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COVID-19 DIAGNOSTIC FAILURE TRUTH BEHIND DIAGNOSTIC KIT IN PAKISTAN.

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Abstract

Background of the Study.

Rapid, and efficient diagnosis of Covid-19 during the pandemic enables the timely treatment of the virus. For the surveillance, and the efficient diagnosis of the infection, assessment of the reliability, and accuracy of the diagnostic method is important.

Objective.

This study aimed to determine the hidden aspects that make Covid-19 diagnostic failures in Pakistan.

Methodology.

This research was conducted at Sadu Teaching Hospital, for the duration of 6 months from October 2021 to February 2022. The target population was the patients who had the symptoms of Covid-19. 3400 participants were selected. Their consent regarding research participants was taken at that time. Sample of saliva, blood and other body fluid was taken from the patients. For further diagnosis 2 diagnostic kits were selected from China, and U.S. Participants were first tested with the U.S kit, the rest of them

who were not tested positive with this kit were further tested with the China Covid diagnostic kit. Whole-genome analysis was done on the 7 patients by using Illumina sequencing, and then results were correlated with the diagnostic kits.

Results.

In this study, 2000 patients were suspected as covid-positive using the U.S based kit. The rest of the participants with common symptoms were tested by using a China kit which confirm 1000 people as Covid- positive. To determine this difference ./hows by the diagnostic kits whole genome analysis was performed which shows that the samples that confirm the covid positive with the U.S based covid-diagnostic kits have the similarity with the Delta B strain of U.S. Whereas samples which shows positive with the China diagnostic kit has the similarity with the Wuhan strain of Covid-19.

Conclusion.

Each strain of Covid-19 has distinctive morphology. Therefore, the diagnostic kit for each strain should be specific to have an accurate, and efficient diagnosis of the virus.

Keywords.

Diagnosis failure, Covid-19 genome, diagnostic kits, and SARS-CoV-2.

INTRODUCTION.

Coronavirus disease 2019 (COVID-2019) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which appears as a highly communicable viral sickness, and has had a disastrous impact on the worldwide socio-economic and intimidating in immensely 3.8 million deaths worldwide. This pandemic emerges as the most dangerous global health crisis since the era of influence pandemic of 1918. Predominantly the first case was reported in Wuhan, Hubei Province, China, in December 2019. Subsequently Covid-19 rapidly distribute to the whole world in a very short period, entrancing the world health organization (WHO) to proclaim it a global pandemic in March 2020. (T, 2020)

Similar to other RNA viruses, SARS cov-2 also transform into their new human hosts susceptible to novel genetic evolution. This virus develops rapid mutations with time and results in multiple classes of mutants with completely different characteristics than its ancestral class. In the pandemic era, numerous variants have been identified, and among all of the novel variants, only a few of them contemplate by the WHO as the variants of the concerns (VOCs). Based on them some of the classes are Alpha

(B.1.1.9), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron (B.1.1.529), and others. (Lauring AS, 2021)

The virus contains four major proteins like nucleocapsid, glycoprotein spike surface, matrix core protein, and envelope. Covid-19 employs spike surface glycoproteins to bind with the receptor-binding site angiotensin-converting enzyme that manifests on the epithelial alveolar type 2 progenitor cells of the alveoli lungs, the surface of the artery, surface of veins, salivary glands, kidney, and heart tissues. By using the independent endocytic pathway of clathrinid and caveolae SARS-Covid enters the human host cells. (Singh Y, 2020)

The SARA covid 2 generates proteolytically active fragments of RAS protein that activate angiotensin 2 receptor type 2. These receptors bind with the ACE-2 and with using the host cell-directed network of the G-protein coupled receptors trigger the Jun N Terminal Kinase (JNK). Junk along with other signal transducers mediate the biochemical activity in host cells for viral dissemination. (Saeed U, 2021).

