Current Scenario of Covid-19 with Epidemiological and Phylogenetic Analysis of Pakistani Coronavirus: A Review

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Cover Page Footnote
Footnote of abbreviations: CoVs, Coronaviruses; SARS, Severe Acute Respiratory Syndrome; MERS, Middle East Respiratory Syndrome; WHO, World Health Organization; 2019-nCoV, 2019-novel Coronavirus; PHEIC, Public Health Emergency of International Concern; ICTV, International Committee on Taxonomy of Viruses; COVID-19, Coronavirus Disease-2019; αCoV, Alpha-coronavirus; δCoV, Delta-coronavirus; βCoV, Beta-coronavirus; γCoV, Gamma-coronavirus; R₀, Basic Reproduction Number; UTRs, Untranslated Regions; S, spike; N, nucleocapsid; E, envelope; M, membrane; ACE2, Angiotensin-Converting Enzyme 2; HTD, Hydrophobic transmembrane Domain; LKR, Linker Region; CTD, C-Terminal Domain; NTD, N-Terminal Domain; NSPs, Non-structural Proteins; RTC, Replication Transcriptional Complex; APC, antigen presenting cells; MHC, major histocompatibility complex; HLA, Human Leukocyte antigen; CTLs, Cytotoxic T-Lymphocytes; ARDS, Acute Respiratory Distress Syndrome; G-CSF, Granulocyte-colony stimulating factor; M-CSF, macrophage colony stimulating factor; HGF, hepatocyte growth factor; NHC, National Health Center; PD, Parkinson disease; CHD, Coronary Heart Disease; GSK, GlaxoSmithKline; CVD, cardiovascular diseases; DB diabetes mellitus; HTN, hypertension; RSD, respiratory system disease; BUN, blood urea nitrogen. Acknowledgement: We acknowledged efforts of different countries of the World, as they participated in knowing about 2019-nCoV & COVID-19 as well as in reducing the impact of this pandemic on susceptible populations.

This article is available in Journal of Bioresource Management: https://corescholar.libraries.wright.edu/jbm/vol7/iss3/4
CURRENT SCENARIO OF COVID-19 WITH EPIDEMIOLOGICAL AND PHYLOGENETIC ANALYSIS OF PAKISTANI CORONAVIRUS: A REVIEW

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ABSTRACT

A novel corona virus named as 2019 n-CoV was identified to be the actual cause of an outbreak of respiratory illness in Wuhan, China beginning in late December 2019. This respiratory disease was named as COVID-19 by WHO. There are many countries affected by COVID-19 including Pakistan. In this review we have provided a brief introduction of COVID-19 and discussed epidemiological and phylogenetic analysis of Pakistani novel corona virus strain. Our epidemiological analysis of data till 16th May 2020 showed rapidly increasing number of new cases, while at the same time a higher recovery rate than number of deaths in Pakistan. Moreover, phylogenetic analysis indicated that 2019-nCoV strain of Pakistan shared 100 % bootstrap value with various countries’ novel corona virus strains. Similarly, phylogenetic analysis was also conducted in comparison with SARS species to confirm our results. In this review, current knowledge of pathogenesis, diagnosis, treatment of COVID-19 and comorbidities which could be helpful in offering novel understanding and possible therapeutic targets for fighting against the COVID-19 infection are discussed.

Keywords: COVID-19, 2019-ncov, phylogenetic analysis, epidemiological analysis, Pakistan n-cov strain.

INTRODUCTION

Coronaviruses (CoVs) are the part of the family Coronaviridae with subfamily and order Orthocoronavirinae and Nidovirales (Banerjee et al., 2019). CoVs are familiar human pathogens, with an association to mild acute respiratory illnesses known as the common cold (Wilson et al., 2020). CoVs mainly cause enzootic infections in birds and mammals but prior outbreaks of CoVs in the last decades, including the Severe Acute Respiratory Syndrome (SARS)-CoV in 2002 and the Middle East Respiratory Syndrome (MERS)-CoV in 2012, have confirmed the deadliness of CoVs when they infect humans by crossing species barrier (Bogoch et al., 2020). A group of patients, with a primary diagnosis of pneumonia of ambiguous origin, were admitted to hospitals in late December 2019. Several of the early patients visited a wet seafood and animal market in Wuhan, Hubei province, China (Lu et al., 2020a). Successive virus isolations from human patients and their molecular analysis revealed that the disease-causing agent was a novel coronavirus, first named as 2019-nCoV. The WHO (World Health Organization) revealed the outburst of a Public Health Emergency of International Concern (PHEIC) on January 30, 2020. Another name SARS-CoV-2 was suggested by a study group of the International Committee on Taxonomy of Viruses (ICTV) based on taxonomy phylogeny, and established practice on February 11, 2020, afterwards this disease was renamed by WHO as COVID-19 on February 12, 2020. This review is focused on
ETIOLOGY

