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Research Article: Target Testing and Specificity of Nucleic Acid Based Diagnostics for COVID-19

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Abstract

Objective: The seek of this study is to provide an indication on the features of diagnostic testing of SARS-CoV-2 by RT-PCR, including parameters of sensitivity, specificity, positive and negative likelihood ratios.

Background: Coronavirus Disease is the fifth international emergency after 1918 Spanish flu pandemic, triggered by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2). On 30 January the WHO acknowledged COVID-19 to be a global health disaster of international importance and a pandemic on 11 March 2020. In vitro analyses of the data shows that for SARS-CoV-2 the RT-PCR test is highly specific, as it is not counter react with nucleic acid of other viruses.

Methods: Oropharyngeal and nasopharyngeal swabs were collected into a 3 ml viral transport media (VTM) and transported to Laboratory. Extraction of the viral RNA was done by Qiasymphony DSP Virus/ Pathogen mini kit (Qiagen GmbH, Germany). For amplification process of RT-PCR qualitative detection of SARS-CoV-2 RNA utilizing with SYSTAAQ 2019-Novel Coronavirus (COVID-19) Real time PCR kit using a BIORAD-CFX 96.

Results: Out of 15,049, 3195 samples were positive for covid-19 qPCR. Ratio of the Males patients were greater than females. 63.7% males and 36.3% females were effected with Covid-19. Symptom wise analysis shows 62% patient were asymptomatic, 22.7% mild, 1.7% moderate, 12.7% stable, 0.6% severe and 0.2% were critical. Our analysis reveals age group 1 (4.9%), group 2 (55.5%), group 3 (27.5%), and group 4 (12.1%) were effected with SARS-nCoV-2. Our result shows 3.0 % patients were deceased and 97% were recovered.

Conclusion: Our findings contribute to the evolving understanding of the sophisticated interaction between this emerging SARS-CoV-2 virus and nucleic acid based target testing of COVID-19.

Keywords: Pandamic, Covid-19, RT-PCR, RNA

Introduction: Coronavirus Disease is the fifth international emergency after 1918 Spanish flu pandemic, triggered by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2)(1). SARS-CoV2 known as COVID-19 has done significant harm to the public health and the environment across the globe (2). In the last two years, the rise of coronavirus-associated infections, Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome (MERS and SARS) have raised global threats to the public health services (3). The most recent addition to this emerging list of novel agents is the SARS-CoV-2 (that is the causal agent for coronavirus disease COVID-19) (4).SARS-CoV-2 can easily spread between humans and has a strong potential for pandemic (5-7). In comparison, the strong propagation capacity of SARS-CoV-2, the development and ease of global travel may make the problem worse (8). On 30 January the WHO acknowledged COVID-19 to be a global health disaster of international importance and a pandemic on 11 March 2020.
Coronaviruses are small in size (65–125 nm in diameter) and carry a single-stranded RNA as a nucleic material, varying in size from 26 to 32kbs in length. Coronavirus subgroups include alpha (α), beta (β), gamma (γ) and delta (d) coronavirus. Coronaviruses that are associated with human diseases belong to the alpha- or

the beta- types. Numerous of these Coronaviruses can often affect a variety of animal species. In 2002, SARS-Coronavirus infected humans and infected civet cats were found and in 2012 MERS-Coronavirus is found in infected humans and in dromedary camels. It is accepted that SARS-Coronaviruse-2 has animal origin and is not a constructed or manipulated virus. The ecological reservoir of SARS-CoV-2 virus are bats (9).It is revealed by whole genome sequence study that the novel virus collected in Yunnan province, China, has 96.2% similarity with bat SARS related coronavirus (SARS-CoV; RaTG13) (10, 11), but has little similarity to that of SARS-CoV (about 79%) or MERS-CoV (about 50%)(12, 13).

During the COVID-19 pandemic, Health Ministry of Pakistan was working to enhance the testing capabilities for this virus so that this could help in rapid diagnosis. The most frequently used test for SARS-CoV-2 detection is a nasopharyngeal swab to identify viral RNA by using a reverse transcriptase-polymerase chain reaction (RT-PCR). In vitro analyses of the data shows that for SARS-CoV-2 the RT-PCR test is highly specific, as it is not counter react with nucleic acid of other viruses (14). Correspondingly, the in vitro RT-PCR sensitivity is high, but in diagnosing COVID-19 the sensitivity of the nasopharyngeal RT-PCR swab is uncertain. For the detection of SARS-COV-2 real-time RT-PCR test provides both sensitive and specific method, including diagnosis protocols such as the sequences of target primer exist in the World Health Organization public database (15). On the other hand, if the quantity of the viral genome is inadequate or sample not be collected at right time then this test can give false results (16). The seek of this study is to provide an indication on the features of diagnostic testing of SARS-CoV-2 by RT-PCR, including parameters of sensitivity, specificity, positive and negative likelihood ratios.