In developed countries, the Covid-19 vaccine is commercially available. More vaccines are on trial in clinical, and pre-clinical trials. However, to control the viral pathogenicity in the human host early detection is the most primed factor. Employing this nasopharyngeal swab (NPS) is used which is further followed by RNA extraction with the use of the real-time reverse transcription-polymerase chain reaction (RT-PCR). This method is considered the gold standard method for the diagnosis of Covid-19. The major challenge in this pandemic is to detect the large population suffering from Covid-19, by using the RT-PCR it is not possible to detect such a wide ratio within the limited time duration which eventually causes a technical, and financial burden. (Singh R, 2021)

In Pakistan limited research labs are present. All of these country research labs are unable to meet the rapid, accurate, and cost-effective diagnosis of Covid-19 with the limited research facilities. The diagnostic procedure with the MPS is invasive and it may end with bleeding which enhances the probity of the Covid-19 transfer to the healthcare workers. (Hobbs EC, 2021)

In the saliva or the MPS-based diagnostic of Covid-19, the major challenge is to govern the most appropriate diagnostic assay without incorporating the reliability of the test results. By the years 2021-2022, there is a sudden increase or decrease in the

cases of Covid-19. Symptoms are similar in the patients, but the diagnostic kits give the results of negative of diseased patients. (Wu K, 2021)

This study is the need for Pakistan, and all other countries to understand that the kit that is present for a viral genome is not for each type of strain. To make it clear, and simple it is like the covid-19 kit will not detect another disease. So, the virus with other strains of covid-19 is like a disease from another virus with completely distinctive morphology and characteristics. For instance, some of the studies report that the patient with covid-positive is determined as the covid-negative by the healthcare professionals. But the symptoms make them diagnose the disease with another hospital or the research lab which gives the results of covid-positive. It is because the patient affected with covid-19 strain similar to the diagnostic kit of 2nd healthcare hospital, while 1st hospital is using the diagnostic kit for some other strain. So, the kit is not specific to the strain which affects the patients.

Therefore, for effective diagnosis of the virus, it is crucial to understand that each kit is specific for the virus mutants. This article will shed light on the diagnostic failure of the covid-19 in Pakistan and will help in the future to develop effective diagnostic procedures. So, to meet with viral strains effectively in less duration, and adopt the best treatment approach to minimize the mortality rate.

METHODOLOGY.

To suspect the diagnostic accuracy of SARS Covid-19, Saliva, or antigen samples were collected. To determine the differences in the same patients 2 kits were used. The patients who were not suspected as diseased with the 1st kit were diagnosed with another kit. Among 3400 patients' the study was conducted at sadu Teaching Hospital, for October 2021-February 2022. firstly pretesting was conducted which involve counseling. After that diseased individuals with the symptoms of fever, international travel history, respiratory symptoms, flue, loss of smell, and taste, vomiting, were confirmed as Covid-19 patients. In all of the duration patient's consent regarding the study was taken. For further evaluation, we select the patients, and we detect them with the 1st covid-19 kit. Among them, 2000 were confirmed as diseased individuals. The rest of them also have symptoms that we use another kit which confirms that 1000 people as the suspected. For further investigation, we diagnose the above 2000 confirmed patients with the 2nd kit. This kit provides negative results for most of them.

For more analysis, we compare the genome of randomly selected samples from the 7 patients. 3 were from the U.S based kit, 3 were from the China-based kit, and 1 was from the patient who had the symptoms, but both of the kits were not detected as the covid-positive. We extract the RNA from the oropharyngeal swabs. For Illumina MEBNext Ultra w RNA library prep kit was used. This kit was selected for the library preparation of whole-genome sequencing. We put the Wuhan strain as the reference genome, and we assemble the in-house scripts by using the mapping software bwa-mem. We align the genome of the sample with the reference strain of Wuhan. Sam tool is used to provide final consensus sequences. Sam tools generate the pileup of alleles from the bam file. For this following parameters are set; maximum depth of reads, dial base quality, orphan read pair, minimum base quality, and others.

This whole genome of samples is compared to verify that the patients are affected by the different strains.

