Original Article

VIRAL SHEDDING PATTERN AND CLINICAL FEATURES OF PAKISTANI COVID-19 PATIENTS

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ABSTRACT

Objective: This study was aimed to analyze the pattern of viral shedding and correlate it with clinical demographic characteristics.

Material and Methods: The nasopharyngeal swabs of suspected patients with COVID-19 were collected. The clinical symptoms and medical history were recorded. RNA was detected by using Real-time PCR for N and Orf1b genes. The viral load was determined by the CT method.

Results: Out of a total of 2000 patients, 400 (20%) were found to be positive. Only 22.5% (90/400) patients had variable clinical symptoms, 77.5% (310/ 400) were asymptomatic. Fever, fatigue and loss of taste were the common symptoms in in-patients. Majority of the patients, 52%, were between 21 to 40 years and had viral load between CT values 21 to 30. Male gender was predominant in all the age groups except the oldest age group above 60 years. Orf1b based assay was more sensitive to detect SARS CoV-2 as compared to N gene.

Conclusion: This is the first report from Pakistan describing that males of intermediate age were more prone to COVID-19 infection and the Orf1b gene was more sensitive for diagnosis. The findings may be helpful for diagnosis and management of COVID-19 patients.

Key Words: SARS CoV-2, Viral shedding, Clinical characteristics.

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INTRODUCTION

The corona virus disease 2019 (COVID-19) has claimed over 2.7 million lives across the globe and has infected more than 100-million-world population. Pakistan reported its first COVID-19 patient in February 2020, since than 0.9 million confirmed cases and over 20,000 deaths have been reported across the country [1]. The progression of the infection, clinical severity and mortality rate is low in Pakistani region as compared to Europe and America. Several theories and scientific reasons have been postulated to underline the scientific link responsible for the low clinical severity and mortality of COVID-19 in this region. The pattern of infection and course of pathogenicity of COVID-19 is still not clear. The variation of the viral load and detection of different viral genes at different stages of infection may be helpful to understand and correlate the viral detection and disease progression.

The SARS-CoV-2 is RNA zoonotic corona virus, rapidly evolved in the humans and caused COVID-19 disease [2,3]. It is composed of a single positive stranded genome of 30Kb long RNA, containing nucleoprotein capsuled in a protein matrix. The viral genome encodes 9860 amino acids, which form different functional and structural proteins [4]. Its genome is organized into 5 distinct regions in the following order; 5' replicase (ORF1a/b)-spike (S)- envelope (E)-membrane (M)-nucleocapsid (N)-poly(A)-3 [5,6]. The RT-PCR (Real-time Polymerase Chain Reaction) based testing of viruses is based on the detection of highly conserved regions of the genome for the maximum specificity. It may include the structural S and N genes and non-structural RdRp and Open Reading Frame (ORF) 1a/b genes [6,7]. The sensitivity and specificity of different target genes of SARS-COV-2 virus is variable [6]. The patients diagnosed with different stages, the severity of infection and the results of RT-PCR based detection of targeted genes are also variable. This study has been proposed to correlate the viral load of SARS-COV-2 based on two targeted genes, Orf1b and N, with the demographic clinical findings of the patients. The findings may be helpful to find any link with the viral load of specific gene and stage of infection.

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MATERIAL AND METHODS

A total of 2000 suspected patients of COVID-19, belonging to different areas of Sindh province were enrolled at Diagnostic and Research Laboratory, Liaquat University of Medical and Health Sciences, Jamshoro. The nasopharyngeal swabs were collected from deep nasal cavity and stored in tubes containing Viral Transport Medium (VTM) as per World Health Organisation guidelines. The

demographic characteristics including age and sex were recorded. The clinical presentation including the onset of the disease and initial symptoms of the infection were noted on a preformed questionnaire.

Total RNA was extracted from all the collected swabs by using the Qiagen Viral RNA extraction kit as per manufacturer's instructions. The 10ul of extracted RNA was used for further detection of SARS CoV-2 RNA. One Step RT-PCR was used to amplify two targeted genes, ORF1b and N gene of COVID-19. Both target genes were labelled with two different fluorophores, FAM and ROX, for simultaneous detection. In addition, an exogenous internal control labelled with VIC dye was also amplified in multiplex master mix to validate the RT-PCR reaction and to rule out presence of any inhibitors in the reaction. The RT-PCR reaction was performed as per kit manufacturer's instructions. The assay was performed on Quant Studio 5, Real-Time Thermal cycler by Applied Biosystems (USA). The CT values of both target genes were recorded and the viral load was determined by CT values, which was inversely proportional to viral load [8].