Methods

This study was performed at Molecular Research Laboratory, Lahore General Hospital Lahore. We collect the data of all patients (n=1949) from electronic health record who underwent PCR testing for COVID-19 between April 28, to Aug 07, 2020.

Sample collection

Healthcare workers were collected the oropharyngeal and nasopharyngeal swabs and put into a 3 ml viral transport media (VTM) and transported to Molecular Research Laboratory, Lahore General Hospital, Lahore. A volume of 200 µl of the sample was additionally run for viral nucleic acid extraction by Qiasymphony DSP Virus/ Pathogen mini kit (Qiagen GmbH, Germany) as per the producer's protocol in elutes of 50 µl each (17). Each sample was exposed to the addition of 10µl of extraction control (EAC) at the time of extraction itself, to check the validity of the extraction procedure.

Performance of RT-PCR in the laboratory

For the qualitative detection of SARS-CoV-2 RNA utilizing with SYSTAAQ 2019-Novel Coronavirus (COVID-19) Real time PCR kit using a BIORAD-CFX 96, the 5 ul elute/sample was used for amplification process of RT-PCR. In first step reverse transcription was done at 50˚C for 30 minutes. In second step denaturation was done at 95˚C for 3 minutes and in third step 50 cycles of amplification were done at 95˚C for 15 seconds and 60˚C for 60 seconds by using FAM channel for B gene and HEX channel for Internal Control (6). This kit Target the detection of both A gene (SARS) and B gene (nCoV-2). Separate regent vials are provided within kit for A gene and B gene detection. The PCR mix preparation details are given in Table 1 and PCR reaction condition details are in Table 2. This study received institutional review board (18) approval.

Table 1. PCR Mix Preparation

Contents	For A gene	For B Gene
nCov BioAmp RT Mix	10 µl	10 µl
nCov BioAmp RT Enzyme	2 µl	2 µl
nCoV BioAmp Enhancer A	1.5 µl	-
nCoV BioAmp Enhancer B	-	1.5 µl
nCoV BioAmp Enhancer C	1.5 µl	1.5 µl
nCoV BioAmp Enhancer D	1 µl	1 µl
Total =	16 µl	16 µl
Mastermix Volume in a Reaction=	14.5 µl	15 µl
Purified Sample Containing Internal Control =	5.5 µl	5 µl
Total Reaction Volume	20 µl	20 µl

Table 2. PCR Reaction Condition

Temperature	Incubation Time	Cycles
50	30 min	-
95	3 min	-
95	15 sec	50
60	60 sec	

Results

Overall Performance of RT-PCR for SARS-nCoV2:

Total 15,049 samples were performed from different areas of Punjab. Out of 15049, 3195 positive and 11854 were negative. Institute wise negative and positive details are mention in Table 3 and also graphical view is showed in Figure 1.