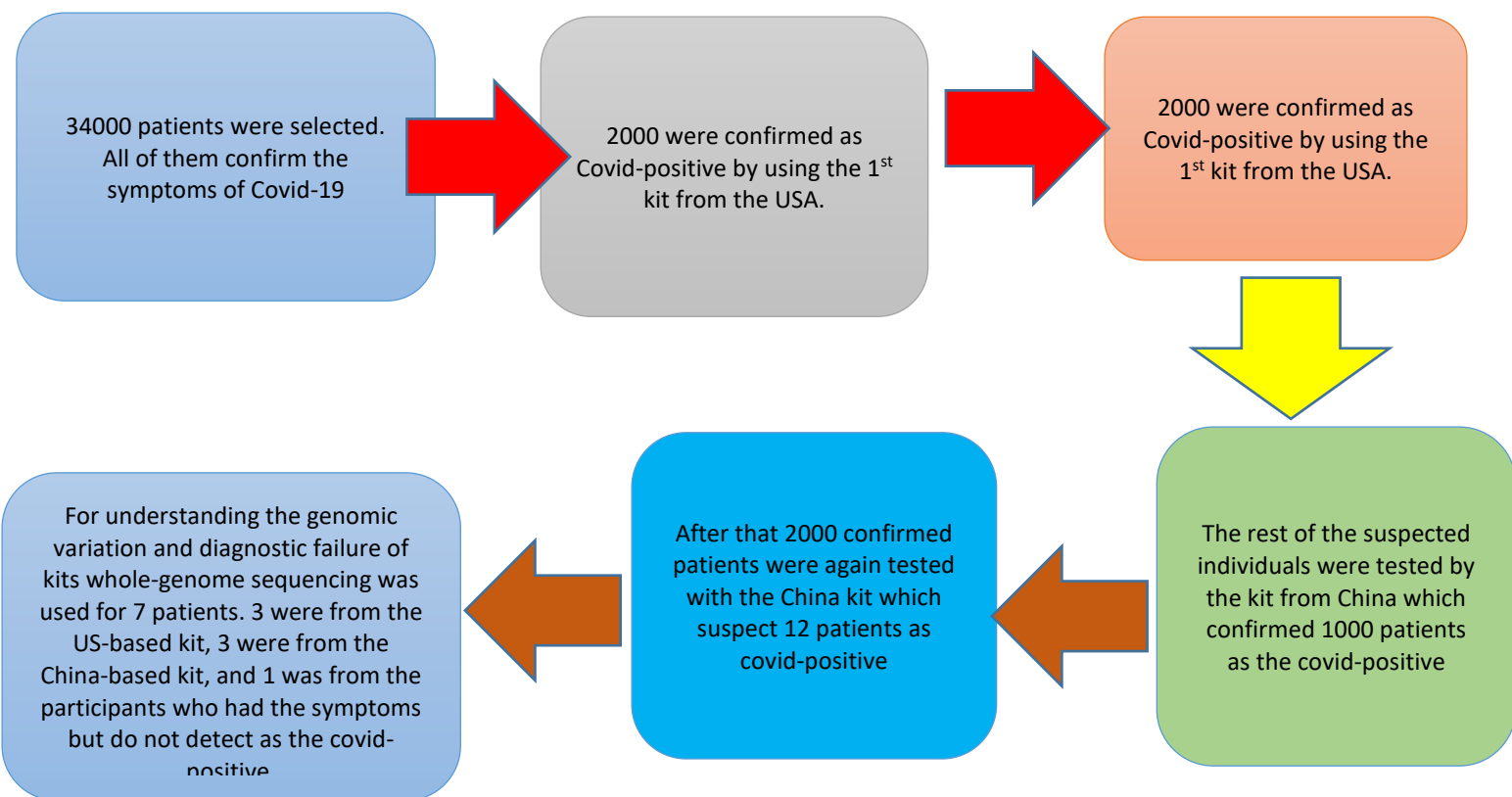


Figure 1: Flow chart of the whole methodology.

RESULTS.

Common symptoms among all the suspected patients include cough, fever, tiredness, loss of smell, loss of taste, and others. Among all of the symptoms higher frequency has the fever. Almost all of the patients show that they are suffering from a fever.

Table1: Common symptoms and their frequency.

Symptoms	Frequency among suspected Individuals
Fever	98%
Cough	60%
Tiredness	76%
Loss of taste.	40%
Loss of smell.	55%
Difficulty breathing	10%
Chest pain	11%
Irritated eyes	5%
Headache	12%
Lack of speech	1%
Others	23%

The above symptoms enhance the probability of having Covid-19. Therefore, healthcare professionals recommend Covid-19 testing.

Table 2: The kit from China and the other one is from the U.S present the number of patients diagnosed with Covid-19.

