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Middle East Respiratory Syndrome Corona Virus (MERS-CoV): A Narrative Review

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Abstract

Middle East Respiratory Syndrome (MERS) is a disease of the respiratory system caused by a coronavirus (CoV) named as MERS-CoV. MERS has been reported in various Arabian countries, mainly Saudi Arabia, where it is transmitted to human through dromedary camels. People having other health conditions like renal failure, diabetes, heart diseases and above the age of 60 are at higher risk of getting infected. MERS-CoV is an RNA virus that interacts with receptors on host cells via components of its spike proteins. After entry of viral RNA into host cells, the viral RNA is translated to form viral proteins. Viral spike proteins have been found to undergo mutations that make it difficult to design vaccines for this infection. Current methods of diagnosis include molecular and serological methods. Antiviral therapies for treatment of this MERS are still being accessed. There is an urgent need to gather the sequencing data from infection outbreaks to highlight any possible mutations in the virus. Additionally, simple diagnostic tools should be developed for early diagnosis. Suitable animal models can prove to be useful not only for diagnosis of MERS infection, but also for accessing antiviral therapy and vaccine development.

Keywords: *MERS-CoV; Respiratory Disease; Middle East; Dromedary Camels; Corona Virus*

Introduction

Middle East Respiratory Syndrome (MERS) is a respiratory disease caused by a coronavirus named as Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Six human coronaviruses have been reported so far, including the human coronavirus (HCoV)-NL63, HCoV-229E, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV. Coronaviruses were known to cause mild respiratory diseases until 2002 - 2003 when around 8000 cases of severe respiratory infection were reported in a SARS-CoV epidemic. Later on, MERS-CoV was also found to cause severe respiratory disease and various outbreaks are still reported in various countries [1].

Prevalence of MERS-CoV

The first case of MERS-CoV infection was reported in 2012 in Jeddah, Saudi Arabia. According to World Health organization, 2229 laboratory confirmed cases of MERS-CoV were reported between 2012 and June 2018. Around 83% of the infections in this period were reported in Saudi Arabia. The largest outbreak outside the Middle East was reported in the Republic of Korea that caused 39 deaths out of the 186 reported cases. MERS-CoV infection has caused total 791 deaths to date. Since 2012, MERS-CoV infection has been reported in 27 countries from the Middle East, Africa, Europe, Asia and America [2]. Table 1 shows the number of laboratory confirmed cases reported from various countries.

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Country	No. of cases
Saudi Arabia	1854
United Arab Emirates	86
Jordan	28
Qatar	19
Oman	11
Kuwait	4
Lebanon	2
Bahrain	1
Egypt	1
Yemen	1

Table 1: *Laboratory confirmed cases of MERS infection in Arabian countries (Adapted from [2]).*

Symptoms and risk factors

Symptoms of MERS infection include cough, fever, shortness of breath and in some cases, pneumonia. Some patients also develop gastrointestinal symptoms like diarrhoea. However, MERS have also been detected in patients who did not show any symptoms (asymptomatic) of the disease but the laboratory tests showed that they had the infection. Blood analysis of infected patients show increased white blood cells and decreased lymphocytes, platelets and red blood cell count. Severe infection causes death due to respiratory failure [3]. The incubation period of MERS is 2 - 16 days and symptoms of disease begin to appear between 13 - 14 days. The patient may live from 5 - 27 days and the median time to death is 11 - 13 days [4].

MERS-CoV may be a serious risk for males who are more than 60 years old and suffer from other medical conditions like hypertension, diabetes and renal failure [5]. Alraddadi., *et al.* (2016) found in a multivariable analysis that diabetes mellitus, heart disease and smoking are independently associated with MERS-CoV infection [6]. Based on public health emergency, MERS-CoV infection has been listed in the WHO Blueprint list of priority viruses [7].

