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Evolving trends in molecular virulence, pathogenesis, symptomatology and epidemiology of recent Middle East respiratory syndrome coronavirus (MERS-CoV)

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ABSTRACT:

The Middle East respiratory syndrome coronavirus (MERS-CoV) is a recently reported virus that is associated with severe, life-threatening and rapidly spreading primarily respiratory illness called the Middle East respiratory syndrome (MERS). MERS-CoV possesses a unique positive sense single-stranded (ss) RNA and can undergo rapid mutation in the viral genome. This results in antigenic switching and genetic variation finally leading to the emergence of novel and new MERS-CoV subtypes which are uncontrollable by vaccines. Researchers are also finding difficulties to sort out therapeutic intervention strategies for MERS-CoV. This virus can spread from human to human but transmission from dromedary camels to humans plays a crucial epidemiological role. Dromedary camel acts as "gene mixing vessels" for MERS-CoV and these viruses undergo rapid genomic change. Viral receptors called dipeptidylpeptidase 4 are important receptors for attachment and spread of MERS-CoV in humans. The current method of laboratory confirmation is through Real Time-Polymerase Chain Reaction (RT-PCR) on broncho alveolar lavage, sputum and tracheal aspirates. Unfortunately, till today there are no definite antiviral drugs available for MERS-CoV.

Keywords: MERS-CoV, +ss RNA, Virulence, Genetic rearrangement, RT-PCR.

INTRODUCTION

The Middle East respiratory syndrome coronavirus (MERS-CoV), is a unique positive sense single strand (ss) RNA virus belonging to the genus Betacoronavirus[1]. Previously it was called novel coronavirus 2012 because it was isolated in 2012 by genomic sequencing from the sputum of a patient, during the 2012 outbreak of new flu[2].

Until August 2015, MERS-CoV outbreaks were reported in more than 21 nations like Saudi Arabia, Jordan, Egypt, Qatar, UAE, Turkey, Kuwait, Oman, Bangladesh, Indonesia, Algeria, South Korea, Philippines, Thailand, USA, UK, and Austria[3]. According to the European Center for Disease Prevention and Control (ECDC), it was estimated that out of a total of 1082 MERS-CoV cases, there were 439 deaths, with a mortality rate of 41%[4]. As per the World Health Organization (WHO) estimates, the fatality rate was 37%. MERS-CoV patients have acquired this deadly infection through various sources, namely, infected humans, camels, bats other domesticated animals and pets[5]. The special feature of coronaviruses is its ability to undergo rapid genetic rearrangement and profound molecular variation in its ssRNA. These rearrangements and variations of the viral genome, resulting in new antigenic subtypes, lead to difficulties in developing vaccines and therapeutic interventions[6]. Hence, MERS-CoV poses serious threat not only to the Middle East region but throughout the world, as a whole.

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Genomes of MERS-CoV are phylogenetically grouped into clade A and clade B. Initially, reported MERS-CoV cases belonged to clade A (Jordan-N3/2012), recently, the newly reported cases were shown to be genetically different, as such, they were named as clade B. Based on molecular, genetic and antigenic studies it was established that MERS-CoV is unique and different from SARS coronaviruses and common cold coronaviruses. Due to this fact, earlier MERS-CoV was called "Saudi SARS"[7]. This notorious virus was first identified and isolated in Saudi Arabia by Dr. Ali Mohamed Zaki from a patient suffering from pneumonia-like illness and these isolates exhibited cytopathic effects (CPE) like syncytia formation. Through molecular clock analysis, zoonotic transmission was established from dromedary camels (*Camelus dromedaries*) particularly prevailing in Middle East countries.