Diagnostic Kits	The number of patients.
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U.S based	2000 (59%)
China-Based	1000 (29%)
Non-infected	400 (12%)

Whole-genome sequence analysis of the 7 patients shows the major proteins, and domains involved in the infection. The percentage of these proteins is different in each sample.

Table 3: Major proteins involve in causing infection among all of the patients.

Proteins Name	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Spike (S)	98%	54%	32%	10%	45%	54%	31%
Membrane (M)	89%	90%	35%	45%	38%	15%	45%
Envelop (E)	20%	28%	36%	90%	30%	35%	45%
N terminal domain	20%	21%	55%	98%	54%	32%	10%
Receptor binding domain	35%	45%	38%	15%	45%	20%	28%
Nucleocapsid	85%	48%	89%	90%	35%	45%	38%

Table 4: samples sequences, and their coverage average, compared with the reference genome, and missing bases.

Sequence name	Coverage average	Compared to the	Missing Bases
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		reference genome	
Sample NIH-1	15.90	97.90	13
Sample NIH-2	19.2	99.66	33
Sample NIH-3	86.7	99.99	12
China Kits detection results with Wuhan reference genome.			
Sample NIH-4	11	54.55%	40
Sample NIH-5	10	40.55%	39
Sample NIH-6	9	34%	34
US Kits detection results with Wuhan reference genome.			
Sample NIH-7	213	74%	41
Non-infectious suspected person			

These tables show the majority of similarities with the reference genome are with the Chian strain. So, the person who is diagnosed with the China diagnostic kits is suffering from the chain strain. Differences are majorly observed in the sequences of the U.S strain (Delta-B). Therefore, the U.S kits are particularly to the strain of Delta B. Therefore, they do not detect the covid-19 patients suffering from the Wuhan strain. This table presents after sequencing results and shows the values of similarity with the reference genome, average, and missing bases values in the sequences.

DISCUSSION.

In third world countries, Covid-19 diagnosis is a serious problem reported by many healthcare studies. China, Italy, United States, India, and many other countries had the highest viral attack. (Paital B, 2020) Meanwhile, some of the diagnostic kits were not providing efficient results. By December 25, 2020, the whole genome sequences of Covid-19 available on the GISAID. (To KK, 2021) In the present study, we predict that the kit is specific to each strain of the Covid-19. Therefore, making the detection kit genome sequence should be understood properly. The infection rises and decreases with time because of the standard operating procedures followed by the public. There are some cases in which the symptoms are similar to viral infection. However, the Covid-test is negative for the patients. Some patients were further confirmed by the antibodies test and their reports show the antibodies to the viral infection. (La Marca A, 2020) This myth of having covid-negative but antibodies report positive makes the researchers, doctors, and scientists very worried about the situation. This article provides the similarities, and differences in the genomes of different strains, and further confirmed that the patients with the Wuhan strain will only be successfully detected with the diagnostic kit based on the Wuhan strain.

CONCLUSION.

As most healthcare professionals were worried about the patients who had severe symptoms of Covid-19, but the test shows them negative. As Scientists work on multiple domains to understand the virus completely. Therefore, this research will be helpful for them in understanding that kit efficiency, and accuracy is based on the type of mutants that attack the patients. It is crucial to understand which kind of strain infects the host. For this, if the patients have severe symptoms, healthcare professionals will go for multiple tests by using different kits. They do not predict the result based on one kit from China, Italy, or some other countries. Pakistan was in the phase where the cases were not reported but many of the patients were on the ventilator. Doctors were suggesting that the patients are suffering from pneumonia or some other disease. This article will be very helpful for understanding the proper diagnostic approach so, as to have accurate, and efficient treatment plans in case of again pandemic attack. In Pakistan limited research labs are present. All of these country research labs are unable to meet the rapid, accurate, and cost-effective

diagnosis of Covid-19 with the limited research facilities. Therefore, for effective diagnosis of the virus, it is crucial to understand that each kit is specific for the virus mutants. This article will shed the light on the diagnostic failure of the covid-19 in Pakistan and will help in the future to develop effective diagnostics procedures. So, to meet with viral strains effectively in less duration, and adopt the best treatment approach to minimize the mortality rate.

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