CoVs have mainly four genera namely, αCoV (alpha coronavirus), δCoV (Deltacoronavirus), βCoV (Betacoronavirus) and γCoV (Gammacoronavirus). Their evolutionary investigations indicated that gene sources of αCoV and βCoVs were bats and rodents while avian species were the gene sources of many δCoV and γCoVs. These viruses gradually crossed species barrier and have become evident human pathogens (Chan et al., 2020a). The first complete genome of the novel β genus coronavirus (later named SARS-CoV-2) was discovered in a patient from Wuhan by taking broncho alveolar lavage fluid as a sample on January 3, 2020 (Wu et al., 2020b). The experimental report declared that the virus had 88 % sequence similarity with two bat-derived SARS (severe acute respiratory syndrome) like CoVs, but recognizably different in nature from SARS-CoV (Lu et al., 2020b). On the support of present data, it appears that COVID-19 might be originally hosted by bats, and it is a possibility that it was transmitted to humans through pangolins (Lam et al., 2020) or various other wild animals that were being sold at Huanan seafood market and successive spread occurred through human to human transmission (Zu et al., 2020).

EPIDEMIOLOGY AND PREVALENCE

On 31 December 2019, the WHO China Country Office was informed of cases of pneumonia with unknown etiology, detected in Wuhan City, Hubei Province of China. From 31 December 2019 through 3 January 2020, a total of 44 case-patients with pneumonia of unknown etiology were reported to WHO by the national authorities in China. During this reported period, the causal agent was not identified. Data from 20 January 2020 to 8 April 2020 was observed. On 20 January 2020, 278 confirmed cases of 2019-nCoV were reported in China out of 282 total reported cases. While on 8 April 2020, 1,353,361 confirmed cases (73,639 new) with 79,235 deaths (6,695 new) were reported globally and 83,157 confirmed (86 new) cases and 3,342 deaths (2 new) were reported in China, (“Coronavirus disease 2019 (COVID-19) Situation Report – 79”, 2020). Data reported on 16th May, 2020 of most affected countries is represented in the form of graph (Figure 1A). It consists of total confirmed cases (affected individuals), deaths and recovered patients of different countries from onset of the disease till May 16, 2020.

Data of COVID-19 cases in main areas (including five provinces) of Pakistan is depicted in the form of graph (Figure 1B). As is shown in the graph, the highest total confirmed cases were in Sindh, while lowest numbers of cases were present in Azad Jammu Kashmir (AJK). Total deaths and total recoveries in Pakistan, are presented in the form of graph (Figure 1C). Data till 16th May, 2020 showed higher recovery rate than number of deaths as presented in graph. Numerical data of newly identified cases on daily basis versus newly recovered patients is presented in graphical form as shown in graph (Figure 1D). Number of new cases was increasing on a daily basis and was greater than that of recovered individuals as indicated in the graph. Data of daily new COVID-19 cases in Pakistan is presented in graph (Figure 1E).

R₀ (Basic Reproduction Number) is the mean amount of secondary infection that patient may develop in an entirely susceptible population without any intervention (Remais, 2010). Estimation of R₀ of 2019-nCoV (2.0-
3.30) indicated that 2019-nCoV has high transmissibility as compared to other CoVs.

Figure 1: (A) Graphs of COVID-19 cases of most suffered countries, (B) total cases of Pakistan till May 16, 2020, (C) total deaths versus total recoveries in Pakistan, (D) new cases versus newly recovered cases in Pakistan, (E) new cases of COVID-19 reported in Pakistan on daily basis.

PHYLOGENETIC ANALYSIS

Spike protein analysis of 2019-nCOV from different countries with Pakistani novel corona virus (n-CoV) strains

Here we aimed to reveal evolutionary position of Pakistani n-CoV strain with respect to n-CoV strains of different countries based on spike (S) glycoprotein gene analysis. For phylogenetic analysis the dataset used included (n=17) S glycoprotein gene sequences of current epidemic from different countries, plus (n=2) n-CoV strains from Pakistan (Gilgit, KPK). These sequences were retrieved from NCBI database (http://www.ncbi.nlm.nih.gov/genbank/).

Evolutionary examination was conducted in MEGA 7.0 version applying ML (maximum likelihood) method based General Time. The tree was constructed to scale, with the branch lengths in same units as those of evolutionary distances used to deduce phylogenetic-tree (Zuckerkandl and Pauling, 1965).

This analysis suggests that novel coronavirus S protein sequences from Gilgit and KPK share 100% bootstrap value with n-CoV protein sequences from various countries which means that COVID-19 in Pakistan was caused by the same n-CoV which targeted other countries (Figure 2A). Emergence of this virus in Pakistan might be
due to the tourists visiting, which were already infected or by people which were deported by corona infected countries. Thus, these infected people spread the disease in natives.

