FROM SARS-CoV TO MARS-CoV

Editors:
Kamal Niaz
Muhammad Sajjad Khan
Muhammad Farrukh Nisar

Bentham Books

Genetic Diversity of Coronaviruses

(Volume 1)

From SARS-CoV to MARS-CoV

Edited by

Kamal Niaz
Department of Pharmacology and Toxicology
Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur-63100, Pakistan

Muhammad Sajjad Khan
Cholistan University of Veterinary and Animal Sciences
Bahawalpur-63100, Pakistan

&

Muhammad Farrukh Nisar
Department of Physiology and Biochemistry
Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur-63100, Pakistan

Genetic Diversity of Coronaviruses

(Volume 1)

From SARS-CoV to MARS-CoV

Editors: Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar

ISBN (Online): 978-981-5274-94-3

ISBN (Print): 978-981-5274-95-0

ISBN (Paperback): 978-981-5274-96-7

First published in 2024.

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

PREFACE

Coronaviruses, such as severe acute respiratory syndrome-coronavirus (SARS-CoV) and Middle East respiratory syndrome-coronavirus (MERS-CoV), have posed significant public health threats in the last two decades. It has been revealed that bats act as natural reservoirs for these viruses, and periodic monitoring of coronaviruses in bats, dogs, civets, and other wild animals may thus provide important clues about emergent infectious viruses that transfer to humans. The Eastern bent-wing bat *Miniopterus fuliginosus* (*M. fuliginosus*) and genus *Rhinolophus* are distributed extensively throughout China and other countries. Therefore, there is a need to analyze the genetic diversity of coronaviruses transmitted to humans. The only coronavirus genus found was *alphacoronavirus*. The established *alphacoronavirus* genome sequences showed high similarity to other *alphacoronaviruses* found in other *Miniopterus* species and other animals. It suggests that their transmission in different *Miniopterus* species may provide opportunities for recombination with different *alphacoronaviruses*. The genetic information for these novel *alphacoronaviruses* will improve our understanding of the evolution and genetic diversity of coronaviruses, with potentially important implications for the transmission of human diseases. This virus is different from the previously isolated MERS-CoV and SARS-CoV, which are the seventh ones that can infect humans.

In Volume 1 of this book proposal, we consolidated the genetic diversity/mutation that occurred in 2002-12. Since both SARS-CoV and MERS-CoV are closest, the approaches discussed here will be similar and/or varying by a slight degree. In the last 18-19 years, this is the third outbreak of the same coronavirus with a slight mutation that shocked the whole world. This book should be prioritized as up-to-date literature on genetic mutations that have occurred in the form of SARS-CoV and MERS-CoV. It will act as a suitable reference if any such outbreak appears in the near future. Volume 1 of this proposed book proposal has been classified into two parts: Part I: Genetic Mutation of SARS-CoVand Part II: Genetic Mutation of MERS-CoV.

With the emergence of new coronavirus variants, different host tropism permits a thorough analysis of their genomic diversity/mutations that acquired adaptability to their host. Thus, in Part I, we start the book with chapters dealing with mutations in SARS-CoV, the host genetic diversity of SARS-CoV, newly emerging variants of SARS-CoV, the genetic architecture of host proteins involved in SARS-CoV, and the landscape of host genetic factors correlating with SARS-CoV. In Part II, a critical analysis of the MERS-CoV involves the potential to mutate its genome by opposite genetics and to get better recombinant viruses with described mutations. Such processes will assist in studying the capabilities of particular genes and their effects on virus survival and pathogenesis. These strategies can even help in determining host factors correlating with MERS-CoV genome growth and proliferation.

This book will appear as a baseline for scientists and health professionals to better understand the genetic diversity of SARS-CoV and MERS-CoV. However, this single book would not have succeeded without the enthusiasm and determination of publishers and investigators to take time from their hectic schedules and endow on time. We thank the scrutineers who contributed, directly and indirectly, to bring it to realism.

Kamal Niaz
Department of Pharmacology and Toxicology
Faculty of Bio-Sciences
Cholistan University of Veterinary and Animal Sciences
Bahawalpur-63100, Pakistan

Muhammad Sajjad Khan
Cholistan University of Veterinary and Animal Sciences
Bahawalpur-63100, Pakistan

&

Muhammad Farrukh Nisar
Department of Physiology and Biochemistry
Faculty of Bio-Sciences
Cholistan University of Veterinary and Animal Sciences (CUVAS)
Bahawalpur-63100, Pakistan

List of Contributors

Amjad Khan	Department of Epidemiology and Public Health, The University of Haripur, Pakistan
Arooj Fatima	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan
Atteeqah Siddique	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan
Amjad Islam Aqib	Department of Medicine, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan
Asghar Abbas	Department of Parasitology, Muhammad Nawaz Sharif University, Multan, Pakistan
Asif Javaid	Department of Animal Nutrition, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan
Abdul Basit	Department of Microbiology, University of Jhang, Punjab, Pakistan
Chen Shanyuan	School of Life Sciences, Yunnan University, Kunming 650091, China
Etab Saleh Alghamdi	Department of Food and Nutrition, King Abdul-Aziz University, Jeddah, Saudi Arabia
Firasat Hussain	Department of Microbiology, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan
Faisal Siddique	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan
Hamid Majeed	Department of Food Science and Technology, Faculty of Biosciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan
Hamid Ali	Department of Biosciences, COMSATS University, Islamabad, Pakistan
Hidayat Ullah	Department of Agriculture, The University of Swabi, Anbar-Swabi - 23561, Khyber Pakhtunkhwa, Pakistan
Hayat Khan	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan
Inam Ullah Khan	Institute of Microbiology, Faculty of Veterinary and Animal Sciences, Gomal University, D.I. Khan 29220, Khyber Pakhtunkhwa, Pakistan
Imtiaz Ali Khan	Department of Entomology, The University of Agriculture, Peshawar 25000, Pakistan
Ihtesham ul Haq	Department of Biosciences, COMSATS University Islamabad (CUI) 45550, Pakistan
Imtiaz Ali Khan	Department of Entomology, The University of Agriculture, Peshawar 25000, Pakistan
Kashif Rahim	Department of Microbiology, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan

Khawar Ali Shahzad	Department of Zoology, Baghdad Campus, The Islamia University of Bahawalpur, Pakistan
Muhammad Nisar	Department of Epidemiology & Public Health, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan
Muhammad Shuaib	School of Ecology and Environmental Science, Yunnan University, Kunming, China
Mukhtar Alam	Department of Agriculture, The University of Swabi, Anbar-Swabi - 23561, Khyber Pakhtunkhwa, Pakistan
Muhammad Adnan	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan
Muhammad Naveed Nawaz	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan
Muhammad Kalim	Department of Microbiology and Immunology, Wake Forest University Winston-Salem, NC 27101, USA Houston Methodist Hospital Research Institute, Houston, Texas, 77030, USA
Muhammad Saeed	Department of Poultry Science, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan
Muhammad Zeeshan Iqbal	Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan
Muhammad Sajid	Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur
Muhammad Bilal Bin Majeed	Department of Animal Breeding and Genetics, Faculty of Animal Production and Technology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan
Muhammad Safdar	Department of Breeding and Genetics, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan
Muhammad Sajjad Khan	Department of Breeding and Genetics, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan
Muhammad Naveed	Department of Clinical Pharmacology, School of Pharmacy, Nanjing Medical University, Nanjing 211166, China
Muhammad Javed	Department of Microbiology, The University of Haripur, Pakistan
Rao Zahid Abbas	Department of Parasitology, University of Agriculture, Faisalabad, Pakistan
Saigha Marriam	Department of Microbiology & Molecular Genetics, Faculty of Life Sciences, University of Okara, Punjab, Pakistan

Sana Tehseen	Department of Food Science and Technology, Faculty of Science and Technology, Government College Women University, Faisalabad, Punjab, Pakistan
Sidra-Tul-Muntaha	Department of Food Science and Technology, Faculty of Science and Technology, Government College Women University, Faisalabad, Punjab, Pakistan
Safdar Abbas	Department of Animal Breeding and Genetics, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan
Tehmeena Nousheen	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan
Umair Younas	Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan
Wen-Jun Li	State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, College of Ecology and Evolution, Sun Yat-Sen University, Guangzhou 510275, PR China
Zia-ud-Din Basit	Department of Community Medicine, Kohat Institute of Medical Sciences, Pakistan

CHAPTER 1

History of SARS-CoV

Muhammad Zeeshan Iqbal[1], Muhammad Bilal Bin Majeed[2], Muhammad Saeed[3], Muhammad Safdar[4], Kashif Rahim[5], Firasat Hussain[5], Hamid Majeed[6] and **Umair Younas[1,*]**

[1] *Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

[2] *Department of Animal Breeding and Genetics, Faculty of Animal Production and Technology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan*

[3] *Department of Poultry Sciences, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

[4] *Department of Breeding and Genetics, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

[5] *Department of Microbiology, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

[6] *Department of Food Science and Technology, Faculty of Biosciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

Abstract: Severe acute respiratory syndrome-coronavirus **(**SARS-CoV) is a viral disease of the respiratory system with zoonotic importance. It was initially reported in Southern China (province: Guangdong) in mid-November (2002). This disease showed a viral spread to more than thirty countries belonging to five different continents and infected 8098 people, out of which 774 died. The emergence of SARS has been found to be due to human-animal contact. SARS-CoV is not harmful in children, and there is no vertical transmission from mothers to newborns. In pediatric age groups, no death has been reported. Most SARS autopsies cases showed extensive spleen and white pulp necrosis with severe depletion of lymphocytes. The genomic sequence of SARS-CoV is detected through RT-PCR in some specimens of the brain and cerebral spinal fluid. The pathogenesis of SARS is very complex as multiple factors are involved. With the prevalence of SARS-CoV, many diseases are associated with and cause damage to different organs and systems of the body. Some strategies that can help treat SARS-CoV are host-directed therapies, the use of antibiotics, inhibitors of viral and host proteases, and interferons. The World Health Organization (WHO) issued an alert on 12th March 2003 about new deadly infectious diseases globally. After three days, the WHO named these diseases SARS. China, Singapore, Taiwan, and Hong Kong were the most severely affected areas.

[*] **Corresponding author Umair Younas:** Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan; E-mail: umairyounas@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

Keywords: China, History, SARS, WHO.

INTRODUCTION

Primary epidemiological investigations revealed that severe acute respiratory syndrome-coronavirus (SARS-CoV) has an animal origin. The emergence of SARS has been found to be due to human-animal contact [1]. Horseshoe bats belonging to the genus *Rhinolophus* were found to be the natural reservoirs of SARS coronaviruses. Samples were collected from a live animals' market, and closely related viruses were found in palm civets. Chinese scientists also found the same virus in Asian palm civets and cave-dwelling horseshoe bats in China [2]. Retrospective studies on human populations prove the absence of antibodies against SARS-CoV in humans before the onset of the SARS-CoV outbreak [3]. Genetically diverse SARS-CoVs were identified in Chinese horseshoe bats as natural reservoir hosts [4]. However, no evidence had been reported regarding the transmission of SARS-CoVs from bat to human. The epidemiological investigations prove the zoonotic origin of SARS-CoV [5]. The isolation and identification of SARS-CoV from masked palm civets and its detection in the serum of people involved in the trade of civets suggest that masked palm civets could be a possible source of infection initially in Guangdong people and later in the world. Furthermore, culling drastically decreased the number of infected animals in the marketplaces of Guangdong [6].