The mean and median values were calculated for all the continuous variables and categorical variables were presented as frequency and percentages. The p-value <0.05 was considered significant. The statistical analysis was performed by using SPSS ver 17.

RESULTS

Total 2000 patients were tested for SARS CoV-2 and 20% (400/2000) were found positive. Major proportion of the positive patients, 77.5% (310/400) were asymptomatic, whereas 22.5% (90/400) patients had mild to moderate symptoms. Only two patients were admitted in ICU but stabilized after necessary interventions. The median age was 35 years (IQR, 27-45, Range 02-80) and 60.5% (242/400) were males and 39.5% (158/400) were females. The common clinical symptom was fever along with fatigue and loss of sense of taste (Table-I). Majority of the patients, 53.5% (214/400), were in their third and fourth decade of life, ranging from 21 to 40 years, followed by 26.75% (107/400) patients included in the age group from 41 to 60 years. It is noteworthy that, only 7.25% COVID-19 infected patients were over 60 years. Further, it was noticed that male gender predominates in all the age groups except the oldest age group above 60 years (Table-I). There were 131/214 males in the largest group of patients aged from 21 to 40 years, whereas 16/29 were females in the group of patients having age above 60 years.

Table-I: Demographic, clinical and PCR test findings of COVID-19 patients.

Demographic Parameters	All patients (n=400)	P Value
Age, Median (IQR) Years	35 (27-45)	
Group Wise Age Breakup		
0 to 20	50 (12.5%))	
21 to 40	214 (53.5%)	
41 to 60	107(26.8%)	
Above 60	29 (7.3%)	
Gender		
Male	242(60.5%)	P<0.05
Female	158(39.5%)	
Clinical Parameters	90/400 (22.5%)	
Fever >=38℃	90/90	
Sputum production	30/90	
Dyspnea	5/90	
Fatigue	90/90	
Cardiopulmonary disease	0	

PCR test findings	Mean CT Values	
	ORF1b gene	N gene
1st PCR test	25(n=400)	25(n=400)
2nd PCR test after 10 days	29(n=80)	30 (n=80)
3rd PCR test after 20 days	32(n=8)	34(n=8)

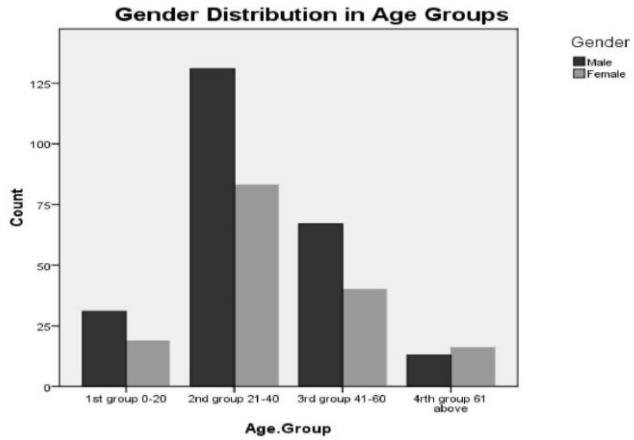
The patients found positive on first RT-PCR test for SARS CoV-2 were quarantined as per standard procedure and were retested for the active infection after 10 days. The 80% (320/400) patients became negative on retesting by second RT-PCR test, where as 20% (80/400) were remained positive and quarantined for further period and subjected to 3rd RT-PCR test after 20 days. Seventy-two patients, 17.5% (72/400) became negative on third RT-PCR test while 8 patients, 2% (8/400) were positive and remained in isolation for the period over 30 days and became negative on the subsequent RT-PCR tests. Only 2 patients 0.5% (2/400) required extensive care in the ICU and no patient expired in this cohort of COVID-19 positive patients.

The RT-PCR results and viral load showed that ORf1b and N gene both were positive simultaneously in 92.75% (371/400) patients, while 29 patients (7.25%, 29/400) were negative for N gene. It is notable that only 10.75% (43/400) patients had the highest viral load between CT 15 to 20 for Orf1b gene and 13% (52/400) for N gene. The major proportion of the patients 51% (205/400) had moderate viral load between CT 21 to 30, whereas 38.25% (153/400) patient had the low viral load from 30 to 40 CT values of ORF1b1 gene and 27% (108/400) of N gene. The details of CT values in different age groups of COVID-19 patients have been described in Table-II.