**Phylogenetic analysis of Pakistani n-CoV strains V/S previously known SARS species spike (S)-gene sequence**

We further broadened the analysis by targeting the S glycoprotein gene of the CoVs from human SARS, bat-like CoVs, animal-origin CoVs together with MERSV and the current outbreak of n-CoV in Pakistan. These sequences were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/genbank/) database. Evolutionary examination was conducted in MEGA 7.0 version applying ML (maximum likelihood) method based General Time. In this phylogenetic analysis, Pakistani nCoV strains clustered in a monophyletic clade. Our analysis indicates that two bat SARS-like CoVs (Bat-SL-RsSHC014, and Rs3367) and two β-CoVs (BtRs-BetaCoV/YN2018C and BtRs-BetaCoV/YN2018D) are sharing 100% bootstrap support with Pakistani 2019-nCoV isolates. Our analysis confirms that this is same coronavirus strain that infected other countries as represented in Figure 2B. However, spread of COVID-19 is less severe in Pakistan as compared to other countries according to the data till 9 April. One reason of this less prevalence could be strong immunity of Pakistani but further research is needed to confirm this fact.
Figure 2 Phylogenetic analysis of Pakistani n-CoV isolates (A) Phylogenetic analysis of spike glycoprotein gene sequences of 2019-nCoV strains (17 different countries and 2 Pakistani). The solid-red circles are for n-CoV isolates from Pakistan. (B) Phylogenetic analysis of spike glycoprotein gene sequences of 2019-nCoV strains (17 different SARS species, 2 Pakistani). The solid-red circles are for n-CoV isolates from Pakistan.
ORGANIZATION OF GENOME

2019-nCov has single stranded RNA genome of 29891 bases encoding 9860 amino acids. Novel corona virus genome, similar to other βCoVs, has two UTRs (untranslated regions) and a polyprotein encoding single long open reading frame. The G+C content was found to be 38 %. Hemagglutinin-esterase gene which is primarily present in lineage A β-CoVs is absent in 2019-nCoV and its genome is organized in sequence of 5′-replicase (orf/ab)-proteins of structural nature, nucleocapsid (N)-Envelope (E)-Spike (S)-Membrane (M)-3′. Notably, orf3b encodes a completely new short protein. Moreover, new orf8 probably encodes a secreted protein with α-helix, following with a β-sheet(s) consisting six strands as shown in Figure 3A.

**Spike glycoprotein**

Spike glycoprotein consists of two subunits S1 and S2. The components in these subunits are shown in Figure 3B. It was found that S2 subunit of novel coronavirus is largely reserved. It is 99% identical to human SARS-CoV and two bat SARS-like CoVs (ZC45 and SL-CoV ZXC21). Coronavirus proteins called spike (S) binds to ACE2 (angiotensin-converting enzyme 2) receptor and initiates successive fusion between the host cell membrane and envelope and in turn help in viral entry into host cell (Kirchdoerfer et al., 2016).

**M protein**

The most abundant protein present in virion particle is the M protein and it provides particular shape to viral envelope (Neuman et al., 2011). M proteins of CoVs are very much diverse, M-M interaction is involved in maintaining viral scaffold (Arndt et al., 2010). In contrast to SARS-CoV the M protein of 2019-nCoV does not have any kind of amino acid substitution (Wu et al., 2020a).

**E protein**

Among the major structural proteins, E protein is the most mysterious and smallest. It plays a multi-functional role in assembly, pathogenesis and release of virus (Nieto-Torres et al., 2014). Deletion or inactivation of this protein cause changes in tropism and morphology which in turn lead to variation in virulence of coronavirus (DeDiego et al., 2007). The E protein of 2019-nCoV presents a comparable amino acid constitution without any type of substitution (Wu et al., 2020a).

**N protein**

Multi-purpose N protein of coronavirus has role in increasing transcription efficiency of virus, formation of complex with viral genome and aids M protein interaction required for virion assembly (Chang et al., 2006). It consists of three recognizably different and highly conserved domains which are linker region (LKR) or RNA-binding domain and a (CTD) C-terminal domain (McBride et al., 2014). The N protein of 2019-nCoV, in contrast with SARS-CoV, has five amino acid mutations. Where two are present in IDR (intrinsically dispersed region; 26 and 25 positions), and other in LKR (217 position), NTD (103 position) and CTD (334 position).

**NSPs and accessory proteins**

Besides the crucial structural proteins, 2019-nCoV genome also contains 8 accessory proteins (3a, 7a, 9b, 3a, 8b, p6 and orf14) and nsp 12-16, 15 nsps, nsp1-nsp10. 2019-nCoV genome does not contain longer 8b and 8a proteins in comparison with SARS-CoV. Matrix, envelope, nsp13, nsp7 or
accessory proteins 8b and p6 have not been found with any amino acid substitution with respect to CoVs as shown in Figure 3C (Wu et al., 2020a).