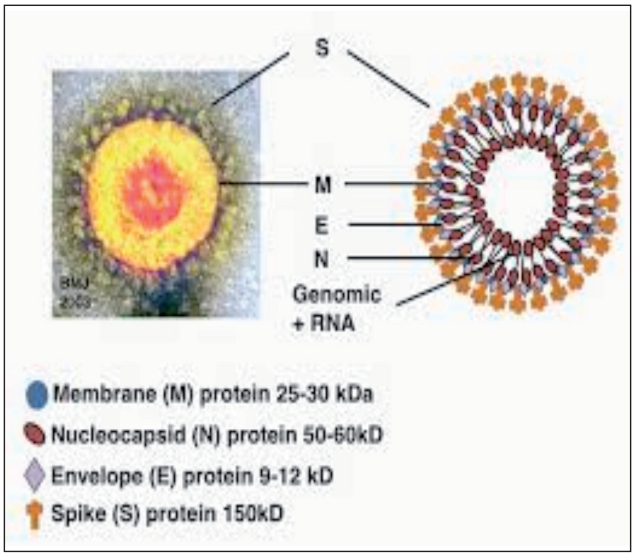

Figure 1. Molecular structure of MERS-CoV.

MOLECULAR VIRULENCE

Both MERS-CoV and SARS-CoV have similar ss positive strand RNA in their genome.

This ssRNA in MERS-CoV encodes structural proteins (SP), membrane protein (M), envelope (E), nucleocapsid (N), two non structural, replicasepolyproteins (ORF1a & ORF1b) and spike (S)[8]. The two non-structural proteins (NSP) initiate genomic replication and RNA synthesis. The huge replicase gene surrounds 5' proximal side of RNA genome. Translation of ORF 1a give rise to polyprotein 1 a (pp1a), ribosomal frame shifting makes translation of ORF 1b to pp1b. MERS-CoV utilize one or two papain like protease to release NSPs. It is established that MERS-CoV possess 16 NSP[9].

Viral components like NSPs, SPs, M, E, N, S and other proteins, glycoproteins and enzymes play a crucial role in virulence, in establishing and exaggerating the disease (Figure 1).

MERS-CoV's E protein is responsible for attachment of virus particles to host cell receptors. S protein is in charge of fusion and entry in to the respiratory epithelia[10].

Viral proteases help the spread to lower parts of the lungs, thus enhancing the severity of the infection. It results in inflammation and suppression of anti viral interferons (IFN). At this point of the infection, there is excessive anti viral immune responses like interleukins (IL-6, IL-8 and TNF-α), humoral IgG and IgM are inefficient to control the viral spread and replication in the lungs. The very nature of MERS-CoV virus ability to overcome and suppress host immune challenge is due to its +ssRNA, viral structural (SPs) and non structural proteins (NSPs)[11].

PATHOGENESIS AND SYMPTOMATOLOGY

MERS-CoV pathogenicity is based on the extent pathogen-host interaction. It elicits maximum pathogenic potential especially in humans. This is due to the fact that MERS-CoV shows a strong tropism for bronchial non-ciliated epithelia. Furthermore, the virus arrests host bronchial interferon (IFN) synthesis[11,12]. It should be noted that most of other viruses causing respiratory diseases attack and damage epithelial cilia, including Influenza type A. Molecular studies revealed that cellular receptors for MERS-CoV are exopeptidase (angiotensin converting enzyme 2)[12]. Moreover, it was found that neutralization of angiotensin converting enzyme 2 by specific antibodies did not arrest the spread of infection into bronchus and lung alveolus. Extensive investigations showed that another functional cellular receptor called dipeptidylpeptidase 4 were also involved in the severity of MERS-CoV disease spread into the lungs[13]. Of note, receptors for dipeptidylpeptidase 4 are also located in nephrons of kidneys and heart. During the acute stage of the MERS-CoV infection, there is a severe viremia, leading to spread of MERS-CoV viral particles in the blood stream. Hence, MERS-CoV leads not only to the damage of lungs but also kidneys and heart, thereby resulting in respiratory, renal and cardiac failure, ultimately ending to coma and death[14]. The severity is worsened by concurrent secondary bacterial infections. Recent research showed that bacterial infections due to *Staphylococcus aureus, Group A Streptococcus, Streptococcus pnuemoniae* and *Haemophilus influenzae type b* augment the pathogenic potential of MERS-CoV, particularly in humans. These bacteria particularly dwell in the oral cavities, tonsils and pharynx of humans[15]. Another interesting fact is that the reservoir host dromedary camels and other animals do not exhibit the presence of dipeptidylpeptidase 4 receptors in their kidneys and heart. In humans, after the entry of MERS-CoV viral particles in to lung alveoles, alveolar macrophages fail to contain the spread of infection. The strong host cellular immune response and cytokine release leads to inflammation and fluid accumulation in lungs. This will result in the characteristic symptoms of MERS-CoV: high fever, chills, rigors, severe cough, dyspnea, and hypovolemic shock[16]. Many patients exhibited common symptoms of pneumonia, whereas, a significant number of patients expressed symptoms of chronic obstructive pulmonary syndrome (COPD) like disease. Another large number of patients also revealed laryngo-tracheo-bronchi-