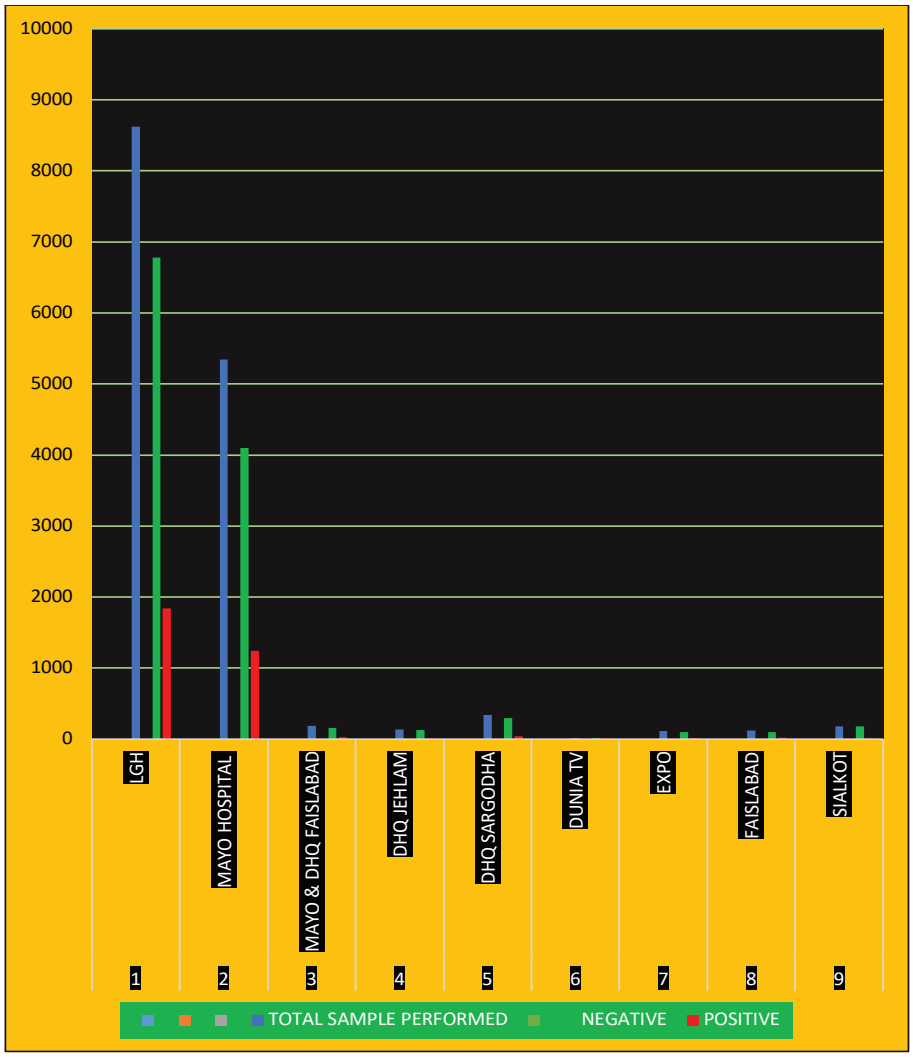

Figure 1. Graphical View of Institute Wise Positive and Negative Samples

Table 3. Institute Wise Positive and Negative

LAHORE GENERAL HOSPITAL COVID PCR LAB TOTAL SAMPLES RECEIVED AND REPORTED TILL TO DATE 27 JULY 2020					
SR:#	NAME OF INSTITUTION	TOTAL SAMPLE PERFORMED	NEGATIVE	POSITIVE	COUNTER CHECKED
1	LGH	8624	6781	1843	8624
2	MAYO HOSPITAL	5344	4102	1242	5344
3	MAYO & DHQ FAISLABAD	184	159	25	184
4	DHQ JEHLAM	133	128	5	133
5	DHQ SARGODHA	338	294	44	338
6	DUNIA TV	15	14	1	15
7	EXPO	112	101	11	112
8	FAISLABAD	118	97	21	118
9	SIALKOT	181	178	3	181
TOTAL		15049	11854	3195	15049

Statistical Analysis

Gender Wise Analysis

Out of 15,049, 3195 samples were positive for covid-19 qPCR. 1947 are follow up cases others are neglected from the data. Ratio of the Males patients were greater than females. In this research 63.7% males and 36.3% females were effected with Covid-19. Gender wise calculation of percentages and frequency are shown in Table 4 & Figure 2.