Figure 3: (A) Organization of βcoronavirus. The genome is composed of ORF (open reading frame), 1ab (Steel blue box), nsp (non-structural proteins), structural proteins including envelope (dark khaki box), spike (Slate gray box), membrane (chocolate box), accessory proteins (Corn flower blue box) such as orf 9b, 7b, 7a, 8, 3 and 6, similarly nucleocapsid proteins (dark slate blue box) and 5'-UTR in the 2019-n CoV. Example of lineages is from A to D. The length of orfs and nsps are not drawn to scale. (B) Components of S1 and S2 subunits. S1 subunit has S-peptide (single peptide), RBD (receptor binding domain), NTD (N-terminal domain) while S2 subunit has F-peptide (fusion peptide), HP (heptad repeat), CM (cytoplasmic domain), TM (transmembrane domain). (C) Structure of 2019-nCoV. “S” (spike glycoprotein), “M” (membrane protein), “N” (nucleocapsid protein), “E” (envelope protein), “ssRNA” (single stranded positive sense RNA).

MOLECULAR COMPARISON BETWEEN ACE2 RECEPTORS OF HUMAN AND ANIMAL SPECIES

The recognition of residues of contact between human ACE2 and 2019-nCoV receptor binding domain permit estimation of whether 2019-nCoV is able to infect other species. To evaluate this Sun et al., (2020) aligned amino acid sequences of all available ACE2 with human ACE. They focused on N-glycosylation of motifs near the binding site, as they may affect attachment of S (Sun et al., 2020). The molecular differences they found are given in Table 1. Particularly, binding of S from SARS-CoV to rat is prevented by glycosylation of residue 82 (Li et al., 2005). Further Sun et al., (2020) found that amino acid differences in ACE2 affect binding of 2019-nCoV in various species. Studies of S binding site of ACE2 from various species disclose that ten and nine amino acid differences are found in rodents (mouse and rat respectively), eleven in chicken, and only three are present in cats as compared with ACE2 of human. ACE2 of human is identical to ACE2 from chimpanzees and macaque. So, these amino acid and glycosylation differences make these animals reservoirs for 2019-n CoV. A structure model examination shows that 2019-nCoV binds with ACE2 of host with above 10 folds higher affinity than SARS-CoV (Wrapp et al., 2020). These findings explain the faster transmission ability of 2019-n CoV in humans than SARS-CoV, and this may be reason of much higher confirmed COVID-19 cases than SARS-CoV infection. Taking into consideration the high affinity of 2019-nCoV binding with ACE2, soluble ACE2 might be a possible approach
for treatment of COVID-19 treatment. Patients with COVID-19 exhibit similar clinical signs and symptoms with those of SARS-CoV and MERS-CoV infections (Huang et al., 2020). Since pathogenesis of COVID-19 is not properly understood by scientist, the somewhat similar mechanism of MERS-CoV and SARS-CoV can give us much information about pathogenesis of 2019-nCoV infection to facilitate our understanding about COVID-19.

Table 1: Glycosylation differences of ACE motifs in different species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Glycosylation Sites</th>
<th>Sites at which glycosylation does not occur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>N53, N90, N322</td>
<td></td>
</tr>
<tr>
<td>Mouse, pig, raccoon, fox, chicken, <em>N. procyonoides</em>, ferret, <em>E. telfairi and civet</em></td>
<td>N90</td>
<td></td>
</tr>
<tr>
<td>Sheep, rat, cattle, pangolin, <em>E. telfairi</em>, mouse</td>
<td>N322</td>
<td></td>
</tr>
<tr>
<td>Additional glycosylation motif in this region of ACE2 in some species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>L79</td>
<td></td>
</tr>
<tr>
<td>Rat, <em>Rhinolophus sinicus</em>, and pangolin</td>
<td>M82</td>
<td></td>
</tr>
</tbody>
</table>

* N322 common in all species

PATHOGENESIS OF THE CORONAVIRUS

Entry and replication of coronavirus

Cell receptors, ACE2, for 2019-nCoV are found in lower respiratory tract of humans (Jia et al., 2005). S-glycoprotein on the surface of virion is capable of attachment to these (ACE2) receptors (Tortorici and Veesler, 2019). The entrance of SARS-CoV into the cell was primarily recognized to be accomplished by face to face fusion between the plasma membrane and virus. Belouzard et al. (2009) discovered that membrane fusion and viral infectivity of SARS-CoV is mediated by critical proteolytic cleavage event at S2' position of ‘S- protein’. In addition to membrane fusion clathrin-independent and dependent endocytosis mediated entry of SARS-CoV has also been observed (Wang et al., 2008). After the virus entry into the cell, the viral genome is released into the cytoplasm and is translated into two (pp1a, pp1ab) polyproteins (de Wilde et al., 2017), which encodes NTP (non-structural proteins) and form RTC (replication transcriptional complex) (Sawicki and Sawicki, 2005). Newly synthesized envelope glycoproteins, genomic RNA and nucleocapsid proteins arrange and from viral particle buds. At the end, viruses are released by the fusion of these virus containing vesicles with plasma membrane (de Wit et al., 2016).