Initially, the SARS coronavirus species were reported as SARS-CoV. There was a great epidemic in China due to SARS-CoV in 2002-2004. Later, around 2017, Chinese scientists found the SARS-CoV virus in Asian palm civets and horse how cave-dwelling bats and reported them as intermediate hosts of this virus [2]. Since 2004, no new cases have been reported anywhere in the world. SARS-CoV is not harmful in children, and there is no vertical transmission from mothers to newborns. In pediatric age groups, no death has been reported [7]. The teenage patients showed symptoms of myalgia, malaise, and rigor similar to adults, while younger children showed runny nose and cough, and none showed myalgia, rigor, or chills. In younger children, there was a mild clinical course with a probable short duration.

Similarly, the radiological findings were also not serious, and these cases were resolved quickly compared to the teenager group [8]. From the histopathological findings perspective, the SARS-CoV that affected the patient's lungs showed diffuse alveolar damage (DAD). During the first 7-10 days, extensive lung edema occurs. Then, the hyaline membrane is formed, which leads to the collapse of alveoli and the desquamation of alveolar epithelial cells. Fibrosis occurs, and fibrous tissue is formed in alveolar spaces. If the disease persists longer, DAD

appears after 10-14 days [9 - 11]. The periarterial sheaths in the spleen decrease more sharply. The presence of infection in T lymphocytes and macrophages in the spleen shows a high viral load in splenic cells [12 - 14]. The kidneys of SARS-CoV patients were autopsied, and focal necrosis was found along with small-vessel vasculitis in renal and intestinal tissue [15]. The most commonly reported cases were gastrointestinal manifestations. SARS-CoV indirectly affects certain other organs of the body [12]. It is reported that more than 20% of patients had diarrhea, and up to 67% showed signs of developing diarrhea [16 - 18]. During illness, it infects the tubular epithelial cells of the kidney, mucosal cells of the intestines, several types of immune cells, and brain neurons.

This disease's estimated case fatality ratio was 15% [19]. This disease has caused a huge negative impact on population health, China's economy, and national and international security. Its outbreak was critical for the country's economy and society [20]. The impact of SARS-CoV was quite serious, both socially and psychologically. This disease has profoundly impacted human society, particularly in China. Mental stress was developed in society. By March 2003, no information was confirmed officially, but the epidemic was spreading, and people started to believe rumors and purchase anti-viral drugs in Guangdong and Beijing [20]. The projected macroeconomic influence of SARS-CoV was around 3-10 million US dollars per case globally [21]. The losses caused by the 2003 SARS-CoV were about 12.3-28.4 billion US$. The projected downfall of the GDP of China and South Asia was 1% and 0.5%, respectively [22].

Animal Perspective History of SARS-CoV

The emergence of infectious diseases is primarily an ecological process. Most infectious diseases (75%) that affect human health are zoonotic. The reservoir of these diseases is direct contact of humans with wildlife or domestic animals. The zoonotic disease can be attributed to habitat fragmentation/deforestation, agricultural extension, global trade, and urbanization. These factors enhance the interaction between humans and domestic/wildlife populations, thus increasing the chances of the occurrence of spilling-over events. The emergence of SARS-CoV in China in 2003 took place due to human-animal contact [1].

The SARS-CoV was initially reported in Southern China (Guangdong) in mid-November (2002). This disease showed a viral spread to over thirty countries belonging to five different continents and infected 8098 people, out of which 774 died [23]. Primary epidemiological investigations revealed that SARS-CoV has an animal origin. Samples were collected from a live animals' market and found the closely related virus in palm civets. Horseshoe bats belonging to the genus *Rhinolophus* were found to be the natural reservoir of SARS-CoVs. Chinese

CHAPTER 2

Molecular Epidemiological Analysis of SARS-CoV

Sana Tehseen[1], Sidra-Tul- Muntaha[1], Muhammad Sajjad Khan[2], Muhammad Saeed[3], Muhammad Naveed[4], Muhammad Safdar[2], Firasat Hussain[5] and **Umair Younas[6,*]**

[1] *Department of Food Science and Technology, Faculty of Science and Technology, Government College Women University, Faisalabad, Punjab, Pakistan*

[2] *Department of Breeding and Genetics, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

[3] *Department of Poultry Science, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

[4] *Department of Clinical Pharmacology, School of Pharmacy, Nanjing Medical University, Nanjing 211166, China*

[5] *Department of Microbiology, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

[6] *Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

Abstract: Coronaviruses **(**CoVs) are a large and distinct group of RNA viruses that can affect a wide range of animal species. These are spherical-shaped viruses with spike (S) proteins positioned from the virion surface. The severe acute respiratory syndrome-coronavirus (SARS-CoV) has a 30 kb RNA genome with 14 open reading frames flanked by 5′ and 3′ UTR sections. The 5′ untranslated region is 265 bp long, while the 3′ end is 342 bp long. Normally, the coronavirus S protein is fragmented into 2 subunits, S1 and S2, though in the context of SARS-CoV, an un-cleaved type one transmembrane S protein with S1 and S2 subunit homology has been discovered. CoVs are classified into four genera based on genetic and antigenic characteristics: α, β, γ, and δ. α and β CoVs only infect mammals, whereas primarily γ and δ infect birds, though some can infect mammals as well. In 29 regions and countries, many deaths and cases were reported due to the outbreak of SARS. Initially, the cases were reported in 2002 in China. In 2003, the outbreak of atypical pneumonia was first time reported by WHO, and five deaths and 306 cases were reported in China due to this outbreak. Molecular epidemiology studies revealed that the virus from the 2002–2003 South China pandemic was distinct from the unique virus isolated in similar areas in the late 2003 and early 2004 epidemics, presenting distinct species-crossing events. SARS-CoV has a wide host range. Different studies also showed that this virus can proliferate in ferrets and macaques; however, in cats, no symptoms of this virus were found.

* **Corresponding author Umair Younas:** Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan; E-mail: umairyounas@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

Keywords: RNA viruses, SARS-CoV, Molecular epidemiology, Coronavirus, A typical pneumonia, Spike (S) proteins.

INTRODUCTION

Coronaviruses (CoVs) are 125 nm large distinct groups of RNA viruses that can affect a wide range of animal species [1, 2]. The viral enclosure is mandatory for the attachment of the target cell, which promotes the subsequent steps of replication [3]. Once the envelope of the virus is disintegrated, coronavirus is unable to reproduce [4]. The CoVs are spherical-shaped viruses with spike (S) proteins positioned from the virion surface, like solar crowns, as shown by transmission electron microscopy images, hence the name "CoVs" [5]. SARS-CoV has a 30 kb RNA genome with 14 open reading frames flanked by 5′ and 3′ UTR sections [6]. The 5′ untranslated region is 265 bp long, while the 3′ end is 342 bp long. SARS-CoV is considered particularly susceptible to temperature, chemicals, sanitizers, soaps, and disinfectants [7].

Normally, the coronavirus S protein is fragmented into 2-subunits, S1 and S2, though in the context of SARS-CoV, an un-cleaved type one transmembrane S protein with S1 and S2 subunit homology has been discovered [8 - 10]. The 193 amino acid segment of S protein is active in infection, and its 318–510 residues specifically engage with the angiotensin-converting enzyme2 (ACE2) receptor [11 - 13]. The receptor-binding domain (RBD) on S aids virus binding to the human ACE2 peptidase domain [14 - 16]. The RBD ultrastructure revealed that it is structurally modified and that its concave area cradles the peptidase N-terminal, giving a site for SARS-CoV attachment [17, 18]. In 29 regions and countries, many deaths and cases were reported due to the outbreak of SARS. The first cases were reported in 2002 in China. In 2003, the outbreak of atypical pneumonia was first time reported by WHO, and by this outbreak, five deaths and 306 cases were reported in China [19]. When people travel, this disease spreads rapidly throughout the world. But after its appearance, within seven months, it was controlled. After that, it did not appear, but it is still present in some wild animals and can re-emerge in humans, like Ebola, which, after a small outbreak, re-originated after 15 years [20, 21]. Molecular epidemiology studies revealed that the virus from the 2002–2003 South China pandemic is distinct from the unique virus isolated in a similar area in the late 2003 and early 2004 epidemics, presenting distinct species-crossing events [22].

CoVs are classified into four genera based on genetic and antigenic characteristics: α, β, γ, and δ. α and β CoVs only infect mammals, whereas primarily γ and δ infect birds, though some can infect mammals as well. The 1st two proteins are usually directed through the ER (endoplasmic reticulum) and the

Golgi apparatus. The RNA protein complex then forms a composite with M protein. The nucleocapsid (N) particle buds into ER following the Golgi apparatus, and by exocytosis, it emerges outside the cell. With these proteins, a significant number of auxiliary protein sets are generated, and their sequence varies within the coronavirus family, giving it the particular importance of having huge polyproteins [23]. CoVs are only slightly shorter than planarian secretory cell nidovirus (41 kilobases [kb]) among RNA viruses, with genome sizes ranging from 26 to 32 kb [7, 24]. It is reported that there are 1,255 amino acids and 23 N-linked glycosylation convention signals in the SARS-S protein, making it a type I transmembrane protein [25]. It is stated that endogenous ACE2 expression is linked to cell line susceptibility to SARS-CoV infection [25 - 27]. The ectopic expression of ACE2 makes it easier for SARS-S to infect previously non-susceptible cells [28]. Ultimately, it was discovered that knocking down ACE2 in mice reduced vulnerability to SARS-CoV infection [3]. ACE2 appears to be a genuine SARS-CoV receptor, as it is both essential and appropriate for the viral entrance into cells of the host [3].

Animal viruses have no receptor-binding motif (RBM)-like sequence in their S protein, and they do not utilize ACE2 for cellular entrance [29]. Therefore, SARS-CoV was most likely transmitted to humans through palm civets, and an intermediate host that carries viruses along with a high sequence resemblance to human SARS-CoV could be a candidate [30, 31]. The fatality rate of SARS is low compared to the Middle East respiratory syndrome-coronavirus (MERS-CoV), as SARS had an 11% death rate while MERS had 35%. SARS has a high reproductive number and survives for a duration of 8 months, while MERS has a low reproductive number but spreads irregularly for 4 years [9, 32 - 34]. Studies also showed that besides humans, coronavirus has a wide host range. From the civet market, it is also possible that the virus directly transfers into human beings and then emerges in bats [35, 36]. Different studies also showed that this virus can produce and germinate in ferrets and macaques, but in cats, no symptoms of this virus were found [37, 38]. The serological evaluation was used to dictate the existence of antibodies against accessory proteins in patients affected by SARS-CoV [39]. According to a study, anti-ORF8a antibodies were present in 2 out of 37 patients, indicating that ORF8a was expressed during infection *in vivo* [40]. Just 5.4% of patients found that they had antibodies of anti-ORF8a, and it was shown that ORF8a is not highly immunological or is expressed at very low levels [40].