Table-II: Cycle threshold of ORF1b and N genes in different age groups.

Age Group	CT. ORF1b. Gene				CT N Gene				
Years	15-20	21-30	31-40	Total	15-20	21-30	31-40	Neg	Total
1st group 0-20	3	32	15	50	7	31	8	4	50
2nd group 21-40	27	97	90	214	29	108	62	15	214
3rd group 41-60	10	58	39	107	12	57	29	9	107
4rth group 61 above	3	17	9	29	4	15	9	1	29
Total	**43**	**204**	**153**	**400**	**52**	**211**	**108**	**29**	**400**

Figure-I: Bar graph showing the gender wise distribution of different age gourps of COVID-19 patients.



DISCUSSION

The World Health Organization has declared COVID-19 as a pandemic, affecting over 100 million people worldwide and has caused 2.7 million fatalities across the globe. The Pakistani COVID-19 infected patients have demonstrated early recovery, less clinical severity and low mortality rate as compared to the patients of USA and Europe. The risk factors, pathogenic effects and associated symptoms along with comorbidities of COVID-19 are not clear. It varies among patients belonging to different ethnicities and geographical regions. This study was aimed to study the demographic credentials, clinical course and results of RT-PCR test findings of COVID-19 patients.

The findings of our cohort of patients reveal that major proportion of the patients is below 60 years. Moreover, significant numbers of patients were asymptomatic and did not develop any pathogenicity till the clearance of virus. Only 22.5% (90/400) patients developed COVID-19 related symptoms, Fever, fatigue and loss of taste and smell were consistent among all symptomatic patients (Table -I). It is also noteworthy that, 90% became negative on second RT-PCR test after 10 days of confirmation of infection. Previously, the patients with old age, greater than 60 years, possibly having weak immunity, been found more prone to COVID-19 infection [9-11]. The differences of clinical presentation may be due to genetic differences of immune related genes of the population. It needs further studies to delineate the causes of mild and negligible clinical consequences in the patients of the region.

Majority of the COVID-19 patients in our cohort were males 60.5% (242/400) (Table-I). This supports the previous studies, describing the male gender as risk factor for COVID-19 infection [10,12, 13]. It is interesting to note that males were more infected in all age groups except the patients with age above 60 years, where females were more infected than males (Figure-I). This has not been described previously. Though the number of patients with age above 60 years was not significant in our cohort (7.2%, 29/400), but the ratio of infected females was higher, 55.17% than other age groups. The older females were less resistant to COVID-19 infection and pathogenicity; this finding requires more studies with statistically significant ratio of females to males with age above 60 years for confirmation.

The RT PCR assays are based on the amplification of highly conserved target sequences to attain the maximum specificity [14]. The conserved genomic regions of SARS-CoV-2 includes replicase (ORF1a/b), structural gene spike (S)-envelope and nucleocapsid (N) genes [6]. Dual target RT- PCR assay of SARS CoV-2 showed that Orf1b gene and nucleocapsid N gene are not always positive simultaneously in same patients. Orf1b was detected in all positive patients, whereas N gene was detected in only 92.5% of the same patients (Table-II). Recent studies showed that N gene based assay is 10 times more sensitive than ORF1b gene assay, thus N gene is more sensitive in detecting SARS CoV-2 assay [15]. This is contradictory to our finding, which emphasizes that only N gene-based assay may not be used for the COVID-19 detection. The Orf1b based assay may be more sensitive and may have priority for the confirmation of COVID-19 infection. Furthermore, majority of the patients, 80% (320/400), became negative after 10 days of infection and all the patients became negative on 4th RT-PCR test after

30 days. The mean CT values of remaining positive patients were gradually increasing for both target genes (Table-I).

This is the first report describing the dynamics of RT PCR findings, clinical and demographic characteristics of Pakistani COVID-19 patients. The majority of the patients were below 60 years and without the COVID-19 symptoms. This is opposite to the findings from other populations and need further studies to understand the underlying causes. These findings may be helpful to better understand the course of pathogenicity and clinical outcome of COVID-19 patients.

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AUTHOR CONTRIBUTION

Ikram Din Ujjan: Designed the study and helped in critical review.

Yar Muhammad Waryah: Drafting and collection of data.

Ali Raza Rajput: Performed laboratory tests.

Ali Muhammad Waryah: Designed the study and helped in critical review.

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