Genome structure and function of MERS-CoV

MERS-CoV is the largest (30 kb) single stranded (positive sense) RNA virus. Two-third of MERS-CoV genome is contains replicase complex i.e. ORF1a and ORF1b while the remaining one-third encodes for spike, envelope, membrane, nucleocapsid and five accessory proteins. The flanking regions of MERS-CoV genome contain 5' and 3' untranslated regions [8]. MERS-CoV contains an RNA genome that codes for 4 proteins including envelop protein, nucleocapsid protein, membrane protein and spike protein (Figure 1). The viral spike (S) protein is known to interact with DPP4 receptors on the host cell surface. The S protein is made up of two subunits called S1 and S2 subunits. The S1 subunit is made up of receptor binding protein (RBP) and N-terminal domain (NTD). The spike protein binds host receptors through the receptor binding domain (RBD). The spike protein undergoes conformational changes after binding the host receptor that promotes membrane fusion. Due to the significant role of spike protein in interaction with the host, various components of spike protein may serve as excellent targets for developing vaccines [9].

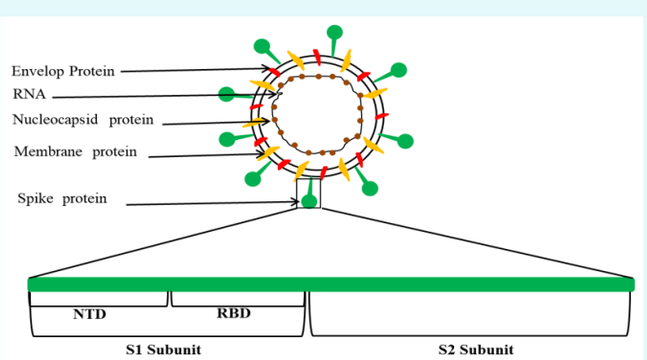

Figure 1: Genome structure of MERS-CoV. NTD: N-Terminal Domain; RBD: Receptor Binding Domain. Adapted from [7].

Pathogenesis

The receptor binding domain of spike glycoprotein of MERS-CoV attaches itself to the host through DPP4 receptors on the host cells. The successful virus and host cell fusion results in the release of viral RNA into the cytoplasm of host cell. The ORF1a and ORF1b of viral RNA are translated into viral polyproteins. These polyproteins are then cleaved by viral 3C-like protease and papain-like protease to form 16 non-structural proteins. Then the proteins involved in transcription and translation assemble at endoplasmic reticulum membranes to form replication-transcription complexes. New viral RNA are generated and enclosed in nucleocapsid proteins in cytoplasm. This encapsidated RNA is transported to endoplasmic reticulum-golgi intermediate compartment where other viral proteins including spike, membrane and envelope proteins are inserted to assemble viral particles. These assembled viral particles are matured and released from the buds of golgi bodies [10].

After entering the host cells, the MERS-CoV evades the host defence mechanisms to cause the disease. An autopsy reported obtained from a case in the United Arab Emirates in 2014 revealed that MERS-CoV primarily affects respiratory multinucleated syncytial cells and type 2 alveolar pneumocytes [11]. Generally, viral infections illicit an immune response in the host cells that involves production of type I interferon (IFN) including IFN-α and IFN-β. Viruses escape the host immune response by producing IFN antagonist proteins that block one or more components of IFN and NF-κB pathways. Coronaviruses also produce proteins that help them evade host immunity by blocking the host immune signalling pathways. MERS-CoV produces membrane protein, ORF4a, ORF4b and ORF5 that act as strong IFN antagonists. For instance, ORF4b is the first reported RNase L antagonist that inhibits the IFN and NF-κB signalling pathways thereby avoiding host immunity. The replicase proteins of MERS-CoV including nsp1, nsp3 and nsp14 also help the virus to bypass host immune response through different mechanisms. Therefore, MERS-CoV spreads rapidly in the host by preventing the host immune response in various ways [8].