SARS-CoV Genome Structure/Organization

CoVs are 125 nm large and distinct groups of RNA viruses that can affect a wide range of mammals, amphibians, reptiles, and birds [1, 2]. A positive sense RNA

CHAPTER 3

Mutations in SARS-CoV

Firasat Hussain[1,*], Muhammad Javed[2], Amjad Khan[3], Kashif Rahim[1], Muhammad Naveed Nawaz[1], Amjid Islam Aqib[7], Muhammad Shuaib[4], Khawar Ali Shahzad[8], Umair Younas[6] and **Imtiaz Ali Khan[5]**

[1] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[2] *Department of Microbiology, The University of Haripur, Pakistan*

[3] *Department of Epidemiology and Public Health, The University of Haripur, Pakistan*

[4] *School of Ecology and Environmental Science, Yunnan University, Kunming, China*

[5] *Department of Entomology, The University of Agriculture, Peshawar 25000, Pakistan*

[6] *Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[7] *Department of Medicine, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[8] *Department of Zoology, Baghdad Campus, The Islamia University of Bahawalpur, Pakistan*

Abstract: The coronavirus family is named for the large spike protein molecules found on the pathogen exterior, which give the virus a crown-like appearance, the coronavirus genome is the biggest among RNA viruses. There are about seven viruses capable of infecting humans: in the alpha genus, there are 229E and NL63, and in the beta genus, there are OC-43, HKU1, MERS-CoV, SARS-CoV, and SARS-CoV-2. The severe acute respiratory syndrome coronavirus (SARS-CoV) is a positive-stranded RNA virus. In humans, the virus is transmitted through respiratory tract droplets or discharges from diseased persons. The reservoir hosts for MERS-CoV are camels, while those for SARS-CoV are most likely bats. SARS-CoV-2 infecting a snake may have been transmitted by zoonotic transmission in a palm civet. The Chinese viruses SARS-Co--2 and SARS-CoV have many things in common, including contact with wild animals.However, both SARS-CoV-2 and MERS-CoV have the ability to persist and spread the illness even when the infected individuals are untreated. SARS-S1 CoV-2's components of the spike proteins have 75% structural commonality with SARS-like CoVs in bats and SARS-CoV. According to genetic comparisons, the latest investigations have proven that SARS-CoV-2 targets angiotensin-converting enzyme type-2 (ACE-2) in humans. However, SARS-CoV-2 possesses an identical receptor-binding domain (RBD) pattern to SARS-CoV, with differences in amino acid sequences at certain vital positions. The RBD is also found in the C-domain S1

* **Corresponding author Firasat Hussain:** Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan;
E-mail: firasathussain@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

component of MERS-CoV's S protein (Spike). Conversely, in contrast to SARS-CoV, MERS-CoV uses a dipeptidyl-peptidase-4 (DPP-4) helix as its binding site. Similarly, MERS-RBD coronaviruses (CoVs) have an extra subdomain that functions as the receptor-binding motif (RBM).

Keywords: SARS-CoV, SARS-CoV-2, MERS-CoV, Mutations, Emergence, Phylogeny, Intermediate host, Conserved region.

INTRODUCTION

Humans and other animals are at risk from coronavirus (SARS-CoV) as it had affected 8,000 people between 2002 and 2003, with a 10% death rate [1]. CoVs (Coronaviruses) belong to the order Nidovirales and the family Coronaviridae [2, 3]. *Alphacoronaviruses*, *Betacoronaviruses*, *Gammacoronaviruses*, and *Deltacoronaviruses* are the four genera, and SARS-CoV belongs to beta-CoVs. In the Chinese region of Guangdong, where SARS-CoV was first reported in November 2002, an epidemic ran from January 2003 to April 2003 [4]. The inaugural case of the SARS epidemic in the Hong Kong Special Administrative Region dates back to February 2003. Since then, the infection has spread worldwide and infected 8098, out of which, 744 deaths were reported in more than 30 nations [5]. Chills, lingering fever, lethargy, muscle aches, breathlessness, a dry cough, and headaches are the most typical clinical signs. Difficulty swallowing, sputum generation, runny nose, vomiting, diarrhea, and anxiety are among the less common symptoms [6, 7].

The SARS-CoV was spread *via* the palm civet as an intermediary carrier, which had its origins in bats of the Hipposideridae family [8]. SARS-CoV is a capsulated, positive-sense RNA with one strand virus. The genome, which is nearly 27,000 bp, encodes the replicas along with the spike, nucleocapsid, membrane, and envelope protein [9, 10]. The gene of spike protein is split into carboxyl and amino subunits (S2 & S1) in most animal and human coronaviruses; the amino subunit is involved in binding with the receptor, while the subunit with carboxyl region is a transmembrane protein with conserve sequences that mediate viral fusion to the cell membrane [11]. Many coronaviruses (CoVs) originate from animals that go through homologous mutation and adaptation, ending in changed CoVs that are extremely dangerous and could be fatal to humans [12].

The SARS-CoV genomic alteration ratio was assumed to be 0.80-2.38 103 nucleotide replacements per site annually, with non-synonymous and synonymous modification rates of 1.16-3.30 103 and 1.67-4.67 103 per site yearly, respectively. These values are similar to those of other RNA viruses [13]. Many coronaviruses originating from animals go through genetic variation and transformation within their host organism or when they cross species boundaries.

As a result, such modifications may result in variations with high pathogenicity when transferred to humans [14, 15].

First Reported Genome of Coronavirus

Virions were isolated and cDNA was synthesized using viral RNA recovered from the cells. To proceed with rtPCR, a primers pair covering the whole viral genome was utilized, with a product size category of 400-800 base pairs. PCR-amplified pieces were replicated into amplicon libraries, and two hundred or more clones were analyzed for each PCR-amplified component to assure sequencing integrity and prevent problems and cloning techniques. Although every reading was generated and specific sequence differences were discernible, only consensus sequences with overall majority votes were selected for genomic sequences and extra processing.

The RNA genome, which is positive sense in a spheroid capsid, is roughly 30 kb, making it the biggest of its kind [16]. The structure of the 29,740 bases of the genome of the SARS-CoV was uncovered by sequence data, revealing the typical hallmarks of coronaviruses [17]. A projected RNA leader sequence precedes a 192-nucleotide untranslated region (UTR) on nucleotides 1–72. The UTR is followed by two overlapped coding sequences covering around two-thirds of the genome. A translating read-through enables the translations of the overlapped transcriptional regions in one protein complex by a 1 ribosomal frameshift method [18]. The protein is broken down into separate peptides by virus-encoded cysteine proteases called 3C and papain, which could produce all the polypeptides required for translation and proliferation [18, 19]. A few of these proteins, combined with host proteins, may constitute the viral replication–transcription complex, which is linked to the affected cells' membrane structures [20, 21]. The four structural components encoded by the remaining 3′ half of the genome in all coronaviruses are E (envelope), N (nucleocapsid), S (spike), and M (membrane proteins). Moreover, the SARS-CoV genome's structural protein section includes numerous genes encoding non-structural proteins termed “optional extra genes”. Many of these compounds appear to be unnecessary for viral survival in *vitro* and in *vivo*; their absence results in weakened viruses [22]. Eventually, a secondary 340-nucleotide UTR was discovered at the 3′ ends of the genome, which is preceded by a poly(A) region (Fig. **1**). Moreover, astroviruses, one equine rhinovirus, and the avian infectious bronchitis virus (IBV) have been shown to contain a 32-nucleotide stem-loop-like domain [23] (Fig. **2**). The existence of a sequence that regulates transcription is critical in RNA expression, and control is a common characteristic of coronaviruses.

CHAPTER 4

Host Genetic Diversity of SARS-CoV

Firasat Hussain[1,*], Hamid Ali[2], Ateeqah Siddique[1], Kashif Rahim[1], Umair Younas[3], Faisal Siddique[1], Muhammad Nisar[4], Muhammad Shuaib[5], Muhammad Saeed[6], Wen-Jun Li[7] and **Arooj Fatima[1]**

[1] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[2] *Department of Biosciences, COMSATS University, Islamabad, Pakistan*

[3] *Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[4] *Department of Epidemiology & Public Health, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[5] *School of Ecology and Environmental Science, Yunnan University, Kunming, China*

[6] *Department of Poultry Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[7] *State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, College of Ecology and Evolution, Sun Yat-Sen University, Guangzhou 510275, PR China*

Abstract: SARS-CoV has an RNA genome that is categorized in the family *Coronaviridae* and the order *Nidovirales*. Similarly, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) also belongs to this family and order. A significant degree of variability was observed in SARS-CoV-2 in individuals, which could be influenced by genetic variations in the host. This may impact the progression of sickness and the efficacy of treatment approaches. Individuals who carry certain mutants of genes (ACE2 and TMPRSS) directly linked to viral illness or who have a distinctive expression of those genes may be more vulnerable (SARS-CoV-2). These alterations may explain the enormous diversity of symptoms and severity of Coronavirus Disease-19 (COVID-19)-related disease in various people. Regarding variation, the D614G spikes gene is the most varied among hosts. Moreover, single nucleotide polymorphism (SNP) and single nucleotide variants (SNVs) are causes of host genetic diversity, according to some studies. The structure of SARS-CoV is made up of structural and accessory proteins. These accessory proteins (3a, 3b, 7b, ORF, *etc*.) show missense mutations in their sequence. Both types of proteins undergo rapid mutations. Point mutations and genetic recombination of SARS-CoV participate in its adaptations and variations among hosts of different species. Middle East Respiratory-

* **Corresponding author Firasat Hussain:** Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan;
E-mail: firasathussain@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

Coronavirus (MERS-CoV) and SARS-CoV-1, SARS-CoV-2 have striking differences among their proteins. Mannose-binding protein (MBL) takes part in countering viral infections. A decrease in MBL increases the exposure of host cells to SARS-CoV infections.

Keywords: Host genetic diversity, Host susceptibility, Protein, Phylogenetic analysis, Pathogenesis, SARS-CoV.