Antigen presentation during coronavirus infection

The direct infection of T cells and macrophages by SARS-CoV has been reported, but it is still unknown whether 2019-nCoV infects immune cells
Prompetchara et al., 2020). In the central part of the body’s anti-viral immunity, after the entry of virus in the cells, its antigen would be presented to APC (antigen presenting cells). MHC (major histocompatibility complex) or HLA (human leukocyte antigen) in humans presents the antigentic peptides, which are then recognized by the CTLs (cytotoxic T lymphocytes). Thus, comprehension of 2019-nCoV antigen presentation will help our understanding of COVID-19 pathogenesis.

**Humoral and cellular immunity**

Presentation of antigen then activates the body’s cellular and humoral immunity, virus-specific B and T cells are responsible for this immunity. IgG and IgM production occurs against SARS-CoV virus, similar to some common acute viral infection. IgG antibodies may principally play protective role, as they can last for longer time, while SARS-specific IgM antibodies vanish at the end of week 12 (Li et al., 2003). SARS-specific IgG antibodies are mainly N-specific and S-specific antibodies. A recent report showed that the number of CD8+ and CD4+ T-cells was remarkably reduced in peripheral blood of 2019-nCoV infected patients, although its status is too much activation, as revealed by high fraction of CD38 (CD8 39.4 %) and HLA-DR (CD4 3.47 %) double-positive proportions (Xu et al., 2020). In the same way in patients with SARS-CoV, the acute phase response is related with a serious decrease of CD8+ and CD4+ T-cells. In SARS-CoV recovered individuals, even if there is no antigen present, the memory T-cells will remain present for four years and can carry out T-cell multiplication, production of IFN-γ and DTH response (Fan et al., 2009). The particular CD8+ T cells also exhibit a similar effect on MERS-CoV clearance in mice. These previous findings may give valuable details for the rational design of vaccines against 2019-nCoV.

**COVID-19 and Cytokine storm**

In case of CoVs infection (SARS-CoV, 2019-nCoV and MERS-CoV) acute respiratory distress syndrome (ARDS) is the usual immunopathological event (Xu et al., 2020). The uncontrolled deadly inflammatory response is produced due to release of a very large amount of IL-16, IL-18, IL-12, IL-33, IFN-γ, IL-1β, TNF-β, IFN-α and TGFα, etc. (pro-inflammatory cytokines) and CCL3, CXCL8, CXCL10, CCL2, CCL5, CXCL9 etc. (chemokines) in SARS-CoV infection, by immune effector cells (Williams and Chambers, 2014). Some plasma chemokines and cytokines were also found to be increased in case of COVID-19 patients, these include different types of interleukin (IL-2, IL-1, IL-10, IL-7, IL-17, IL-13, IL-12, IL-1), G-CSF (Granulocyte-colony stimulating factor) M-CSF (macrophage colony stimulating factor), HGF (hepatocyte growth factor), MCP-1α, MCP-1, IP-10, TNF-α and IFN-γ (Chen et al., 2020). Accumulatively, the virus particles occupy the respiratory mucosa at first and then infect other cells of the body, they initiate the synthesis of cytokine storm in the body and a series of immune responses as shown in Figure 4. This cytokine storm will activate an intense attack to the body by immune system causing multiple organ failure and will at the end lead to the death of severe cases of 2019-nCoV infection (Xu et al., 2020).
Figure 4: Pathogenesis of 2019-n-CoV; 2019-nCoV entry and replication. 1: Attachment of virus with ACE2 receptors. 2: Vesicle formation. 3: Virus entry in cell. 4: Replication of genome. 5: Newly synthesized viral particle buds. 6: Released viral particles, Antigen Presentation MP (macrophage particles), DC (dendritic cells), EC (epithelial cells), APC (antigen presenting cells), CK (cytokines), Cellular immunity CK (cytokines), TC (T-cells/lymphocytes), Humoral immunity PC (plasma cells), M-BC (memory B-cells), Th2-C (helper -T cells).

Incubation Period of 2019-nCoV

The average incubation period of 5.2 days was reported by a study of initial transmission kinetics of 2019-nCo (Backer et al., 2020). In a later study, performed based on appearance of symptoms and travel history of 88 confirmed patients, a comparable average incubation period of 6.4 days was described. A unique case was reported having an incubation period as long as 19 days (Bai et al., 2020). Particularly, a long incubation time indicates acclimatization in screening and control policies (Jiang et al., 2020). In the recent guidelines provided by Chinese health authorities, a mean incubation period of 7 days, varying from 2-14 days was declared.