tis, which is called "croup". The patterns of disease due to MERS-CoV also differed among different age groups. Clinical cases, morbidit

y and mortality rates due to MERS-CoV infections are more common and higher (80%) in the age group of 35-45. Astonishingly, the severity of MERS-CoV is lower (40%) in the higher age groups, particularly above fifty five[17].

EPIDEMIOLOGY

Epidemiology and mode of spread of MERS-CoV play a crucial role in morbidity and fatality due to these life threatening viruses. Different ways of transmission of MERS-CoV have been documented[18]. They include dromedary camel to human mode, bats to camels, among camels, cattle to man, dogs to humans, cats to man, bats to humans and finally man to man transmission. Human to human spread is very effective and common in close contacts and crowded surroundings. Nosocomial, hospital borne outbreaks have also been reported in many instances. There have been reported incidences of spread from patients to health care workers[19]. In MERS-CoV epidemiology dromedary camels play an important role because these animals act as reservoir host. Dromedary camels also act as "gene mixing vessels". When two different strains of MERS-CoV, from two different sources, infect dromedary camels, these two genetically different MERS-CoV exchange their +ssRNA resulting in development of new subtypes of MERS-CoV. The stunning features of these new subtypes of MERS-CoV are new antigens and novel virulence genes. In Saudi Arabia there were possible cases of human transmission after drinking camel milk. But there are no cases documented in humans through ingestion of camel meat[20].

RECENT LABORATORY TESTING TECHNIQUES

Patients suffering from MERS exhibited low white blood cell count particularly low lymphocytes. Confirmation and rapid molecular testing can be performed using Real time-polymerase chain reaction (RT-PCR) test. RT-PCR testing can be performed on bronchoalveolar lavage (BAL), sputum and tracheal aspirates. Serological test like immunofluorescence technique (IFT) is also useful. But the main issue with IFT is that many other serologically related viruses may cross react with MERS-CoV antibodies. So IFT is unsuitable for confirmation. The World Health Organization recommends RT-PCR for confirmation of MERS-CoV in clinical samples[21].

PREVENTION AND TREATMENT

Since the main transmission is through camels, people should be advised to handle camels with precautions especially camels with severe nasal discharge. Other general health care measures should be taken to arrest nosocomial hospital borne MERS-CoV outbreaks. For the treatment of MERS-CoV cases, administration of ribavirin and interferon (IFN-α2b) was found to be promising but not very effective. Researchers are recently investigating many ways to overcome the outbreak of MERS-CoV. Many drugs are currently under evaluation (IFN, chloroquine, chlorpromazine, loperamide, camostat, mycophenolicacid and lopinavir[22]).

CONCLUSIONS

MERS-CoV causes severe, life threatening and rapidly spreading Middle East respiratory syndrome not only in Middle East region, but throughout the world. Dromedary camels and bats play an important role in the spread of MERS-CoV. Several nosocomial infections due to MERS-CoV have also been reported worldwide. Several structural viral proteins and non-structural proteins are critical for the molecular virulence of MERS-CoV. Due to the +sense ssRNA, MERS-CoV can undergo molecular rearrangement leading to antigenic change, genetic variation and mutation. These viral characteristics contribute to the emergence of new and novel sub types of MERS-CoV in the human populations. These unique subtypes are difficult to control, and new strategic therapeutic intervention are needed. Another important issue faced by scientists and molecular virologists is related to these new MERS-CoV viral particles which possess enhanced molecular virulence, thus leading to increased pathogenicity and a more rapid spread than their parental strains.

Prevention is mainly based on avoiding contact with dromedary camels and focusing on nosocomial infection outbreaks. Although no drugs are currently available, researchers are trying to develop antiviral molecules to combat MERS-CoV.

Conflict of Interests:
The Authors declare that they have no conflict of interests.

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