INTRODUCTION

Viruses have likely been infecting cells for billions of years, evolving alongside cellular life forms since the earliest days of life on Earth. The oldest known viruses date back at least 300 million years, but some evidence suggests they may have existed even earlier [1]. In Wuhan city of China, a unique type of pneumonia was reported in patients suffering from SARS in December 2019 [2]. SARS-Co--2 is the 7th identified serotype of the *Coronaviridae* family. While the SARS-CoV, the MERS-CoV, and SARS-CoV-2 are capable of posing severe sickness, the NL63, HKU1, 229E, and OC43 are capable of inflicting only minor symptoms, and their ancestral hosts are not humans in all cases [3]. SARS-CoV-2 causes coronavirus disease-19 (COVID-19) and is the third coronavirus to cause severe disease in humans. The spike (S) protein of SARS-CoV-2, like all other coronaviruses, hooks to host cell membrane angiotensin-converting enzyme-2 (ACE2) to initiate infection [4]. It subsequently stimulates host cell enzymatic machinery to achieve cellular incorporation. After an infection, the immune system response varies from person to person, and in rare situations, a cytokine storm is observed. As a result, researchers have concentrated on genetic risk factors [5]. Genetic studies on virus-host interactions have identified genes encoding virus receptors, receptor-modifying mechanisms, and a diverse range of natural and adaptive immune responses-associated proteins in the progression of illness [6]. Several attempts have already been undertaken to predict gene targets in polymorphism-associated investigations on COVID-19, with various degrees of success [7]. Understanding the genetic variability of the host and its connections with COVID-19 can provide insightful data for disease etiology, the specific propensity to SARS-CoV-2 disease seriousness, consequences, and death prognosis associated with the disease, amongst other things [7]. SARS-CoV-2 infection results in various outcomes, from subclinical to critical symptoms, depending on the individual (6.1%) [6]. As SARS-CoV-1, it has been claimed that SARS-CoV-2 evolves as a quasispecies within an infected host, with several mutations occurring that might be advantageous to the virus [8]. Viruses have exerted considerable selective pressure on humans throughout our extensive co-evolutionary history, both clearly through lethal epidemics and secretly side to side with mysterious immunological responses when a pathogen is inactive [9].

The SARS-CoV-2 pandemic highlights the need for public health research, particularly in terms of understanding human genetic variation in response to viral exposure in the context of infectious disease prevention [10]. Differential host genetic variables affecting the immunological response to COVID-19 may describe the clinical variance in COVID-19 virulence and symptomatic presentation, according to some investigators [1]. The World Health Organization (WHO) declared it a worldwide pandemic and the world's sixth public medical emergency on March 11, 2020 [11]. Some antiviral drugs demonstrated results on clearance of this virus during treatment [12].

Taxonomical Classification Based on Genetic Diversity

SARS-CoV is a +ssRNA virus. The International Committee on Taxonomy of Viruses(ICTV) classification system for SARS-CoVs has been principally established based on the analysis of the comparative degree of the replicative protein-encoding genes, with the help of the DivErsity pArtitioning by hierarchical Clustering (DEmARC) software [13]. ICTV recognized 39 species in 27 subgenera, 4 genera, and two subfamilies that belonged to the sub-family *Orthocoronavirinae*, family *Coronaviridae*, sub-order *Cornidovirineae* and order *Nidovirales* [13]. Coronavirus has four genera (Fig. **1**).

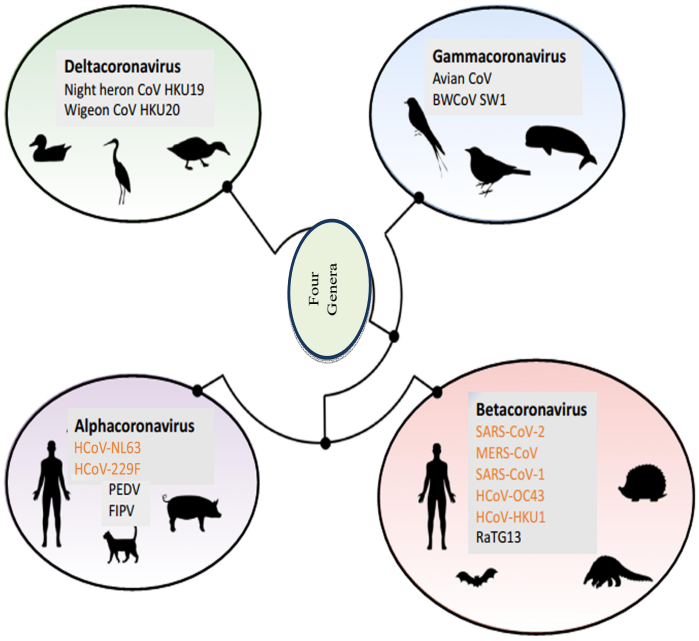

Fig. (1). Illustration of the four genera of coronaviruses, their evolutionary relationship, and their animal hosts [14].

CHAPTER 5

Newly Emerging Variants of SARS-CoV

Firasat Hussain[1,*], Hamid Ali[2], Atteeqah Siddique[1], Tehmeena Nousheen[1], Kashif Rahim[1], Umair Younas[3], Faisal Siddique[1], Mukhtar Alam[4], Hidayat Ullah[4] and **Muhammad Nisar[5]**

[1] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[2] *Department of Biosciences, COMSATS University, Islamabad, Pakistan*

[3] *Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[4] *Department of Agriculture, The University of Swabi, Anbar-Swabi -23561, Khyber Pakhtunkhwa, Pakistan*

[5] *Department of Epidemiology & Public Health, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

Abstract: Severe acute respiratory syndrome-coronavirus (SARS-CoV) is responsible for causing respiratory diseases. Its transmission takes place through saliva droplets. SARS-CoV appeared first in Southern China. It spread quickly across the globe from 2002 to 2003. In the wild, horseshoe bats serve as natural reservoir hosts for SARS-CoV. Palm civets show high susceptibility toward SARS-CoV. SARS-CoV gradually mutates on continuous transmission from human to human, animal to animal, and animal to human. These mutational changes can occur in viral proteins, which bind to the angiotensin-converting enzyme2 (ACE2) receptor of the host cell surface and cause infection. The worldwide spread of infection leads to the survival of of fitter, more spreadable variants with enhanced ability to adapt to their host. In this chapter, we discussed the different angles of variation in SARS-CoV and the impact of these variations on viral pathogenicity. During this study, we observed many variations in virus spike protein, variation in amino acid residues, variation in open reading frames, the interaction of spike with host ACE2 receptor, genetic variability with OC43, the impact of the variation in IL-12, RBI, and the variation in serine protease. No proven treatments, cures, or pre-emptive strategies were available for SARA-CoV. Coronaviruses found in bats show genetic diversity, pointing out our poor understanding of viral zoonosis from wild animals. Viral zoonosis can be prevented by considering the concept of "One Health".

Keywords: SARS-CoV, Foshan, horseshoe bats, palm civets, genetic mutations, genetic diversity, viral zoonosis.

[*] **Corresponding author Firasat Hussain:** Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan; E-mail: firasathussain@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

INTRODUCTION

This virus is responsible for causing respiratory diseases. Its transmission takes place through saliva droplets. Symptoms include pain in muscles, cough, and fever. Ssevere acute respiratory syndrome-coronavirus (SARS-CoV) appeared first in Southern China. It spread quickly globally from 2002 to 2003 [1, 2].

Incidence

In 2002

In November 2002, in Foshan, China, an uncommon epidemic of atypical pneumonia with a high incidence of nosocomial transmission to healthcare personnel occurred, causing widespread concern [3].

In 2003

In March 2003, a novel coronavirus strain was identified as the causative agent of severe acute respiratory syndrome (SARS) and was called SARS-CoV [4]. A nephrologist who was 67 years old traveled in February 2003 to Hong Kong from China to ensure index cases for the large population and healthcare workers during the SARS outbreak in Hong Kong. One hundred and thirty-eight people got infected due to increased infectivity of SARS in Hong Kong at a hospital 2 weeks after exposure. It then spread to 29 countries by international air transport [5, 6]. By the conclusion of the outbreak in July 2003, there were 8,098 cases and 774 fatalities globally (Table **1**) [7].

In 2004

In early 2004, four patients interacted with palm civets when the outbreak of SARS was on a small scale. No confirmed SARS cases have occurred since 2004 [8, 9]. Since 2005, coronaviruses have been isolated from Chinese horseshoe bats, similar to SARS [10].

Prevalence

On February 11, WHO received the first report of the new disease, “SARS”, from the Chinese Ministry of Health, which stated that 305 cases and 5 deaths had occurred in Guangdong province, China [12]. SARS-CoV, a novel coronavirus, was isolated from SARS patients in March 2003 and sequenced later. After inoculation for 5–6 days, a cytotoxic effect was observed. Signature sequences were also discovered in the amino acid sequences of the spike protein (Fig. **1**), which is responsible for viral particle attachment [13]. It is uncertain if these

variations in human reproduction are adaptive mutations. Like all other RNA viruses, coronaviruses also have a high rate of mutation as a result of RNA polymerases' error-prone existence and their characteristically short replicative life cycles [14].

Table 1. Cases of SARS from November 2002 to July 31, 2003 [6, 11].

Country	No. of Cases	No. of Deaths	Fatality
Hong Kong	1755	349	6.6
Singapore	238	33	14
Canada	351	44	17.5
US	27	0	0
Taiwan	346	73	22
Mainland China	5327	349	7

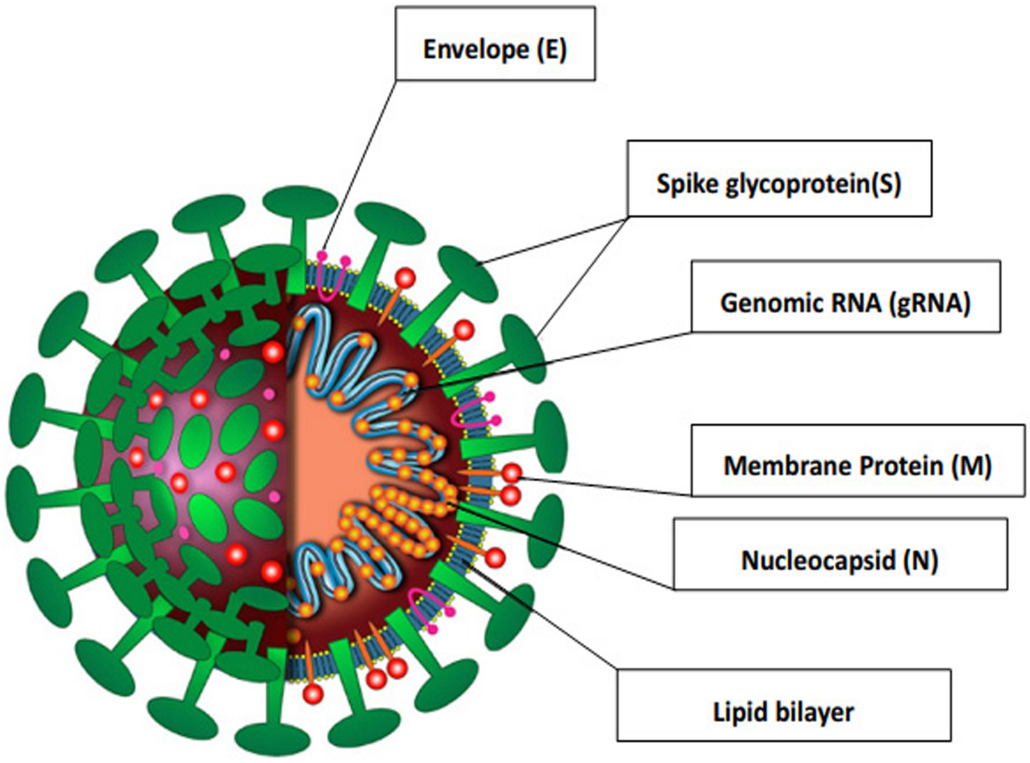

Fig. (1). Structure of SARS-CoV.