Transmission of Coronavirus

Two main transmissions of 2019-nCoV are zoonotic (via contact with animals) and anthroponotic (via direct or indirect contact with affected individual) transmissions. Many wild and tame animals, comprising cats, cattle and bats can function as host for different coronaviruses. Snakes and pangolins at untamed animal stores were seemingly to be intermediary hosts of 2019-nCoV, as shown by latest studies however evidence of human to human viral transmission has also been reported (Liu et al., 2020). Three main, human to human routes for transmission of COVID-19 include direct transmission (respiratory droplets), and indirect routes of transmission (fomites and aerosols). Contact transmission can occur when a person comes in contact with an object or a surface, which is already
contaminated with virus and thereafter touches his/her nose, eyes or mouth (transmission through fomites). Respiratory droplets are formed when an infected individual sneezes or coughs for distances longer than six feet; these droplets travel in the form of aerosols. Digestive system, as another possible route of transmission of 2019-nCoV was described by another study in which it was observed that ACE2 was immensely expressed in absorptive cells of alimentary tract from colon and ileum (Zhang et al., 2020).

Clinical and laboratory findings

After an approximate incubation time of 5.2 days, symptoms of 2019-nCoV viral infection become visible (Li et al., 2020a). Guidelines for treatment and diagnosis of 2019-nCoV (Wang et al., 2020b) are made and updated by NHC (National Health Center) of China, on the basis of broad range of connected ideas of disease severity. Based on the severity of associated symptoms, COVID-19 is now organized into four levels: critical, severe, moderate and mild. Their associated clinical and laboratory features are described in Table 2. Cases ending in death were chiefly middle-aged and old-age patients already having any other disease(s) such as diabetes, PD (Parkinson disease), CHD (Coronary Heart Disease), cirrhosis, hypertension or tumor surgery (Li et al., 2020b).
Table 2: Clinical & Laboratory findings of COVID-19.

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Laboratory features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complicated dyspnea, myalgia, cough, pneumonia &amp; fever are frequently reported systems, while diarrhea is uncommon.</td>
<td>Viral load decreased total lymphocytes, prolonged APTT$^a$&amp;PT$^b$, and increased level of creatinine, ALT$^c$, AST$^d$, blood urea, thrombocytopenia, increased cTnI$^e$&amp; angiotensin II, decreased ALB$^f$.</td>
</tr>
<tr>
<td>Mild patients: mild fatigue, no pneumonia and low fever.</td>
<td>Most patients: increased ESR$^g$, &amp; CRP$^h$ with normal procalcitonin.</td>
</tr>
<tr>
<td>Moderate patients: radiographic features, fever and respiratory symptoms.</td>
<td>Severe cases: Elevated D-dimer, FDP$^i$, decreased lymphocytes in peripheral blood.</td>
</tr>
<tr>
<td>Severe patients: hypoxemia/dyspnea one week after the onset of disease.</td>
<td>Patients of ICU: elevated TNFα$^j$ and other inflammatory cytokines.</td>
</tr>
<tr>
<td>Critical patients: ARDS$^k$, shock, multiple organ failure. Anorexia, dyspnea and abdominal pain were also common.</td>
<td>Non-survivors: elevated blood urea, D-dimer, creatinine and neutrophil count.</td>
</tr>
</tbody>
</table>

$^a$APTT: Activated partial thromboplastin time, $^b$PT: Prothrombin time, $^c$ALT: Alanine transaminase, $^d$AST: Aspartate transaminase, $^e$cTnI: Cardiac troponin I, $^f$ALB: Albumin, $^g$ESR: Erythrocyte sedimentation rate, $^h$CRP: C-reactive protein, $^i$FDP: Fibrin degradation product, $^j$TNFα: Tumor necrosis factor α, $^k$ARDS: Acute respiratory distress syndrome.

ADVANCEMENT IN DIAGNOSIS OF COVID-19

Immediate and precise diagnosis of COVID-19 is critical to control outset in hospitals or in any community (To et al., 2020). Auxiliary assessments like epidemiological history, understanding of clinical symptoms and radiographic characteristics are essential for diagnosing 2019-nCoV and associated irregular symptoms (Li et al., 2020c). Description of different techniques is given in Table 3.

Table 3: Techniques and their description.

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic acid detection methods</td>
<td>RT-qPCR is most ordinary, efficient and simple technique gives 50-79% sensitivity (Yam et al., 2003) also gives *FNR.</td>
</tr>
<tr>
<td>NGS and virus blood culture are authorized methods but cannot be used because of high price &amp; equipment dependency (Zhou et al., 2020).</td>
<td></td>
</tr>
<tr>
<td>RT-LAMP is very precise, has sensitivity similar with rRT-PCR &amp; is used for detection of MERS-CoV (Huang et al., 2018).</td>
<td></td>
</tr>
</tbody>
</table>
From three RT-PCR assays, RdRp/helicase method is highly reactive and is regarded as specific and recommended assay (Chan et al., 2020b).

Cobas 6800 with a good systematic operation takes less time & offers authentic results (Eigner et al., 2019).