Table 4. Gender Wise Frequency & Percentage

Gender	Frequency	Percent
Male	1241	63.7
Female	706	36.3
Total	1947	100.0

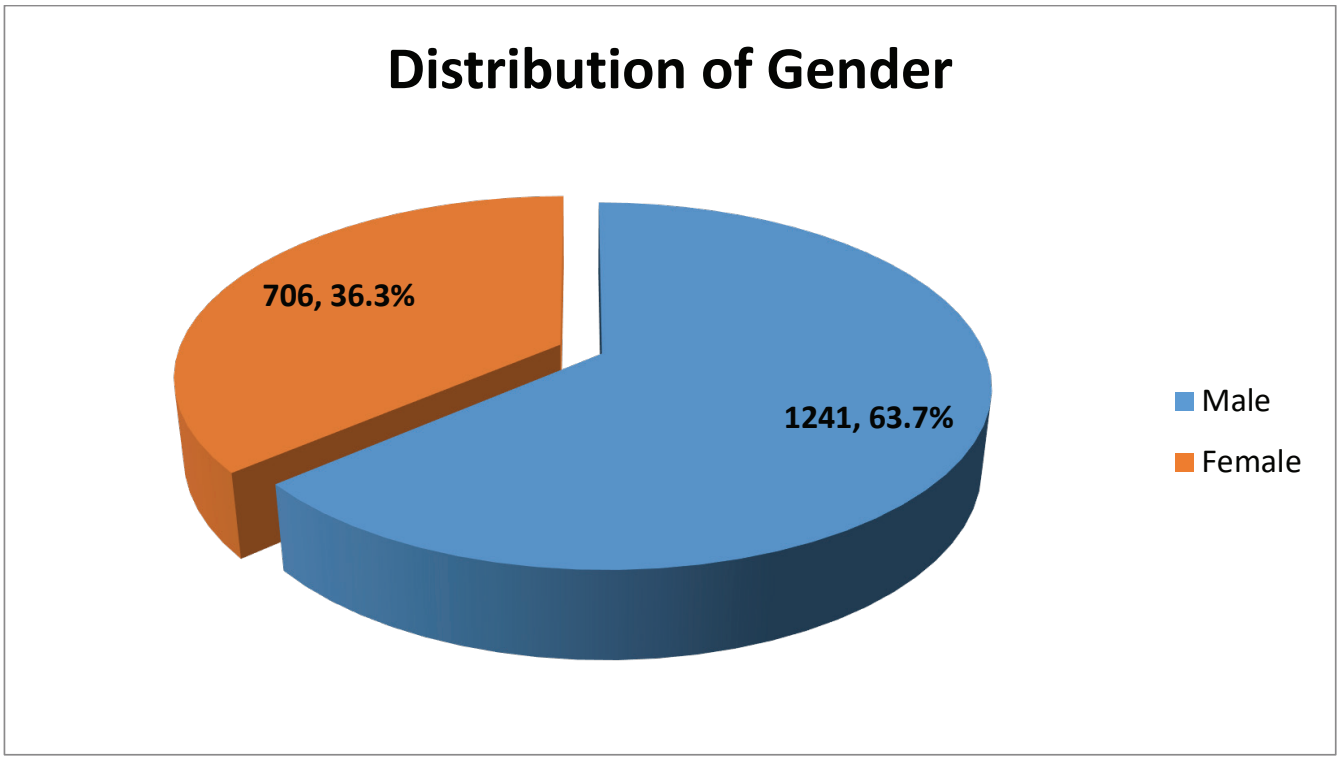

Figure 2. Gender Wise Frequency & Percentage

Condition Wise Analysis

Out of 1947 follow up positive patients, 62% patient were asymptomatic, 22.7% mild, 1.7% moderate, 12.7% stable, 0.6% severe and 0.2% were critical. Symptoms wise calculation of percentages and frequency is shown in Table 5 & Figure 3

Table 5. Symptoms Wise Distribution of Patients

Symptoms	Frequency	Percent
Asymptomatic	1208	62.0
Critical	3	.2
Mild	442	22.7
Moderate	34	1.7
Severe	12	.6
Stable	248	12.7
Total	1947	100.0