CHAPTER 6

Genetic Architecture of Host Proteins Involved in SARS-CoV

Hayat Khan[1], Firasat Hussain[2,*], Muhammad Adnan[2], Muhammad Naveed Nawaz[2], Inam Ullah Khan[3], Umair Younas[4], Muhammad Nisar[5], Imtiaz Ali Khan[6], Muhammad Kalim[7,8], Chen Shanyuan[9] and **Amjad Islam Aqib[10]**

[1] *Department of Microbiology, University of Swabi, Ambar 23561, Pakistan*

[2] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[3] *Institute of Microbiology, Faculty of Veterinary and Animal Sciences, Gomal University, D.I. Khan 29220, Khyber Pakhtunkhwa, Pakistan*

[4] *Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[5] *Department of Epidemiology and Public Health, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[6] *Department of Entomology, The University of Agriculture, Peshawar 25000, Pakistan*

[7] *Department of Microbiology and Immunology, Wake Forest University Winston-Salem, NC, 27101, USA*

[8] *Houston Methodist Hospital Research Institute, Houston, Texas, 77030, USA*

[9] *School of Life Sciences, Yunnan University, Kunming 650091, China*

[10] *Department of Medicine, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

Abstract: The coronavirus spontaneously mutates and produces new strains overtime. A few variants are more infectious and harmful than others. Additionally, certain variations are capable of eluding treatment control. These modifications may have an impact on the virus's features. The novel variations have the power to progress quickly and induce pathogenicity. Vaccines, diagnostic tools, active compounds, and other precautionary care may also be affected by novel variations. At first, it was considered that cells could also ingest and destroy infections in addition to degrading cellular contents. This mechanism was later confirmed for other viruses and given the label xenophagy. Because of the modification of the coronavirus, poor and emerging nations are constantly confronted with new issues. Developing nations must promptly prepare and create a clear direction to vaccinate their entire population. It has frequently been

* **Corresponding author Firasat Hussain:** Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan;
E-mail: firasathussain@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

questioned if vaccination can entirely safeguard someone from a virus that alters its features quickly and produces variations with more powerful alterations. However, much evidence is that immunization prevents the virus from spreading and protects people.

Keywords: Emerging, Genome, Immunization, Immune response, Mutations, New strains, Pathogenicity, Spike, SARS-CoV, Variants, Vaccination.

INTRODUCTION

An illness of the airways brought on by coronavirus is known as severe acute respiratory syndrome, which can cause pneumonia and flu-like symptoms [1 - 6]. SARS-CoV, which causes an atypical lung inflammation that can develop into severe pneumonia, was spotted in China [7]. It escalated swiftly throughout different nations due to its high infectiousness, affected more than 8,000 people, and had an estimated 10% fatality rate [8, 9]. At first, SARS-CoV was thought to naturally infect the palm civet [10]. Genome sequencing, however, demonstrates that the SARS-CoV virus evolved from bat coronaviruses [11].

The SARS-CoV belongs to the Coronaviridae family (order Nidovirales), which is an RNA-enveloped single-stranded virus [12 - 14]. The genome is the longest viral genomic RNA known, at around 30 kb. In comparison to other coronaviruses, it has eight distinct proteins with fourteen open reading frames (ORFs). They are believed to be crucial for SARS-CoV pathogenesis. The translation of ORF 1a 1b produces 16 non-structural proteins (NSPs), including viral RNA-dependent RNA polymerase and protease. The biggest ORFs, 1a and 1b, make up over 60% of the genome of the virus. NSPs have a role in the replication and transcription of viruses. The structural proteins of the SARS-CoV virus are the nucleocapsid (N), spike (S), membrane (M), and envelope (E). Spike protein binds to the host receptor in preparation for future receptor-mediated endocytosis of viral entry in the cytoplasm of the target cell.

The replication cycles of all the viruses are utilized by the genomic machinery of the host [15]. Viruses take over cellular processes by interfering with several cellular pathways, such as those regulating DNA replication and repair, cell cycle regulation, cellular immunity to viral infection, regulation of host transcription, survival, and metabolism [16]. Several cellular pathways essential to the physiological functions of the cell are altered by the coronavirus [17]. Among the pathways that are affected by coronavirus infections include the p38 mitogen-activated protein kinase, phosphatidylinositol 3-kinase, epidermal growth factor receptor (EGFR), interferon, and nuclear factor kappa-light-chain-enhancer of activated B cells. These pathways control how resistant infected and nearby cells

are to viral invasion. For viral pathogenesis, disruption of these cellular processes is essential [18, 19].

SARS-CoV Structure and Life Cycle

The first new viral illness of the twenty-first century was reportedly SARS. SARS was originally recorded in the southern region of China in 2002–2003, with a high fatality and incidence rate. With almost 8000 infections overall, the virus caused 800 deaths in just six months [20]. The new virus SARS-CoV is what causes SARS [1, 2, 21].

Organization of SARS

SARS-CoV is a single-stranded, positive-sense RNA enveloped coronaviruses that spread disease in people and animals by causing respiratory and gastrointestinal diseases. The developed virus has a diameter of around 100 nm [22]. It is present in a free-living state in natural waters. The virus appears spherical under a cryo-electron microscope as opposed to its icosahedral structure [23 - 27]. The effective isolation of enveloped Pithovirus from 30,000-year-old permafrost shows that the virion's envelope does not make it unstable or easily inactivated [28]. Because they remain contagious for one to two days in harsh conditions, coronaviruses are relatively more stable than HIV-1 [29]. Similarly, SARS-CoV viruses are more resilient than MERS-CoV viruses, which have a maximum survival duration of two to three days on rough surfaces. However, under comparable circumstances, the MERS-CoV survives a lot longer than the influenza A virus [30].

Structure of the Genome

The sequencing study shows that coronaviruses have the biggest RNA genomes, with 29,727 bp encoding 14 ORFs. The ORF 1ab gene comprises two-thirds of the genome and is highly conserved across coronaviruses [13]. ORF 1ab is converted into the two polyproteins 1a and 1b *via* ribosomal frameshift. The twelve remaining ORFs encode four structural proteins and eight more auxiliary proteins. These polyproteins were split up into 16 distinct viral proteins by viral proteases [13, 14]. Most of these are thought to be involved but are largely unknown in terms of their function.

Entry and Attachment

SARS-CoV attaches to its receptor in the target cell with the aid of S protein. The S protein has 1255 residues. The S protein's N-terminus contains 13 large signal peptides, followed by an 1182 ectodomain residue and a 28 short cytoplasmic

CHAPTER 7

Landscape of Host Genetic Factors Correlating with SARS-CoV

Firasat Hussain[1,*], Hamid Ali[2], Hayat Khan[1], Ateeqah Siddique[1], Tehmeena Nousheen[1], Inam Ullah Khan[3], Umair Younas[4], Amjad Islam Aqib[5], Mukhtar Alam[6], Hidayat Ullah[6] and **Wen-Jun Li[7]**

[1] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[2] *Department of Biosciences, COMSATS University, Islamabad, Pakistan*

[3] *Institute of Microbiology, Faculty of Veterinary and Animal Sciences, Gomal University, D.I. Khan 29220, Khyber Pakhtunkhwa, Pakistan*

[4] *Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[5] *Department of Medicine, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[6] *Department of Agriculture, The University of Swabi, Anbar-Swabi 23561, Khyber Pakhtunkhwa, Pakistan*

[7] *State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, College of Ecology and Evolution, Sun Yat-Sen University, Guangzhou 510275, PR China*

Abstract: The host's wide range of genetic variation plays an essential role in determining the susceptibility, severity, and overall pathological conditions of coronavirus disease-19 (COVID-19) following infection with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). COVID-19, caused by SARS-CoV, is a zoonotic disease that has affected humans after crossing boundaries. Emerging viral infections typically result from the host when a virus transfers from the very first host into a new species. There is substantial diversity in illness progress among patients infected with SARS-CoV-2. Many do not show any manifestations, while others progress to acquire COVID-19; nonetheless, the intensity of COVID-19 symptoms substantially ranges among people. Host factors such as age, gender, geographical region, diseases, co-morbidities, and various host genetic factors predispose susceptibility to SARS-CoV-2 infection. Individuals who possess certain variations of genes directly implicated in viral infection (*e.g.*, ACE2, TMPRSS2) or who have differential expression of those genes may be more susceptible to SARS-CoV-2. These

[*] **Corresponding author Firasat Hussain:** Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan;
E-mail: firasathussain@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

alterations might account for the wide variety of symptoms and severity of COVID-1--related SICKNESS in various people. Because the behavior of the infectious agent varies so much across strains, the impacts of individual variation are best evident when the same strain of an organism infects previously unexposed people simultaneously. An increase in genetic diversity in host sensitivity to pathogenic agents has been related to the development of major-effect resistance polymorphisms among populations.

Keywords: Host genetic factors, Human leukocyte antigen, SARS-CoV receptors, Susceptible factors, SARS-CoV, TWAS.

INTRODUCTION

SARS-CoV-2, commonly known as severe acute respiratory syndrome-coronavirus-2, is classified as a member of the Betacoronavirus lineage B family. It is an RNA molecule that is enclosed and has a positive sense virus. It has a strong relationship with SARS-CoV, the virus that was found to be responsible for the outbreak of SARS in the human population in 2002 (with which it shares a genetic similarity of 79%), as well as with many SARS-related coronaviruses that are found in bats (with which it shares a genomic resemblance of up to 98%) [1]. Since its appearance in December 2019, a previously unknown RNA virus known as SARS-CoV-2 has been responsible for a worldwide outbreak of coronavirus illness 2019 (COVID-19). Even though the majority of COVID-19 instances are minor, the severity of the illness may vary greatly from patient to patient, and severe cases of certain medical conditions can result in respiratory failure, which in some cases can be fatal. Respiratory failure refers to the inability of the respiratory system to adequately supply oxygen to the body and remove carbon dioxide. It can be caused by various factors, such as acute respiratory distress syndrome (ARDS), pneumonia, or other lung-related complications [2].

The most prevalent viruses in the human body are found in the gastrointestinal system, which are referred to collectively as the gut virome [3]. Retroviruses involve infected cells that are almost half a billion years old, making them important at the medical and economic levels [4, 5]. Viruses have imposed considerable pressure on humans throughout our vast co-evolution, openly during lethal outbreaks and secretly through ambiguous immune interaction while a disease stays dormant [6]. The COVID-19 pandemic has emphasized the importance of comprehending the various responses of humans to viral infections, particularly to genetic diversity. Clinical discrepancies in coronavirus disease-19 (COVID-19) severity and clinical proposal might be attributable to host chromosomes variables of immune response [7]. Understanding the genetic components of how the body's defense system reacts to viruses might shed light on a wide range of challenging diseases [6]. The COVID-19 pandemic has only been underway for a few short months, but researchers have already published

multiple studies demonstrating that genetic variables influence the severity of COVID-19 infection [8] and that gene expression varies depending on an individual's biological gender and age [9, 10]. Identical to SARS-CoV, COVID-19 has been defined by immediate respiratory distress and excessive inflammation that may lead to breathing problems, multi-organ failure, and ultimately, death [1]. In the United States, blood group A was associated with a higher risk of death from illness and larger racial gaps in health outcomes [11, 12]. In early reports, men were more likely to suffer from severe illness than women [13].