Computerized Tomography (CT) Scans

Used in combination with rRT-PCR particularly for early detection & evaluation of severity of disease (Pan et al., 2020). However, contain inadequacies e.g. being indistinct from pneumonia caused by other viruses & hysteresis of unusual CT imaging.

Enzyme Linked Immunosorbent Assay (ELISA)

Exhibit higher diagnostic rates than other techniques & is highly suggested method (Wang et al., 2020a). It has fast turnaround time & comparatively low costs. A warning is that it can give **FPR, because of conservative N-protein of 2019-nCoV.

*False negative result ** False positive result

ADVANCEMENT IN TREATMENT

Drugs and herbal treatment

Treatment of COVID-19 by using different drugs and herbal plants or their extracts is summarized in Table 4.

Table 4 Different drugs and herbal plants used in treatment of COVID-19.

<table>
<thead>
<tr>
<th>Drug name &amp; Composition</th>
<th>Function</th>
<th>Case studies/Findings</th>
<th>Comments (Suggestions/Side effects)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciclesonide (steroid drug)</td>
<td>Represses asthmatic spasms, inhibits eosinophils percolation into respiratory canal, and represses synthesis of IL 4, 5 and TNF-α.</td>
<td>A study shows that respiratory status in patients of COVID-19 can be improved by this drug.</td>
<td>It contains less systemic side effects &amp; is virtuous for additional study in clinical trials.</td>
<td>(Nakajima et al., 2020)</td>
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<tr>
<td>Metronidazole (contain redox operative pro-drugs)</td>
<td>Reduces levels of IL-8, 1B, 6, 12, TNF-α, interferon gamma, neutrophil count &amp; ROS, CRP.</td>
<td>Can serve as possible candidate to prevent most of immunopathological</td>
<td>The need is to do clinical examination with huge sample size to find out its</td>
<td>(Bayraktar et al., 2005)</td>
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<tr>
<td>Drug</td>
<td>Characteristics</td>
<td>Efficiency to Cure COVID-19</td>
<td>Further Analysis</td>
<td></td>
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<tr>
<td>Teicoplanin (Glycopeptides immunoglobulin)</td>
<td>Used to cure bacterial infections, but already efficient against viruses, acts on the initial step of virus life cycle.</td>
<td>Zhou et al reported that it stops liberation of viral genomic RNA &amp; the protraction of virus replication occurs.</td>
<td>Further analysis about its effect against COVID-19 is needed &amp; it suggests that this drug can serve as potential alternative to cure COVID-19. (Colson &amp; Raoult, 2016)</td>
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<tr>
<td>Thalidomide combined with low dose glucocorticoid</td>
<td>Anti-inflammatory, inhibits cell proliferation, decreases pulmonary fibrosis and lung injury.</td>
<td>Clinical condition, absolute lymphocyte value and cytokine level were improved by a study of Chen et al. Improved absorption of pulmonary exudation was also obtained.</td>
<td>It may be used as adjuvant treatment strategy for potentially fatal viral disease. (Wen et al., 2018)</td>
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<td>Hydroxychloroquine &amp; azithromycin</td>
<td>Also effective in removing nasopharyngeal carriage of virus in 3-6 days</td>
<td>A clinical trial performed by P Gautret et al. showed that all patients receiving these drugs were cured at the day 6 of post inclusion</td>
<td>These findings need further research to check whether this combination is effective in severe cases. (Biot et al., 2006)</td>
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<td>Chloroquine (multi-purpose bioactive agent in vitro)</td>
<td>Prevent binding of virus to cell surface, may interfere at the budding step of the replication cycle of virus, can disable viral proteins proper maturation. Reduces level of pro-inflammatory cytokines</td>
<td>Recommended as an effective drug against COVID-19, presently ten clinical trials are trying to test the effectiveness of this drug as an anti-COVID-19 therapy</td>
<td>A survey study is needed to check the adverse effects of therapy done by using this drug. (Randolph et al., 1990)</td>
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<tr>
<td>Indian herbal plants (Giloy, aloe vera, neem, ginger, turmeric, ashwagandha etc)</td>
<td>Protease inhibitors of 2019-nCoV.</td>
<td>In a study by Ambrish K. Srivastava et al. the inhibition capability of their extracts was found.</td>
<td>Can serve as effective therapeutic agents against COVID-19 infection due to their non-toxic nature. (Srivastava et al., 2020)</td>
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Traditional Chinese Medicines

Halt the activity of SARS 3CLpro enzyme, also halt helicase protein of SARS-CoV namely, nsP13 in laboratory by disturbing ATPase activity.

Inhibition of lung inflammation & reduced levels of cytokines along with IL 6 and 3 is obtained by the injection of Shen Fu, as described by Wang, et al.

Convincing evidence (Chen et al., 2002) is that herbal products of some traditional Chinese medicines or their parts possess strong immuno-suppressive impacts.