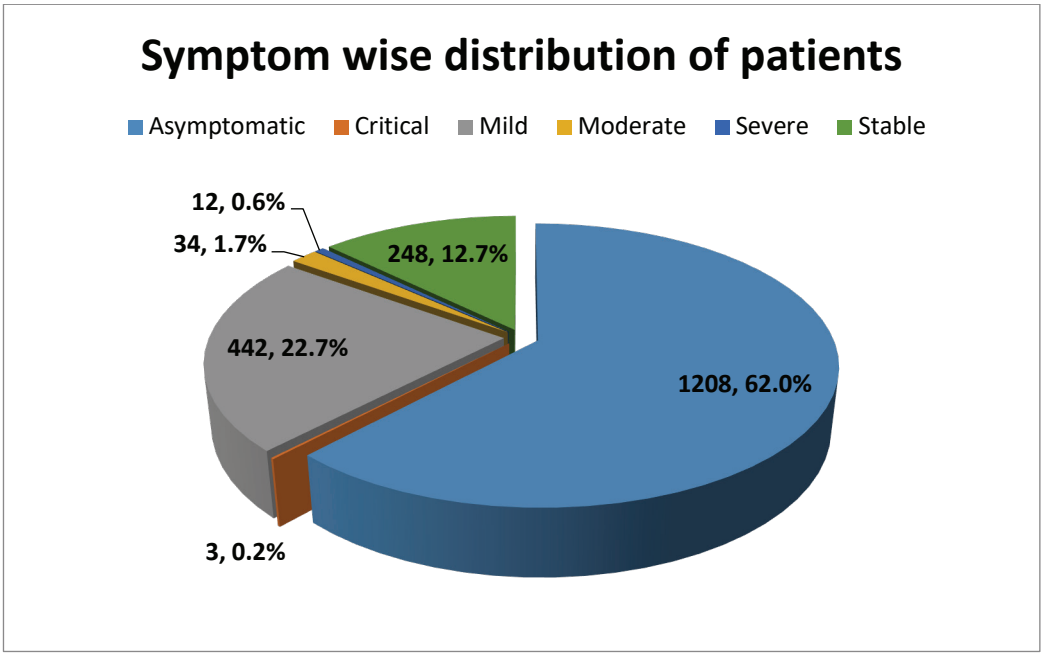

Figure 3. Symptoms Wise Distribution of Patients

Comparison of different age groups

4 Age groups were prepared for comparison of percentages and frequency. Patients <= 20 age were included in group 1, from 21-40 in group 2, from 41-60 in group 3, and > 60 aged patients are included in group 4. Our analysis reveals age group 1 (4.9%), group 2 (55.5%), group 3 (27.5%), and group 4 (12.1%) were effected with SARS-nCoV-2. Results shows that age group 2 are more effected with SARS-nCoV-2 than the other age groups. Percentage of different age groups are shown in Table 6 and frequency are shown in Figure 4.

Table 6. Percentage of Different Age Groups

Age Groups	Frequency	Percent
<= 20	95	4.9
21-40	1081	55.5
41-60	535	27.5
>60	236	12.1
Total	1947	100.0

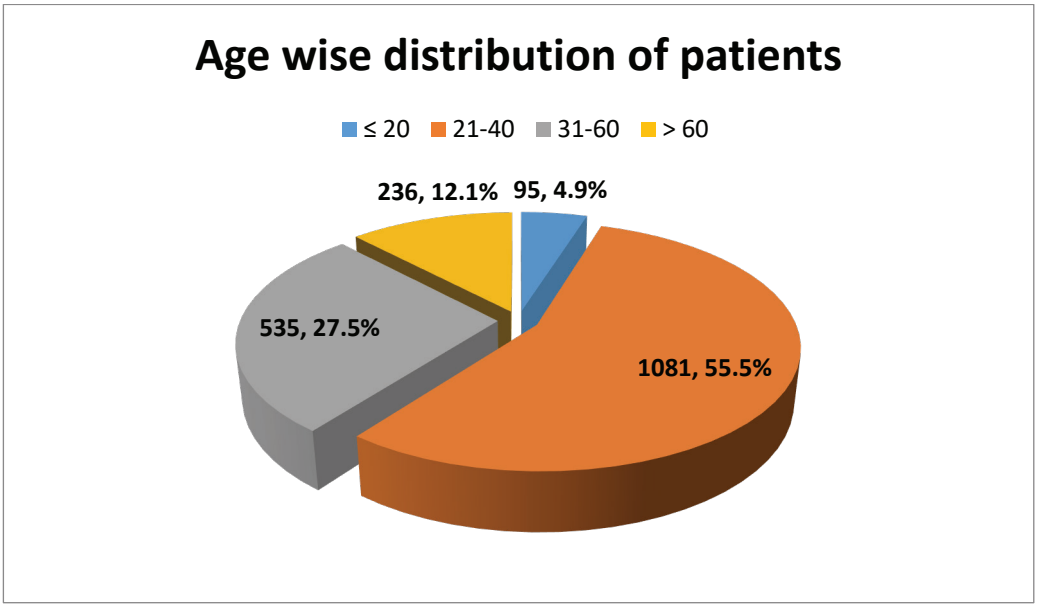

Figure 4. Frequency of Different Age Groups

Recovery & Death Rate Analysis

Total 1947 patients, 1888 patients were recovered, which was about 97 % and 59 patients were deceased which was 3.0 %. Recovery and death based calculation of percentages is shown in Table 7 and Figure 5.