Infection happens swiftly and with a higher antibody level than receptor attacks in males as well [8]. Previous research has implicated human leucocyte antigen (HLA) classes I and II in regulating the immune response to various viral antigens, including the influenza virus [14, 15]. Some people mays be more vulnerable to SARS-associated than others because they contain certain variations of genes directly implicated in viral infection (such as ACE2 and TMPRSS2) [16]. These variations may account for the considerable variation in COVID-19 symptoms and illness severity among affected people [8].

Host Factors and Diseases

Once the WHO declared the SARS outbreak of 2003 a global threat, countries worldwide, recognizing the need for national measures, stepped up their efforts to monitor the outbreak (trends through the WHO data) [17]. In addition, isolated treatment hospitals measuring body temperature upon entry to the country through airports/sea ports were adopted. Similarly, additional isolation measures [18] and follow-up investigations for people who came in contact with infected patients were also mandatory enabling South Korea to gain control and effectively subduing SARS in less than 100 days after the WHO alert [19]. An analysis of the Korean response to the SARS concluded the focus on factors that influence infectious disease response [20]. The analysis also demonstrated the benefits of disease-relevant knowledge, factors, and all the processes having a key influence on achieving targeted goals during infectious disease response [19]. Even some of the questions (the origin and pathogenesis of SARS, the natural history and the best specific treatment of the disease, *etc.*) remained unanswered during that successful response [21]. It illustrated that systems for controlling infectious disease must be tightly linked to systems detecting it. This is highly pertinent when we have to keep in mind factors that contribute to disease severity (they could likely continue if they do not precede re-emergence). In addition, this is also necessary because once an endemic disease has been under-control, the likely return of the infection in the future is also assumed. Some of the vital factors include an increase in international travel, population growth, lack of adequate health care or sanitation, the aging of the population, urbanization, global climate

CHAPTER 8

History of MERS-CoV

Sana Tehseen[1], Sidra-Tul-Muntaha [1], Muhammad Saeed[2], Kashif Rahim[3], Muhammad Zeeshan Iqbal[4], Firasat Hussain[3] and **Umair Younas[4,*]**

[1] *Department of Food Science and Technology, Faculty of Science and Technology, Government College Women University, Faisalabad, Punjab, Pakistan*

[2] *Department of Poultry Science, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

[3] *Department of Microbiology, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

[4] *Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan*

Abstract: The Middle East respiratory syndrome-coronavirus (MERS-CoV) is a rising threat to the whole world's health security. It is considered a new epidemic. It is a fatal respiratory disease with an elevated death rate. In humans, it produces low respiratory tract infections. The virus originated from bats but serological studies were conducted. The evidence of the studies proved that the antibodies of the MERS-CoV were reported in the camels of the Middle Eastern countries that first tested positive for the virus. Thus, these camels were considered the hosts of the MERS-CoV. MERS-CoV may be an animal disease virus that may cause secondary human infections. Camelus dromedarius camels are known as the host. Symptoms include fever (98%), shortness of breath (72%), cough (83%), and myalgia (32%). Other symptoms were also seen: 26% of patients had diarrhea and 21% had vomiting. Diagnosis consists of nasopharyngeal swabs, sputum, tracheal aspirates, and broncho alveolar lavage. There is no vaccine or specific treatment for MERS-CoV, although many vaccines and treatments for the virus are being developed. The patient's health condition determines the type of treatment. The ongoing advancement of technologies to systematically and reliably diagnose asymptomatic MERS-CoV infections will shed light on the virus's true prevalence in the human population.

Keywords: Camels, Diagnosis, Origin, Respiratory disease, Symptoms, Vaccine.

[*] **Corresponding author Umair Younas:** Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, South Punjab, Pakistan;
E-mail: umairyounas@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

INTRODUCTION

The Middle East respiratory syndrome-coronavirus (MERS-CoV) is a rising threat to the whole world's health security. It is considered a new epidemic. MERS-CoV is a respiratory syndrome that originated in Saudi Arabia in 2012. This disease is also known as camel flu. Its main causing agent is MERS-CoV [1]. It is believed that this is a virus that had its origin in bats but its infection is mainly caused by camels. Its infection in humans can be caused directly or indirectly. If an infected person is in direct contact with the other, it can transmit the infection to that individual [2]. The majority of these cases that have been reported are seen in Saudi Arabia [3, 4]. The MERS-CoV is related to two bat coronaviruses. These are HKU4 and HKU5. It is an infection of the lungs, and a high rate of death is recorded. It is a *betacoronavirus* and belongs to lineage C. It causes respiratory illness in humans [5]. The virus originated from bats but serological studies were conducted. The evidence of the studies proved that the antibodies of the MERS-CoV were reported in the camels of the Middle Eastern countries that first tested positive for the virus [6]. Thus, these camels were considered the hosts of the MERS-CoV [7]. In Saudi Arabia, the same virus was seen in humans and camels as well [8]. The study proved that the person who had the infection had direct contact with the infected camel [9]. The people of Saudi Arabia and the countries of the Middle East had infections of the respiratory tract [10]. Almost a 50% death rate was recorded at the initial stage of the spread of the disease. The intensity of the outbreak did not increase in 2013. Only some cases were reported annually. An increase in the number of cases was recorded in April of 2014 when the reported cases reached 200 with 40 deaths. This increase in the number of cases was seen due to the reason that the testing ability of the virus was increased as well as the number of the camels were increased in birth in that year. The European Center for Disease Prevention and Control (ECDC) estimated the cases reported in August 2014. A total of 855 cases were reported with 333 deaths. The death rate was recorded at 40%. In the research data of the WHO, a total of 2519 infected cases have been reported worldwide with 866 deaths. It means the death rate is 34.4% [11].

Origin of MERS-CoV, Bats, and Camel

The infection of the MERS-CoV is now considered a new epidemic. It is a viral infection [12]. The first reported case was from a patient with respiratory disease. The patient was hospitalized in Saudi Arabia (Jeddah) in 2012. The patient died due to infection [13, 14]. The MERS-Cov was identified with the collective efforts [15]. The MERS-CoV was first time identified by Dr. Ali Muhammad Zaki. The unknown virus was isolated from the lungs of the patient, who was 60 years old and was ill with pneumonia and kidney injury [9]. The initial diagnosis

was not successful. At this failure, the doctor contacted another famous virologist Ron Fouchier, at Erasmus Medical Center. Dr. Zaki sent the sample, and Ron was the one to sequence the unknown virus [16]. Fouchier used a real-time reverse-transcription polymerase chain reaction method for testing the distinguishing features of known viruses. This was also used for RNA-dependent RNA polymerase (RdRp) to detect a gene that is present in all the viruses that are responsible for causing infection in humans. The known coronavirus results were negative, while the RdRp screen was found positive [15]. In 2012, the findings of Zaki were published in the Program for Monitoring Emerging Diseases [13]. Meanwhile, the Health Protection Agency of the UK diagnosed another patient with the unknown coronavirus. The patient died in the hospital due to a severe respiratory illness. They named it London1 novel CoV/2012 [17]. The WHO got involved in the characterization of the virus. It also informed all the member States. It led the coordination and provided important health guidance to all the health authorities and agencies related to technical health in September 2012 [18].

In November 2012, Dr. Zaki, along with the co-authors, published an article. He gave the unknown coronavirus a tentative name, HCoV-EMC (Human Coronavirus-Erasmus Medical Center). He also explained the genetic makeup of the virus and other viruses that were found closer to this virus [9]. The official designation was adopted as MERS-CoV by the International Committee on Taxonomy of Viruses in May 2013. The WHO communicated about the disease [19]. Before this official designation as the unknown virus, WHO previously used the simple designation as the novel coronavirus [20]. In the years from 2012 to 2018, the WHO reported 2143 confirmed patients with the virus. They also notified a minimum of 750 deaths due to this infection in 27 countries of the world [21]. The reservoir of the bat was suggested based on the phylogenetic resemblance of many bat coronaviruses with the MERS-CoV. However, no clear evidence of bat infection was observed. Moreover, the history of infection was not seen if the infected one had contact with the bat [22, 23]. The studies later proved that another source of the infection in humans was introduced. The camels were seen to spread infection of the MERS-CoV [24 - 27].

The virus originated from bats but serological studies were conducted. The evidence of the studies proved that the antibodies of the MERS-CoV were reported in the camels of the Middle Eastern Countries that first tested positive for the virus [6]. Thus, these camels were considered the hosts of the MERS-CoV [7]. In Saudi Arabia, the same virus was seen in humans and camels as well [8]. The study proved that the person who had the infection had direct contact with the infected camel. A genomic study was conducted on camels to analyze the origin of the MERS-CoV by Herif El-Kafrawy. The studies indicated that 22.8% of the animals (camels) were found to be infected. It showed that the infection was

CHAPTER 9

Mutation in MERS-CoV

Faisal Siddique[1,*], Rao Zahid Abbas[2], Asghar Abbas[3], Muhammad Saeed[4], Safdar Abbas[5], Muhammad Sajid[6], Kashif Rahim[1] and **Firasat Hussain[1]**

[1] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*

[2] *Department of Parasitology, University of Agriculture, Faisalabad, Pakistan*

[3] *Department of Parasitology, Muhammad Nawaz Sharif University, Multan, Pakistan*

[4] *Department of Poultry Sciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*

[5] *Department of Animal Breeding and Genetics, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

[6] *Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

Abstract: The Middle East respiratory syndrome (MERS-CoV) is a betacoronavirus-borne acute viral illness. Since it first appeared in 2012, multiple cases of animal-to-human transmission of MERS-CoV have been observed, indicating that MERS-CoV has the potential to cause a widespread epidemic. It has been detected in bronchial samples from more than 27 countries, with approximately 2,505 reported cases and a mortality rate of 36%. Genetic heterogeneity of MERS-CoV between different samples may have paved the way for cross-species transmission and changes in the tropics between species and within species. MERS-CoV has many evolutionary genomic origins in spike protein, envelope protein, matrix, and non-structural proteins (nsps) and mutates continuously. In this chapter, we highlighted the causes and significance of mutation in the amino acid sequences of spike protein, envelop protein, matrix protein, nucleocapsid protein, and snp. Among the most enduring obstacles in controlling coronavirus disease is the evolution of the virus, which is influenced by genetic diversity, mutation, and natural selection.

Keywords: Evolution, Epidemic, Human, MERS-CoV, Samples.

INTRODUCTION

The Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel zoonotic human viral infectious agent that is endemic to the Middle East [1].