Animal models for disease pathogenesis and vaccine development

In order to study disease pathogenesis in detail and subsequent development of vaccines and/or drugs, various animal models have been used. For instance, rhesus macaque was infected with MERS-CoV to study the development of disease. However, the animal developed only mild to moderate symptoms of pneumonia. The virus caused self-limiting infection of lower respiratory tract in the rhesus macaque. These results indicated that human MERS-CoV infection is not replicated exactly in the rhesus. Nevertheless, MERS-CoV infected alveolar pneumocytes similar to human beings [12]. Human MERS-CoV infection has been better replicated in marmoset models than rhesus models. Various studies reported that MERS-CoV causes more vigorous viral replication and severe pneumonia in marmosets as compared to rhesus models. Therefore, marmoset models can be useful for studying severe MERS pathology. Mouse models cannot be used to study MERS pathology because the DPP4 receptor of mice does not allow entry of MERS-CoV into the cells. This is due to difference in two amino acids of mouse DPP4 receptors, as compared to humans, that prevents the interaction of mouse DPP4 with MERS-CoV. Scientists replaced mouse DPP4 with an adenovirus mediated transient human DPP4 in the mouse models that resulted in successful entry of MERS-CoV in the mutated mice. Although mice developed the MERS infection but the virus was cleared after eight days of infection. Later on, mouse models carrying full length DPP4 replaced by human counterpart, DPP4 modified through CRISPR/Cas9 or ten to twelve mouse DPP4 exons replaced with human counterpart exons were developed and these models developed severe disease mimicking human MERS infection. These mouse models are also being tested for MERS vaccine development [13].

Transmission of MERS-CoV

MERS-CoV is a zoonotic infection and is endemic in camels in the Arabian Peninsula. The virus likely originated in bats and transmitted to humans through camels. Virus can also be transmitted to the workers during occupational exposure to infected camels. MERS-CoV replicates in bats without showing the symptoms of the disease. Dromedary camels serve as the intermediate host for this virus and these camels have shown high seropositivity for the virus. Seroepidemiological studies on various populations showed high seroprevalence of MERS-CoV in people who had higher exposure to dromedary camels as compared to general population [14]. Human to human

transmission requires prolonged and relatively close exposure [15]. For instance, health care workers who are providing unprotected care to infected patients may get the disease. However, sustained human to human transmission has not been reported anywhere across the globe [5].

The link between camels and humans for the transmittance of MERS-CoV has been established through the genome sequencing data. Genomic data provided a detailed picture of the epidemic and helped to establish the sources of infection [16]. MERS-CoV was first sequenced with the help of random primers that showed various open reading frames. Sequencing data from subsequent infection outbreaks showed mutations in the receptor binding domain of spike protein sequences. Therefore, sequencing data can provide important information about the location of new mutations that may help to improve the accuracy of diagnosis and efficacy of treatments [17].

Diagnosis

Early diagnosis of MERS remains a challenge for the physicians. MERS-CoV can be diagnosed through RT-RCR assays and viral culture in LLC-MK2 and Vero cells. Cell culture is a slow process for diagnosis, however, RCR based methods are preferred because of their accuracy and sensitivity. PCR based diagnosis can be performed with stool, serum and respiratory samples. However, respiratory samples including sputum, tracheal aspirates and nasopharyngeal swabs are considered gold standard for diagnosis of MERS-CoV infection [17]. The WHO recommended an assay kit called The RealStar® kit for diagnosis of MERS-CoV infection. This kit uses primers that target at least two different genomic regions including a region upstream of envelop gene and ORF1. This kit results is still the most widely used diagnosis method of MERS-CoV infection due to its highest sensitivity [4].

In addition to molecular methods like RT-PCR, serological methods can also be used for diagnosis, especially confirmation of MERS-CoV infection. Serological methods detect the antigens and antibodies in the patients. Various ELISA assays have been developed that detect viral nucleocapsid protein and spike proteins with high sensitivity and specificity. In addition, various neutralization assays have been developed to detect the antibodies produced by the host against viral nucleocapsid and spike antigens. Recently, Trivedi., *et al*. (2017) reported a diagnostic algorithm for identification of MERS-CoV specific antibodies that uses indirect ELISA for detecting nucleocapsid proteins followed by immunofluorescent staining and microneutralization titration assays. The authors reported that some patients having MERS-CoV infection never develop spike protein antibodies. Additionally, antibodies against nucleocapsid protein are detected in the earlier stages of infection. However, antibodies against the nucleocapsid may cross react with those for other coronaviruses. Therefore, antibodies against both nucleocapsid and spike proteins should be targeted for sensitive and specific diagnosis of MERS-CoV infection [18]. In short, serological methods can be useful for diagnosis of MERS-CoV infection. Nevertheless, serologic methods are less commonly used as compared to molecular diagnosis methods because the kinetics of antigen production is not well understood and virus cannot be detected in respiratory samples [17].