Table 7. Percentage of Death & Recover Patients

Death	Frequency	Percent
Yes	59	3.0
No	1888	97.0
Total	1947	100.0

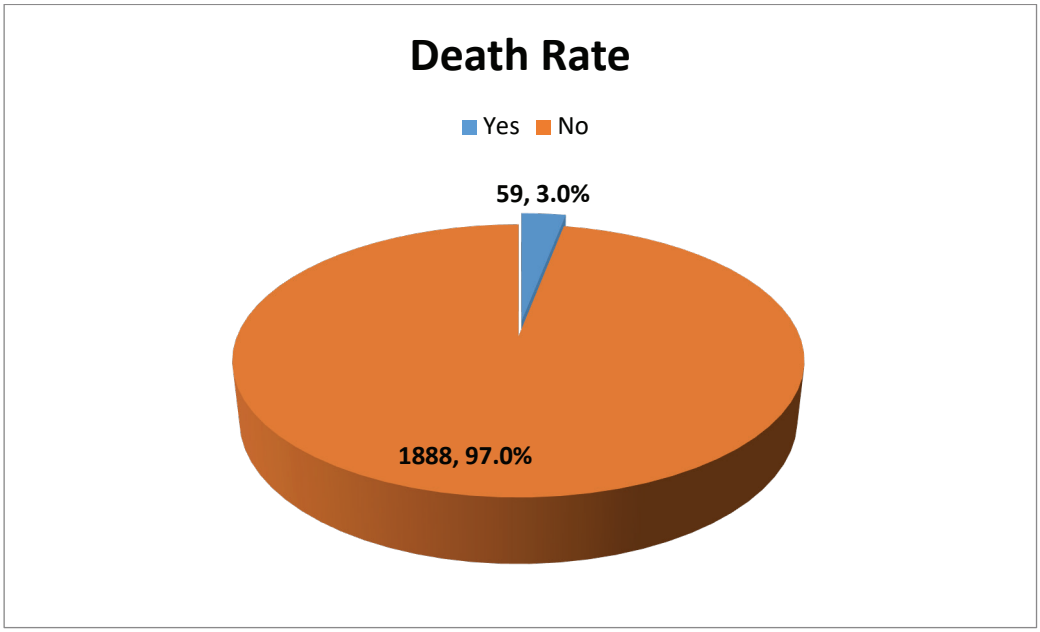

Figure 5. Frequency of Death & Recover Patients

Age and Recovery Rate

Our results shows that total 1947 patients were effected with COVID-19. Male age was higher as compared to female age. Minimum two and maximum 94 years ages were seen. Out of 1947 patients 1858 were recovered. Minimum recovery was 09 days and maximum recovery rate was 40 days. Descriptive statistics with standard deviation are shown in Table 8. Male patients average age was 40 years and maximum were recovered in 21 days, while female average age was 38 years and recovered in 20 days. Gender vs Age and gender vs recovery days are mention in Table 9.

Group Statistics

	Gender	N	Mean	Std. Deviation	p-value
Age	Male	1241	40.74	15.585	0.001*
	Female	706	38.30	15.926	
Recovery	Male	1186	21.12	6.724	0.001*
	Female	672	20.07	6.830	

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Age (years)	1947	2	94	39.85	15.749
Recovery (days)	1858	9	40	20.74	6.780

Gender and Death Correlation

Our results shows that male patients were more deceased then females. Out of 59 deaths, 39 were males and remaining 20 were females. Statistical analysis shows that p-value is 0.701. No Significant difference was observed or no significant association was observed between gender and death. Gender and death cross tabulation are shown in Table 10.

Table 10. Gender and Death Correlation

Gender * Death Cross tabulation

			Death		Total	p-value
			Yes	No		
Gender	Male	Count	39	1202	1241	0.701
		% within Gender	3.1%	96.9%	100.0%	
	Female	Count	20	686	706	
		% within Gender	2.8%	97.2%	100.0%	
Total		Count	59	1888	1947	
		% within Gender	3.0%	97.0%	100.0%	

Correlation of Gender and Different Age Groups

Statistical analysis shows that significant difference was observed or significant association was observed between age and death. Death rate was higher in elder age group as compared to young age group. Age group and death cross tabulation are shown in Table 11.