* **Corresponding author Faisal Siddique:** Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan; E-mail: faisalsiddique@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

MERS-CoV is a rising infectious agent that has been designated as a priority pathogen by the World Health Organization (WHO) because it causes a severe infection with a high fatality rate. The animal source of infection in humans is the host reservoir dromedaries in MERS-CoV [2]. In 2012, in Jeddah (a city in Saudi Arabia), MERS-CoV was discovered in the lungs of a 60-year-old man who had died of respiratory failure. MERS is the name given to the disease caused by MERS-CoV. The mortality rate of MERS-CoV is about 40%. MERS-CoV incubation period is 2-14 days [3]. As reported by WHO as of January 2020, the number of cases of MERS-CoV have been reported in more than twenty-seven countries, with two thousand four hundred and ninety-four laboratory-confirmed cases and eight hundred and fifty-eight deaths since September 2012 [4]. The "Gulf Cooperation Council (GCC)" region, which includes Oman, Qatar, Saudi Arabia, Bahrain, the United Arab Emirates, and Kuwait, is largely dominated by "MERS-CoV". These countries reported the highest rate of MERS-CoV infection, which could be related to the Middle East's massive population of "dromedary camels". According to the present literature, camel farmhouses become a significant source of "MERS-CoV" infections during the mating season. Seasonal variations in the frequency of "MERS-CoV infection" have been identified. From 2012 to 2017, the maximum global seasonal incidence of "MERS-CoV infection" was identified in June, while the lowest incidence was reported in October-January [5].

MERS-CoV belongs to the Coronaviridae family, and the genus betacoronavirus is a single standard positive-sense RNA virus that consists of 16 non-structural proteins (nsps) and four structural proteins. MERS-CoV viral genome consists of 30,119 nucleotides and 26-33 kb in length [2]. MERS-CoV can infect non-human primates such as bats, civets, and rabbits. MERS-CoV causes minor or no respiratory clinical symptoms in camels, making it difficult to spot and diagnose. MERS-CoV is most commonly found in dromedaries under the age of one year [6]. MERS-CoV is frequently transmitted from dromedary camels to humans, as well as through human-to-human contact or due to nosocomial infections [7]. Current epidemiologic research suggests that direct contact with camels or humans with symptomatic MERS plays a significant role in transmission [8]. MERS-CoV spread from camel to human has been documented in Saudi Arabia, where after genome sequencing, it seems that the isolates of both humans and animals were similar and positive for MERS-CoV RNA [2]. Nasal and mucosal secretions in humans and dromedaries, eliminated through coughing and sneezing, are the key transmission pathways in zoonotic and human-to-human transmissions because the virus attacks the respiratory tract [7]. MERS-CoV is closely linked to coronaviruses identified in bats, indicating that bats could be a MERS-CoV reservoir. This encouraged some researchers to do tests on bats, but no antibodies to MERS-CoV were discovered [6]. MERS-CoV has been linked to

temperature, chills, cough, difficulty breathing, and muscle aches [2]. In children and young people, mild cases are found. These include low-grade fever, rhinorrhea, pharyngitis, neuralgia, and abdominal discomfort. Gastrointestinal symptoms such as nausea, emesis, or dysentery have been documented in severely ill patients, and acute renal failure has been described in about half of these patients [6, 9]. A progression to acute respiratory distress characterizes severe MERS-CoV infection. The disease manifests itself more severely in the elderly or immunocompromised patients and people with chronic diseases such as diabetes and lung and kidney diseases [10]. Poor nutrition, deficits, anxiety, parasites, concomitant infections, and other forms of immunosuppression may exacerbate the course of MERS-CoV infection. MERS-CoV infection has been linked to ingesting raw camel milk or improperly cooked meat [6]. RT-PCR also helps in the diagnosis of MERS-CoV [5]. The "MERS CoV" infection diagnosis is based on the clinical history, comprehensive blood analysis, and chest X-ray. Samples of MERS-COV were collected from the tracheal aspirate and phlegm samples from the lungs using bronchoalveolar lavage or by nasal swab from the infected individual [11].

Mutational Concept Regarding MERS-CoV

MERS-CoV, like many human coronaviruses, has a substantial genomic material, such as RNA, which is almost 30kb in size and encodes a broad range of proteins. The mature virus produces many structural proteins and nsps [12]. The MERS-CoV contains four major structural proteins: spike (S) protein, membrane (M) protein, nucleocapsid (N) protein, and envelope (E) protein. ORF1ab generates two main polyproteins, such as pp1ab and pp1ab, which have also been subdivided into 15-16 unique non-structural components. MERS-CoV encodes the necessary proteins similar to other coronaviruses, including ORF3, ORF4a, ORF4b, and ORF5. These viral proteins enhance the host's viral attachment, replication, virulence, and disease outcome [13]. Virus entry into the host cell is helped by the transmembrane protein (Type 1) [14], which fuses to the host cell receptor, such as dipeptidyl peptidase 4 (DPP4). Camel isolates showed a high level of variation and crossover episodes, indicating that they are the primary source of MERS-CoV [15]. The limited genetic variation reflects the minimum standard of immunologic pressure exerted on this coronavirus strain. Specific amino acid changes in MERS-CoV are often linked to the disease's severity or risk of transmission [16]. ORF1ab has the most genetic alterations, followed by nucleoprotein, spike protein, and ORF4b. In ORF1ab replicase, genetic alteration and recombination were seen in nsp3, which encodes a potential papain-like protease [17], nsp12, which encodes a putative RNA-dependent RNA polymerase, nsp13, which encodes a putative helicase, and nsp14, which encrypts a putative exonuclease. Spontaneous substitutions were also observed in the putative

CHAPTER 10

Hosts Genetic Diversity of MERS-CoV

Faisal Siddique[1,*], Etab Saleh Alghamdi[2], Asghar Abbas[3], Muhammad Saeed[4], Kashif Rahim[1], Asif Javaid[5] and **Firasat Hussain[1]**

[1] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*

[2] *Department of Food and Nutrition, King Abdul-Aziz University, Jeddah, Saudi Arabia*

[3] *Department of Parasitology, Muhammad Nawaz Sharif University, Multan, Pakistan*

[4] *Department of Poultry Nutrition, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*

[5] *Department of Animal Nutrition, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*

Abstract: Middle East respiratory syndrome coronavirus (MERS-CoV) is a potentially fatal disease that can be passed from animals to humans. It was first discovered in numerous Arab countries in 2012, including Jordan and Saudi Arabia. Over 2500 people have been impacted by this illness worldwide, with 850 confirmed deaths from 27 nations. Humans, camels, sheep, goats, bats, pigs, rabbits, bovines, horses, and alpacas have all been infected with MERS-CoV worldwide. MERS-CoV keeps a 32 kb positive-sense RNA genome with at least six pathogenic components, including ORF1ab, membrane, envelope, spike, and nucleocapsid. The spike protein promotes virus entrance across the host cell membrane. To initiate the disease, host proteolytic enzymes must separate the MERS-CoV spike protein into two components, S1 and S2. The spike protein receptor-binding domain (RBD) binds to host cell receptors such as dipeptidyl peptidase 4, sialic acid, GRP78, and CEACAM5, which are found on the host cell membrane surface. There is little information available about MERS-CoV infection host genetic diversity. This chapter emphasizes the importance of data related to historical background, host characteristics, the molecular diversity of MERS-CoV host cell entry receptors, and the genetic diversity of MERS-CoVs in bat, human, camel, and civet hosts. These findings will help us better understand the host genetic diversity of MERS-CoV infection.

Keywords: Bat, Camels, Humans, MERS-CoV, Saudi Arabia, Spike protein.

[*] **Corresponding author Faisal Siddique:** Department of Microbiology, Cholistan University of Veterinary & Animal Sciences, Bahawalpur 63100, Pakistan; E-mail: faisalsiddique@cuvas.edu.pk

INTRODUCTION

Coronaviruses can infect both birds and mammals. Even though many coronaviruses have native deposits in bats, birds, and mice, the evolution of the host spectrum in some species has occurred throughout their evolutionary history [1]. There have been seven strains of human coronaviruses reported so far, including HCoV-OC43, HCoV-NL63, Middle East respiratory syndrome-coronavirus (MERS-CoV) HCoV-229E, HCoV-HKU1, severe acute respiratory syndrome-coronavirus (SARS-CoV), and severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), the majority of which are zoonotic. Humans suffer from minor to severe respiratory ailments as a result of them. These aggressive strains pose dangers to human health. It is critical to understand the physicochemical and genetic elements that drive coronavirus host range development [2]. Camel flu (MERS-CoV) was primarily detected in the lungs of a Saudi patient in September 2012 by an Egyptian researcher named Dr. Zaki, and it is still infecting camels and individuals. Simultaneously, a new strain of MERS-CoV (London1 nCoV) was isolated in the UK in a Qatari patient who had traveled from Saudi Arabia *via* Qatar to London [3]. MERS-CoV is a severe respiratory sickness virus that can cause a fatal infection. MERS-CoV has now spread to more than 27 countries, posing a significant health risk to humans and animals worldwide. There have been 2,458 incidents in lab settings, with 848 deaths [4]. Human MERS-CoV infection can be disseminated by bats and camels, which act as reservoirs and potential sources of infection.

MERS-CoV is a viral illness that has infected humans after crossing the species barrier. The most common way for MERS-CoV to infect humans is through direct contact with an infected camel. Several animals tested negative for MERS-CoV antibodies, including swine, lambs, buffaloes, cows, goats, and wild birds [5]. However, the virus was found in the feces of the typhoid perforated bat, which has a 100% identification of nucleotides in which MERS-CoV is isolated from the infected person. It was also found in *Neuromycia capinus* bat. Approximately 85% of these types of coronaviruses (NeoCoV) genome similarities were observed, such as MERS-CoV [6]. The method of direct and indirect dissemination from camels to humans, as well as human-to-human transmission, is still being researched. A significant viral presence in the respiratory tracts of infected camels indicates direct contact infection in humans *via* the nasal cavity [7]. Fragments of MERS-CoV RNA found in air samples from sick camel farms show that a healthy camel and human can be affected by droplets or contact with air. Other exposure methods, such as foodborne transmission, are unknown but may be important. According to a study, MERS-CoV RNA was found in the milk of sick camels that were constantly producing the virus [8]. MERS-CoV is usually transmitted by sneezing and inhalation through the respiratory tract [9]. Although

MERS-CoV is excreted in small amounts in the stomach and vomiting is a common symptom, no evidence of oral transmission has been found. There were no reports of vertical or unexpected MERS-CoV outbreaks [10]. As of 2021, there is no definite vaccination or antiviral remedy for the MERS-CoV cure. Extracorporeal membrane oxygenation (ECMO) has been shown to enhance results considerably. Antivirals, interferons, and corticosteroids did not influence the results [11, 12].