Treatment using antibodies and Vaccines

Monoclonal antibodies target susceptible sites on the surface of viral proteins, first reported monoclonal antibody that reacted with 2019-nCoV was 47D11. In a latest report, use of convalescent plasma was identified as a possible treatment for COVID-19 (Zhang et al., 2020). A meta-analysis from previous study revealed a decrease in death rate by receiving diverse dosage of convalescent plasma in patients having serious respiratory infections, with no unfavorable events or problems after treatment (Mair-Jenkins et al., 2015). One practicable explanation for the efficiency of convalescent plasma therapy is that immunoglobulins from convalescent plasma could reduce viraemia. Hence, it can be beneficial to test the security and effectiveness of convalescent plasma transfusion in COVID-19 infected patients. Antigen S has been involved in various kinds of vaccines against contamination by CoVs (Yu et al., 2020). Moderna declared the commencement of phase#1 testing of mRNA vaccine-1273 on 16 March. On 24 February 2020, Moderna declared the release of first batch of this vaccine for use in humans against 2019-nCoV (Anon., 2020). GlaxoSmithKline (GSK) declared a co-operation with a Chinese company to assess COVID-19 vaccine. This participation was able to produce COVID-19 S Trimer vaccine with adjuvant system. Us20060039926 (patent) application revealed live attenuated torovirus or coronavirus vaccines. Interestingly, GLS-5300 (INO-4700) is already under phase#1 clinical analysis. The vaccine was well-tolerated, inducing immunoglobulin response in 94% of patients after 3 injections. Its immune reactions were not dependent on dose and it was effective through 1 year of follow-up (Modjarrad et al., 2019). It was suggested by a research, that BCG vaccine seemed to significantly decrease number of deaths caused by COVID-19 (Miller et al., 2020). It is necessary to synthesize safe and efficacious vaccines to restrain COVID-19 epidemic, eradicate its spread and eventually stop its recurrence in future.

COVID-19 AND COMORBIDITIES

Comorbidities are risk factors for 2019-nCoV infection. Diseases like CVD (cardiovascular diseases), DB (diabetes mellitus), HTN (hypertension), RSD (respiratory system disease) and their susceptibility states, might be connected to the generation and development of COVID-19. These metabolic diseases can impair the function of macrophages and lymphocytes making a person more vulnerable to complication of other diseases. In a research, among patients having serious symptoms of COVID-19, 25%, 44% and 58% people had heart diseases, arrhythmia and hypertension respectively (Bai et al., 2020). In accordance
with mortality data provided by NHC, 17% patients with COVID-19 had history of CHD, while 35% patients had hypertension.

Similarly, it has been reported that patients with T2DM (type-2 diabetes mellitus) and metabolic syndromes when they were infected with COVID-19, had increased chances of death by up to ten-times (Yang et al., 2006). During the course of SARS in 2003, multiple organ involvement such as gastrointestinal tract, liver and kidney was reported. Recently, quite similar multi-organ involvement has been documented in COVID-19 patients. It has been reported that 13.1% and 14.4% patients in the study had impaired BUN (blood urea nitrogen) and creatinine levels (Cheng et al., 2020). Moreover, in a recent analysis done by China (Sidaway, 2020), 18 out of 1590 COVID-19 infected individuals had a history of cancer. The available evidence is insufficient, but it proposes that symptoms of COVID-19 are very likely more critical in patients having cancer as compared to those without. Hence, we may assume that underlying diseases like DB, HTN, CVD, RSD and cancer may be risk factors for severe patients of COVID-19 in comparison with non-severe patients.

**CONCLUSION**

This review holistically explains the current research done in response to outbreak of COVID-19 and 2019-nCoV, mainly focusing on the phylogenetic and epidemiological analysis of Pakistani coronavirus. Our epidemiological analysis of available data showed less prevalence rate of COVID-19 along with less mortality rate of affected individuals in Pakistan as compared to other countries affected with COVID-19. Phylogenetic analysis confirmed that Pakistani n-CoV strain resembled with bat SARS-like corona virus. Epidemiological history, clinical presentation, and few auxiliary assessments like ELISA, POCT of IgG and IgM, detection of nucleic acid, blood culturing and CT scans are methods for the clinical diagnosis of COVID-19. Until now, specific vaccines or anti-viral medicines against COVID-19 do not exist for potential cure of humans. The only alternative available is utilization of broad spectrum anti-viral medicines and other drugs to reduce associated symptoms like respiratory issues, inflammation etc. It is concluded that COVID-19 associated symptoms and mortality may be reduced mainly by adapting preventive measures as up to now no specific vaccine is available for its cure.

**DECLARATION OF CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**ACKNOWLEDGEMENTS**

We acknowledge efforts of different countries of the world, as they participated in knowing about 2019-nCoV and COVID-19 as well as in reducing the impact of this pandemic on susceptible populations.

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