Table 11. Age group and Death Correlation

Age_cat * Death Crosstabulation

			Death		Total	p-value
			Yes	No		
Age_cat	<= 20	Count	1	94	95	< 0.001*
		% within Age_cat	1.1%	98.9%	100.0%	
	21-40	Count	5	1076	1081	
		% within Age_cat	0.5%	99.5%	100.0%	
	41-60	Count	23	512	535	
		% within Age_cat	4.3%	95.7%	100.0%	
	>60	Count	30	206	236	
		% within Age_cat	12.7%	87.3%	100.0%	
Total		Count	59	1888	1947	
		% within Age_cat	3.0%	97.0%	100.0%	

Correlation of Different Age Groups and Recovery Days

Statistical analysis shows Significant difference was observed or significant association was observed between age and recovery rate. The mean recovery days was higher in elderly age group (41-60 & > 60 years as compared to young age group (≤ 20 and 21 – 40). Age group and recovery days are shown in Table 12.

Table 12. Age Group and Recovery day's correlation

Age group	N	Recovery Days Mean	Std. Deviation	Recovery Days Minimum	Recovery Days Maximum	p-value
≤ 20	88	14.28	1.575	9	21	< 0.001
21-40	1052	16.43	3.077	14	21	
41-60	512	25.96	3.414	14	30	
>60	206	32.50	4.212	30	40	
Total	1858	20.74	6.780	9	40	

Discussion

Covid disease is a developing worldwide wellbeing concern and has infected a critical segment of the total populace. In this study, we explored target testing and particularity of novel COVID-19 RT-PCR. The COVID-19 disease is growing to more than 210 nations and domains. It has contaminated 30,701,535 individuals, and has caused 956,500 (4%) passing's during the period December 29, 2019 to September 19, 2020.

The advancement of new molecular techniques relies upon the information about the structure of protein and genetic composition of the virus or changes in protein expressions during and after interaction with host (19). To design primers and probes for polymerase chain reaction and other molecular tests genome sequencing is important. COVID-19 virus has a Single stranded RNA which is positively charge genome having about 30,000 nucleotides that encodes 27 proteins (19, 20).

During the pandemic, new different RT-PCR kits were discovered for the detection of Coronavirus having the capability to intensify a limited quantity of virus in a sample (21). In this RT-PCR, viral single stranded RNA reverse transcribed in presence of reverse transcriptase enzyme into double stranded complementary DNA strands (cDNA), after cDNA formation amplification process start and specific regions are amplified. The cycle for the most part includes 2 principle steps, the first step involve primer design and sequence alignment, and the second step involve assay optimization and testing, especially because this method requires several temperature changes for each cycle using thermocycling equipment (19).

15049 samples tested from different districts of Punjab from which 3195 sample were positive whereas 11854 were negative. Out of which 63.7% were male, while female positive ratio was 36.3%.This is significantly higher than a study from Wuhan, China, which indicated that 56% of patients with COVID-19 were males (22). Similarly, another study of 140 patients from Wuhan found that 50.7% were males (23). I this study, we enrolled 1947 follow up positive cases that shows males are more affected then females. As indicated by sharma. et al, the MERS & SARS-CoV, were found to infect more men than women (24). In a mouse model study of SARS-CoV infection, male mice were more susceptible to infection than female mice. The enhanced susceptibility of male mice to SARS-CoV correlated with a moderate increase in virus titer and extensive alveolar macrophages and neutrophil amassing in the lungs (25). In this research we found 62% as asymptomatic consistent with another study from east Karachi by Tahir, Shumaila et al. (26) while 22.7% were suffering with mild disease of COVID-19, whereas moderate were only 1.7% and severity of the disease found in 0.6% only. While 12.7% were stable.

Another object of the study was age wise distribution in which we found 4.9% less than 20 years while majority of the cases fall in age group of 21 – 40 years, that is 55.5%, after that 41 – 60 years age positive cases were 27.5% that is 2nd highest. Whereas more than 60 years cases were 12.1%. Severity of the disease mostly fall in this age group.

In this research we found death rate 3.0% whereas 97.0% cases were recovered. As mean value was 39.85 and SD was 15.749 whereas recovery rate mean was 20.74 with SD 6.780.

Statistical analysis show the clear picture of age wise p-value 0.001 that is significant. p-value of all patients of recovered cases was 0.001 that is highly significant.

So, our study focused on group of early and particular diagnosis of COVID-19 by RT- PCR within 6 hours could lead to critical management of COVID suffering patients. We found very high significant ratio of cured and recovered patients, although death rate of the patients was 3.0% with p-value 0.701 that is very non-significant.