MERS-CoV Related to Host Factors

The idea behind a species barrier to viral infections is that the link between viral infections and their hosts is now limited to the virus's genomic adaptation and the combined evolution of its host species and that when these viruses spread, new hosts can become seriously ill because the pathogen is not adapted to them [13]. Coronaviruses are classified into four genera: alphacoronaviruses, betacoronaviruses, gammacoronaviruses, and deltacoronaviruses. Alpha and beta coronaviruses infect exclusively mammalian species, whereas gamma and delta coronaviruses infect primarily avian species [14]. Camels, birds, and bats are the most well-known carriers of coronavirus disease, which can be passed on to other animals living nearby or close to people, such as domesticated animals or feral animals sold for human consumption in the local Chinese market [15]. Organisms may endure gene mutations and gain new genomes, or they may use exchange processes to discover desirable genetic features. These genetic modifications lead to alterations that enable them to breach the species barrier. Such changes have been reported in spike proteins' receptor-binding domain (RBD) that allow MERS-CoV to adapt to human cells [16]. Increased RNA virus mutation rates and coronaviruses' unique ability of genetic recombination in their positive-sense RNA genomes are two mechanisms that generate MERS-CoV outbreaks [17]. MERS-CoV possesses a 32 kb positive-sense RNA genome with at least six pathogenic components, including ORF1ab, membrane, envelope, spike, and nucleocapsid [18]. These huge genomes are responsible for genetic diversity, resulting in increased flexibility and adaptability to diverse hosts, which is advantageous for host jumping and interspecies infiltration. Their genetic diversity has been identified due to two key factors: reduced accuracy of endogenous RNA-dependent RNA polymerase (RdRp) and significant homologous RNA recombination rate [19]. MERS-CoV has a high level of genetic variation, primarily due to fast genetic change induced by the RdRp's low fidelity, which is connected to the pathogen's ability to adapt to a wide range of hosts and explosive proliferation [20]. MERS-CoV has a wide genetic variety, ranging from 10^3 to 10^5, due to the low frequency of the RdRp, allowing them to respond to new environmental stressors and host adaptations [21]. The exoribonuclease (ExoN) enzyme can proofread virus genomic material throughout

CHAPTER 11

Newly Emerging Variants of MERS-CoV

Saigha Marriam[1], Abdul Basit[2], Zia-ud-Din Basit[3], Firasat Hussain[4], Ihtesham ul Haq[5], Muhammad Nisar[6], Umair Younas[7] and **Kashif Rahim[4,*]**

[1] *Department of Microbiology & Molecular Genetics, Faculty of Life Sciences, University of Okara, Punjab, Pakistan*

[2] *Department of Microbiology, University of Jhang, Punjab, Pakistan*

[3] *Department of Community Medicine, Kohat Institute of Medical Sciences, Pakistan*

[4] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[5] *Department of Biosciences, COMSATS University Islamabad (CUI) 45550, Pakistan*

[6] *Department of Epidemiology and Public Health, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[7] *Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

Abstract: The Middle East Respiratory Syndrome (MERS-CoV) coronavirus is an infectious viral disease. It has emerged rapidly from Saudi Arabia and later spread to other countries. MERS-CoV resulted in a 35% case fatality rate and became a global public health priority. The MERS-CoV has been heavily endemic in dromedary camel populations of the Middle East and belongs to the 2C lineage of beta-CoV. This virus expresses the dipeptidyl peptidase 4 (DPP4) receptor and causes severe acute respiratory syndrome in humans. However, the specific mechanism of zoonotic transmission from dromedaries to humans remains unclear. Despite new efforts and significant advancements in the public health care system, numerous gaps exist in understanding MERS-CoV infections. This chapter summarized the molecular virulence of MERS-CoV, associated immune responses, variations in spike proteins, pathogenesis, and genetic differences in MERS-CoV, SARS-CoV, and SARS-CoV-2. Furthermore, new protocols and active surveillance programs are much needed to evaluate future reoccurrence of MERS-CoV infections and test antiviral agents to develop vaccines that can be useful in treating MERS-CoV.

Keywords: DPP4, Dromedary camels, MERS-CoV, Viral disease, Zoonotic.

[*] **Corresponding author Kashif Rahim:** Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan; E-mail: kashifrahim@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

INTRODUCTION

Novel viruses have emerged very rapidly, and coronaviruses are one of the diverse viruses that mainly infect the human respiratory system [1]. Coronaviruses belong to the family of *Coronaviridae*, the enveloped single-stranded RNA viruses of 80-220 nm diameter, with a crown-like appearance and thin pointed projections [2]. Coronavirus is coated with a protein called the capsid, and it protects the genetic material of the virus. Coronaviruses have four glycoproteins: spike (S), membrane (M), nucleocapsid (N), and envelop (E). These proteins are encoded within the viral genome 3' end [3]. Four groups of coronaviruses, alpha, beta, gamma, and delta, have been found in human circulations that cause mild respiratory infections. They are named 229E, HKU1, NL63 and OC43 coronaviruses [4]. Coronaviruses have high mutation rates and higher frequencies of recombination. These properties enable them to play their role in ecological niches and adapt to new hosts [5]. These viruses infect a wide variety of animals, especially humans, and they can also infect other mammals, avians, carnivores, and rodents and cause neurological, hepatic, and enteric diseases [6]. In the last two decades, the human population has faced severe outbreaks of coronaviruses; the severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in the first decade of the 21st century [7]. One other similar virus named Middle East respiratory syndrome (MERS-CoV) was witnessed in 2012, which caused severe respiratory diseases in Middle East regions that later spread to North America, Africa, Europe, and Asia [8].

MERS-CoV is considered the second most lethal and pathogenic zoonotic virus, most probably emerged from bats after discovering highly similar sequences and because of its phylogenetic similarity with bat Betacoronaviruses BtCoV-HKU5 and BtCoV-HKU4. Human-to-bat contact is not common; however, dromedary camels have been considered intermediate hosts in spreading MERS-CoV for the past 20 years [9]. Globally, >2400 MERS-CoV-associated cases have been seen, resulting in >850 mortalities with a 35% case fatality rate [10, 11]. A total of 1,125 confirmed MERS-CoV-associated cases were reported to the World Health Organization (WHO) from 2015 to 2018, of which 157 cases had unknown exposure, 164 reported cases had direct contact with dromedaries, 191 reported cases had indirect contact, and 155 reported cases had contact with unpasteurized camel milk. WHO listed MERS-CoV as a priority disease that requires prompt research [12]. Most known coronaviruses circulate in animals, mainly in bats. Therefore, the emergence of a novel coronavirus is considered the zoonotic origin of disease with animal reservoir [13].

MERS-CoV-associated illness ranges from asymptomatic or mild to severe respiratory infections that lead to death. Symptoms include coughing, nasal

discharge, sneezing, malaise, shortness of breath, loss of appetite, high temperature, lymphopenia, decreased leukocytes, low platelet count, and elevated lactate dehydrogenase levels [14]. The spread of MERS-CoV from camels to humans is thought to be the possible transmission mode, and very limited human-to-human transmissions have been seen. Also, primary and secondary zoonotic transmission from dromedaries was reported. The primary transmission spreads due to contact with non-suspected MERS-CoV patients or an unknown source. If the infection is transmitted due to direct contact with a MERS-CoV-positive patient, that is known as secondary transmission [15]. Raw camel milk was considered a possible mode of transmission and thus investigated, and the first case was detected in a Yemeni pilot who consumed dromedary camel milk [16]. Camel milk-associated MERS-CoV cases were also reported from Qatar and Oman [17]. The reverse transcription-polymerase chain reaction (RT-PCR) was used to detect MERS-CoV that had shown similarity with confirmed human cases after sequencing; thus, the transmission was considered *via* respiratory mode [18]. The pathogenesis of MERS-CoV infections is not fully understood due to a lack of pathological information. Severely infected patients may get acute respiratory distress syndrome (ARDS) and pneumonia and require intensive care with mechanical ventilation. In MERS-CoV infection-causing bacteria, community-acquired bacteria have also been reported [19].

MERS-CoV has emerged from Jeddah, Saudi Arabia, through bats and intermediate hosts to humans [20]. Three different patterns of MERS-CoV transmission, sporadic, healthcare-associated, and intrafamilial transmission, have been reported among Saudi Arabia's population. Among all reported cases, 45% were health-associated, 38% cases were considered primary, and the remaining were among intrafamilial contacts [20]. The infected people had cough, fever, and difficulty breathing. Molecular characterization showed that it was the new form of coronavirus, thus named MERS-CoV [21]. MERS-CoV caused 35% mortality among infected patients, so it has been known as the second major pathogenic coronavirus after SARS-CoV [22]. The SARS-CoV crossed over to humans from bats through intermediary host Asian palm civets that affected many people before being contained with an 11% mortality rate [23]. MERS-CoV has similar phylogenetic characteristics to bat beta-coronaviruses, affecting camels; however, only mild symptoms were seen. The virus was introduced by the infected camels traded from Africa to the Arabian Peninsula. Camel-to-human infection was first seen in 2012. The infection is acquired *via* contact with camel's saliva, respiratory droplets, and respiratory organs during slaughtering, consuming raw milk, or undercooked meat [24]. It has been suggested that transmission occurs through close contact, and many cases were reported among healthcare workers and household members. In Saudi Arabia, 23 healthcare workers got infected in the same hospital, and a family cluster was also seen in three brothers who got

SUBJECT INDEX

A

B

C

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

D

E

F

G

H

I

J

K

L

M

N

P

R

S

T

V

Z

Kamal Niaz

Prof. Kamal Niaz (KN) obtained Ph.D. in the field of toxicology and pharmacology at Tehran University of Medical Sciences (TUMS), Tehran-Iran in 2017. He is working as assistant professor in the Department of Pharmacology & Toxicology, Faculty of Bio-Sciences, Cholistan University of Veterinary & Animal Sciences (CUVAS), Bahawalpur. He has developed excellent research and communication skills and has expertise in the domain of environmental toxicants, diabetes, endocrine disruptor, and genomic disruption. His area of expertise include phytomedicine and ecotoxicity, epigenetic alteration due to toxicants and the role of these chemicals in diabetes. He has published more than 50 articles in national and international journals with h-index-17, and 107+ impact factors along with 40 book chapters in various publishers such as Elsevier, Springer, and Bentham Science. He is working as a volunteer reviewer in different prestigious journals Frontier of Veterinary Sciences, Food and Chemical Toxicology, Scientific Report, Science of the Total Environment, and Current Protein & Peptide Science, etc.

Muhammad Sajjad Khan

Prof. Dr. Muhammad Sajjad Khan did Ph.D. in dairy science (genetics and statistics) University of Wisconsin, Madison, USA. He has over 35 years of research and teaching experience. He published over 650 publications in international journals and 5 books. His main focus is on the development of animal recording and genetic evaluation procedures for improved utilization and conservation of animal genetic resources as well as development of chicken breeds for backyard poultry industry. MS Khan has over 5600 citations with 33 h-index.

Muhammad Farrukh Nisar

Prof. Muhammad Farrukh Nisar completed doctorate from College of Bioengineering, Chongqing University, Chongqing, China in December 2016, and working as an assistant professor in the Department of Physiology & Biochemistry, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, since, December 2018. He is mainly involved in research areas including photo biochemistry, biochemistry, biochemical pathways, oxidative stress, and cellular defense mechanisms, cell biology, cell signaling and pathways biology, screening of natural drugs as potential anti-cancer(skin) and skincare drugs, phytomedicine, photo- and chemo-therapies to treat skin cancer/conditions, natural drugs, and cell signaling, photomedicine and free radicals biology (ROS/RNS). He has published more than 70 research articles so far on multifarious aspects of biomedical/life sciences with a cumulative impact factor of over 120. He is actively involved in exploring novel biomedical materials for drug delivery, photosensitive drug release, and plant isolations to find novel anticancer, antioxidant, and anti-aging drugs.