Prevention and treatment

Since, MERS-CoV is mainly transmitted from dromedary camels, anyone visiting markets, farms or places, where camels are present, should practice general hygiene measures. Contact with sick animals should be avoided and hand should be washed after touching the animals. Animal products including meat and milk should be properly cooked before consumption. Pasteurization of camel milk completely ablates the MERS-CoV. Additionally, cross contamination of uncooked animal products with other food items should also be prevented. People above age 60 or with diabetes, renal failure, heart disease and immunocompromised persons should avoid contact with dromedary camels and their uncooked food products [5].

First incidence of MERS-CoV infection was reported in 2012 but no vaccine has been approved for this disease yet. The process of vaccine development is under-process and efficacy of these vaccines is mostly tested on mouse models expressing human DPP4 receptors. The mechanism that allows MERS-CoV to infect various hosts involves rapid mutations in its viral spikes. A host receptor, dipeptidyl peptidase 4 (DPP4) interacts with the MERS-CoV and change in the DPP4 may prevent the interaction of virus with host thereby preventing the infection. However, studies have shown MERS-CoV rapidly adapts to variations in the host receptor. Various mutations in the

surface charge of viral spike allow it to interact with changed DPP4 receptors. This ability of virus to adapt to various receptors allows successful cross-species adaptation. Therefore, it is still difficult to develop a suitable vaccine for the disease [19]. General supportive care is provided to the patients depending on the severity of symptoms. Fluids, rest and analgesics are used to help the patients. In order to minimize the risk of co-infection with other pathogens, broad spectrum antimicrobials, antifungals and antivirals are used. Antiviral drugs like alisporivir and ribavirin have been tested on animal models but due to the differences of disease development in human and animal models, further studies are required to confirm the effectiveness of these drugs in human MERS infection [8]. Recently, Cong., *et al*. (2018) accessed antiviral activity of three compounds including chloroquine, toremifene and chlorpromazine, in monocyte derived macrophages and dendritic cells upon MERS-CoV infection. Results showed that chloroquine had no activity, chlorpromazine had strong activity with high cytotoxicity while toremifene had marginal activity with high cytotoxicity on the infected cells. This study showed that antiviral drugs can be tested in primary cells before initiating preclinical studies. Additionally, drug screening assays can be used to observe any cytotoxic effect of these drugs [20].

Conclusion and Recommendations

In conclusion, MERS-CoV is mainly prevalent in Arabian Peninsula where it is mainly transmitted from dromedary camels to humans while human to human transmission is rather ineffective. The virus transmission can be controlled by adopting simple preventive measures like pasteurization of milk, proper cooking of meat and practising general hygiene measures at health care centres. It is recommended that educational programmes should be arranged for the awareness of health care workers, workers at camel farms and also for the general public of affected countries. People aged more than 60 or having other health complications like heart disease, renal disease etc. are at higher risk of developing the infection than general population. Therefore, it is recommended that people with high risk of MERS infection should be extra careful about to prevent viral transmission. MERS-CoV is RNA virus that interacts with host cells through its spike protein. Animal models can be useful for studying the pathogenesis of virus *in vivo*. Generally, the animal models do not replicate the entry or pathogenesis of virus as in human beings. Therefore, it is recommended to develop appropriate mutant animal models that can develop disease as human beings. These mutant animal models can be useful for developing both efficient diagnosis and treatment of the disease. Early diagnosis of MERS-CoV infection can be useful for controlling the disease. Currently molecular and serological methods are available to diagnose the infection. Molecular methods including RT-PCR are more appropriate for MERS diagnosis in the earlier stages of infection as compared to serological methods but require complex equipment and expertise for diagnosis. Since the MERS infection develops very fast causing death within a month, a simple, sensitive and quick diagnosis method must be developed. Development of vaccine is a still a challenge especially due to the mutations in the target viral proteins. Therefore, it is recommended to use genome sequencing frequently for any reported cases and keep a record of any possible mutations. Additionally, effectiveness of potential antiviral drugs should be tested in primary cells and suitable animal models before clinical administration.

Declaration of Conflicting Interest

The authors declare that there is no conflict of interest.

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