Conclusion

Collectively, our study of target testing and specificity of nucleic acid based diagnostic of COVID-19 patients indicated that males from age group 2 (21 to 40 are more affected with asymptomatic condition. Our results reveals that death rate was 3% and recovery rate was 97%. Our findings contribute to the evolving understanding of the sophisticated interaction between this emerging SARS-CoV-2 virus and nucleic acid based target testing of COVID-19.

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Conflict of Interests

Authors declare no competing interests.

References

1. Liu Y-C, Kuo R-L, Shih S-R. COVID-19: The first documented coronavirus pandemic in history. Biomedical journal. 2020.
2. Meo SA, Al-Khlaiwi T, Usmani AM, Meo AS, Klonoff DC, Hoang TD. Biological and epidemiological trends in the prevalence and mortality due to outbreaks of novel coronavirus COVID-19. Journal of King Saud University-Science. 2020.
3. De Wit E, Van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nature Reviews Microbiology. 2016;14(8):523.
4. Of the International CSG. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nature Microbiology. 2020;5(4):536.
5. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet. 2020;395(10223):497-506.
6. Carlos WG, Dela CC, Cao B, Pasnick S, Jamil S. Novel Wuhan (2019-nCoV) Coronavirus. American journal of respiratory and critical care medicine. 2020;201(4):P7.
7. Munster VJ, Koopmans M, van Doremalen N, van Riel D, de Wit E. A novel coronavirus emerging in China—key questions for impact assessment. New England Journal of Medicine. 2020;382(8):692-4.
8. Biscayart C, Angeleri P, Lloveras S, Chaves TdSS, Schlagenhauf P, Rodríguez-Morales AJ. The next big threat to global health? 2019 novel coronavirus (2019-nCoV): What advice can we give to travellers?–Interim recommendations January 2020, from the Latin-American society for Travel Medicine (SLAMVI). Travel medicine and infectious disease. 2020;33:101567.
9. Sahin AR, Erdogan A, Agaoglu PM, Dineri Y, Cakirci AY, Senel ME, et al. 2019 novel coronavirus (COVID-19) outbreak: a review of the current literature. EJMO. 2020;4(1):1-7.
10. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. nature. 2020;579(7798):270-3.
11. Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Sourvinos G, Tsiodras S. Full-genome evolutionary analysis

of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. Infection, Genetics and Evolution. 2020;79:104212.
12. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 2020;395(10224):565-74.
13. Gralinski LE, Menachery VD. Return of the Coronavirus: 2019-nCoV. Viruses. 2020;12(2):135.
14. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 2020;25(3):2000045.
15. Wang Y, Kang H, Liu X, Tong Z. Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. Journal of medical virology. 2020;92(6):538-9.
16. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. New England Journal of Medicine. 2020;382(12):1177-9.
17. QIAsymphony D. Virus/Pathogen Kit—QIAGEN Online Shop. 2020.
18. Kirby T. Evidence mounts on the disproportionate effect of COVID-19 on ethnic minorities. The Lancet Respiratory Medicine. 2020;8(6):547-8.
19. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VY, et al. Diagnosing COVID-19: the disease and tools for detection. ACS nano. 2020;14(4):3822-35.
20. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell host & microbe. 2020.
21. Carter LJ, Garner LV, Smoot JW, Li Y, Zhou Q, Saveson CJ, et al. Assay techniques and test development for COVID-19 diagnosis. ACS Publications; 2020.
22. Bai Y, Yao L, Wei T, Tian F, Jin D-Y, Chen L, et al. Presumed asymptomatic carrier transmission of COVID-19. Jama. 2020;323(14):1406-7.
23. Zhang J-j, Dong X, Cao Y-y, Yuan Y-d, Yang Y-b, Yan Y-q, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy. 2020.
24. Alghamdi IG, Hussain II, Almalki SS, Alghamdi MS, Alghamdi MM, El-Sheemy MA. The pattern of Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive epidemiological analysis of data from the Saudi Ministry of Health. International journal of general medicine. 2014;7:417.
25. Channappanavar R, Fett C, Mack M, Ten Eyck PP, Meyerholz DK, Perlman S. Sex-based differences in susceptibility to severe acute respiratory syndrome coronavirus infection. The Journal of Immunology. 2017;198(10):4046-53.
26. Tahir S, Tahir SA, Arif TB, Majid B, Majid Z, Malik F, et al. Epidemiological and Clinical Features of SARS-CoV-2: A Retrospective Study from East Karachi, Pakistan. Cureus. 2020;12(6).