (90.14%) and 2789 (9.86 %) were females. The mean age of participants was found to be 46.27 years ± 6.33. The results of molecular screening tests with respect to gender and age group distribution with positive and negative samples in both gendersare given in Table I.

Our study showed that the overall prevalence of positive individuals was 1722 (6.09%). The data analysis also revealed that the male population had a prevalence of 2.76% while the female population group exhibited a comparatively high prevalence of 13.44%.

Among positively detected males the age group 31-40 years had most patients 424 (31.47%) followed by the age group of 41-50 years with 306 (22.71%). Similarly, among females, the majority of samples from age group 31-40 years with 91 (24.66%) followed by 41-50 years of age group with 70 (18.66%) confirmed cases. In both genders, the lowest contribution of positive samples was reported in the age group from >80 years with confirmed 05 (0. 371%) and 02 (0.533%) samples respectively in both genders. However, within this age group of >80, the positive sample rate was 22.72% and 7.69% concerning male and female gender. The graphical representations of samples percentage within groups and the contribution of each group percentage to overall positive detections are shown in figure 1.

Table 1: Positive and negative results in both genders as per their age groups.

Gender of the patients			Results		p-value	Total
			Negative	Positive		
Male	Age Groups	≤10	246	25	0.06	271
		11-20	284	25	0.12	309
		21-30	6827	252	0.03	7079
		31-40	8148	424	0.31	8572
		41-50	5267	306	0.43	5573
		51-60	3020	215	0.38	3235
		61-70	241	71	0.21	312
		71-80	88	24	0.72	112
		>80	17	5	0.05	22
	Total		**24138**	**1347**	**0.61**	**25485**
Female	Age Groups	≤10	113	21	0.06	134
		11-20	175	29	0.35	204
		21-30	575	63	0.77	638
		31-40	807	91	0.49	898
		41-50	306	70	0.34	376
		51-60	222	58	0.51	280
		61-70	130	30	0.24	160
		71-80	62	11	0.67	73
		>80	24	2	0.56	26
	Total		**2414**	**375**	**0.74**	**2789**
Total	Age Groups	<11	359	46		405
		11-20	459	54		513
		21-30	7402	315		7717
		31-40	8955	515		9470
		41-50	5573	376		5949
		51-60	3242	273		3515
		61-70	371	101		472
		71-80	150	35		185
		>80	41	7		48
	Total		**26552**	**1722**		**28274**

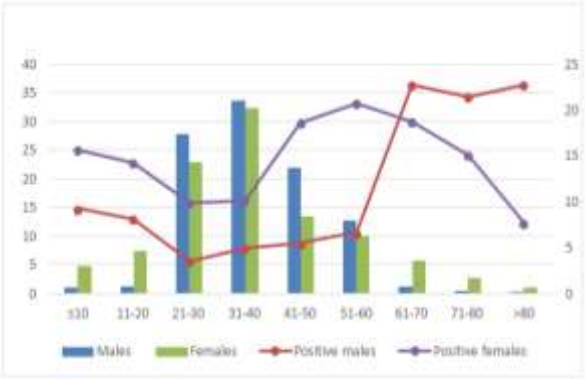

Figure 1: The bar graphs are showing the overall percentage contribution of samples from each group of males and females while the lines are showing the positive detection percentage within that particular group.

Discussion

The first detection of SARS-CoV-2 in humans was made by processing the samples obtained from human bronchoalveolar lavage (BAL) based upon real-time RT-PCR assay that used pan-betacoronavirus degenerate primers. The primers were specifically designed for highly conserved RdRp region.[13] With the global spread of SARS-CoV-2, it was very much essential to screen more and more people to confirm potential cases with reliable and faster techniques.[14] The real-time PCR based tests gives qualitative identification of SARS-CoV-2' by targeting it's RNA.[15] It is well established that the spread of SARS-CoV-2 requires consistent monitoring and the virus may get transmitted from asymptomatic and even presymptomatic infected persons to other healthy individuals.[16] These features of the virus make it very difficult to ensure absolute control of spread however to

acquire the best insight are more screening and identify susceptible age groups and gender.[17] It is very critical to determine the age characteristics of detected individuals as it becomes a tool to design interventions and precautionary measurements to control the disease at local and ultimately global level.[18] The age-related complications of COVID-19 are well reported.[19]

The current study represented the molecular epidemiological data of SARS-CoV-2 in samples collected from province Punjab and capital territory Islamabad Pakistan, till July 2020. Our findings, together with early evidence represent that there is a direct relationship between the age in susceptibility and related clinical manifestation succeeding infection with SARS-CoV-2.

In our study, female samples were found with a higher positive detection rate (13.44%) as compared to the male individuals (2.76%). These results are contradictory to previously reported results from the same province in another study that reported a higher prevalence in males as compare to females *i.e.,* the prevalence of 77.2% in males and 28.2% in females' samples.[20] The possible reasons may include a small number of females samples as compared to males or females only approached hospitals for screening purposes when they suffered from reported symptoms of the disease. The difference between screened samples of males and females is also noticeable which is resulting in less prevalence in males. Generally, in Pakistan males were tested in large numbers as compared to females.[9] The males are exposed more to an open environment than females and also in some organization's males were obligated to go through COVID-19 testing before joining offices/workplaces. Collectively the age groups from 31-50 years have shown a maximum number of patients in both genders. In this age group slot, our data is incoherence reported from other countries.[21] In this study the children of both genders have shown a prevalence of 12.5% which is following the study reported by Jiatong*et al*., stating that there is an increase in positive detections with an increase in children age.[22] The higher ratio of the children maybe because most of the patients belonged to families that were already infected with infection. It is also important to mention that overall children comprised 0.2% of total samples. The expression COVID-19 related symptoms in children are not much prominent as in elders so most of the time they are not screened or seek any clinical support thus most of the time they remain undetected.[23] Our study has also found that the age group >80 in male and female samples made less than 1% of total detections. However, within the same group, they are exhibiting a positive detection rate of 22.72% and 7.69% respectively in the male and female gender. When we determine the overall sample ratio of the age group in the general population that is only 0.8% and 0.9% respectively. A study reported from Spain mentioned a 6% positivity rate in patients older than 75 years of age.[24] A collective data reported from six countries including China, Japan, Italy, Singapore, Canada, and South Korea mentioned that there was a 69% rate of infection in the age group as crosses from 70 years.[25]

It is also important for SARS-CoV-2 infection victims' age and gender-based data from other regions of Pakistan to compare it with the global statistics so that a comprehensive analysis may be made as a way forward to meet this challenge.

Conclusion

Our findings have indicated a 6.09% prevalence of SARS-CoV-2 in samples collected by the end of July 2020, from Punjab province and the federal capital territory of Islamabad, Pakistan. The age-dependent characteristics in infection statistics are very imperative and also provide a pavement to design any intervention leading to control and decrease infection burden in the population at large. The government has taken many measures to control the spread of infection that included imposing strict lockdown at the start of the pandemic as to smart lockdown in areas of high prevalence rate as per guidelines issued by the World Health Organization. These actions have yielded satisfactory results and there is a decline in new cases. However, re-emergence of infection and localized cluster outbreak of COVID-19 infection cannot be neglected. This is a need of time that more data from Pakistan may be published and used to formulate decisive parameters considering any future threat. Moreover, it is also indispensable to conduct further genetic analysis of SARS-COV-2 to determine any mutations in existing strains and phylogenetic characteristics of circulatory strains in Pakistan.

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