Immunopathogenesis and epidemiology of Middle East Respiratory Syndrome Coronavirus

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Abstract

The emergence of a novel coronavirus in the Arabian Peninsula has caused global concerns regarding its spread and control. The virus with nearly 40% case-fatality and with no available therapeutic intervention seems to be another pandemic in making. This is compounded by the fact that Saudi Arabia hosts every year nearly 2-3 million pilgrims for the Hajj season. A number of studies have been carried out on the characterization of the virus, its pathogenesis, epidemiology, clinical presentation and source of infection, yet much remains to be established for the control and management of the disease. The present review tries to summarize the immune response and immunopathogenesis observed in patients and cell lines. We also look at the epidemiological analysis of MERS-CoV cases reported by Saudi ministry of health from August to November 2014, which also comprises of the Hajj season.

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1   Introduction

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a newly described virus, that was first reported in September 2012 from a 60 year old male patient in Jeddah Saudi Arabia, admitted on complaints of respiratory illness [1]. Three years later 550 patients have died [2] and new cases are reported every week by Saudi ministry of health, World Health Organization (WHO) and European Centre for Disease prevention and Control (ECDC). A virus that seems to have emerged in Saudi Arabia has spread to several countries within a short span of two years affecting Middle East, Africa, Asia, Europe and North America. All the cases seem to be linked to Middle East. Affected countries in Middle East include Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, Kingdom of Saudi Arabia (KSA), United Arab Emirates (UAE) and Yemen [3].

MERS-CoV is a new addition to the growing group of emerging viruses that causes the infection of respiratory tract, and comprises of Influenza virus (H1N1, H5N1, H7N9) and Severe Acquired Respiratory Syndrome Corona virus (SARS-CoV). Common symptoms of the disease are fever with chills, shortness of breath and myalgia and in some cases acute renal failure [4,5]. A nosocomial pattern of spread in hospital workers indicates spread through physical contacts [6]. Risk factors of the disease include immunocompromised individuals and diabetics. Until November 2014, 814 cases have been reported from Saudi Arabia alone, out of which 42% (349) have already died making it a highly fatal virus [7]. The epidemic potential of the virus however is low in contrast with SARS virus that infected around 8000 people and caused nearly 800 deaths within a short span of eight months [8,9]. Their does not seems to be a clear source of infection, though presence of MERS-CoV in both nasal and fecal samples that shows 99.9% genomic similarity to human MERS-CoV and the presence of circulating neutralizing antibodies against the virus in dromedary camels points towards dromedary camels as the source of infection [10,11]. Several MERS-CoV isolates have been

analyzed, all of them giving approximately a 30kb sequence. The positive stranded RNA genome encodes at least 11 open reading frames (ORFs) and three different classes of proteins; structural proteins, non-structural proteins and accessory proteins. ORFs1a and 1b encodes two large polyproteins, which could be further cleaved into 16 different non-structural proteins. ORFs-2, -6, -7 and -8a, encodes the structural proteins S, E, M and N [12].   Phylogenetic studies indicate a close relationship between bat coronaviruses BtCoV-HKU4 and BtCoV-HKU5. As for SARS-CoV, where the virus originated from horseshoe bats, and palm civets served as intermediate host, MERS-CoV seemed to have emerged in bats, and camels serving as intermediate source of infection [13]. In camels the virus establishes a relatively benign infection of upper respiratory tract and is shed in large quantities from nasal route, likely resulting in camel-to-camel or camel-to-human transmission [14]. Relatively little is known about the pathology it causes to its host and patterns of its spread. This review tries to summarize the

immunopathogenesis and epidemiological profile of the virus for the past four months that sees nearly 2 million Hajj pilgrims in Saudi Arabia and presents a great risk for its spread.

2 Methodology

The information presented in the review is gathered after an extensive search of the Medline database using the key words 'MERS-CoV', 'Novel coronavirus', 'epidemiology MERS-CoV' and 'pathogenesis MERS-CoV'. Similar searches were carried out on Google scholar. We also gathered information from publicly available data, from the websites of WHO and ECDC. For the epidemiological analysis, cases reported by the Saudi ministry of health from the months of August to November, 2014 were collected and analyzed.

3 Results

3.1 Immunopathogenesis of MERS-CoV

MERS-CoV is considered to be a zoonotic virus that has crossed the species barrier indicated by its ability to successfully infect human airway epithelium, which are the first tissue type to come in contact with respiratory viruses [15]. MERS-CoV spike (S) protein which is a heavily glycosylated membrane protein determines its cell tropism and infectability [16]. For its fusion to host cell the S protein is dependent on cellular proteases like Furin, Trypsin, transmembrane protease serine protease-2 (TMPRSS-2), Cathepsins, human airway trypsin-like protease (HAT) [16]. It has been shown that furin cleaves the S proteins at two different sites at different times in viral life cycle helping in the infection process and also in formation of syncytia facilitating spread of virus without getting exposed to neutralizing antibodies [16,17]. The importance of cellular proteases as facilitators of viral entry is underlined by the fact that inhibiting furin activity decreases the viral entry and infection while increased expression of furin enhances viral entry [17]. The S proteins copurifies with dipeptidyl peptidase-4 (DPP4) showing this exopeptidase as the definitive receptor for the virus [18]. DPP4 is expressed in lung and kidney cells, making these tissues susceptible to viral infection and explains the symptoms of severe respiratory syndrome and renal failure observed in the patients. Human MERS-CoV seems to infect non-ciliated bronchial epithelium and lung alveolar epithelial cells in ex-vivo cultures and could potentially disseminate to lung endothelium also. Unlike SARS-CoV, which infects and release via the apical route, MERS-CoV can do so from both the apical and basolateral surfaces indicating a blood-borne route of infection and spread additional to the respiratory tract infection [19,20]. Despite their efficient replication and cytopathic effects in human lung epithelial cell line Calu-3, they do not induce the production of TNF-α, IP-10 and IFN-β though

other proinflammatory cytokines like IL-1β, IL-6 and IL-8 are induced at a delayed time point (30 h post infection) indicating an attenuated and delayed anti-viral immune response [21].   A global transcriptome analysis of Calu-3 cell lines infected with MERS-CoV shows massive dysregulation of the host transcriptome, where many genes in the pathogen recognition pathway are activated but 22 genes involved in antigen presentation pathways were down regulated implying that adaptive immune response might not function optimally during MERS-CoV infection [22]. The virus is also shown to infect monocyte-derived macrophage and monocyte derived dendritic cells and replicate efficiently inside these cells without the elicitation of a strong antiviral response indicating that virus must have evolved mechanism to actively suppress such responses [23,24].   Analysis of recombinant expressed ORF4a, an accessory protein in 293T cells show that the protein localizes to the cytoplasm and effects negatively the production of IFN-β via the inhibition of IFN-β promoter activation and IRF-3/7 function at transcriptional level and via the inhibition of ISRE promoter element signaling pathways [25].   Another mechanism suggested shows that ORF4a protein has a dsRNA-binding domain that might sequester viral dsRNA rendering it ineffective for binding with the cellular protein kinase PACT required for the activation of downstream molecules RIG-I and MDA-5 thus circumventing host anti-viral innate immune response [26]. A comparison of the immune response between two patients infected with the virus, and giving different outcomes underlines the importance of IFN pathway in clearance of infection. In the patient that showed a poor outcome, there was a decreased expression of RIG-1, MDA-5 and IRF3-7 resulting in lower levels of IFN-α levels in BAL and serum, additionally, there was persistent secretion of IL-10 which could have suppressed the entire immune response. The patient that survived the infection showed consistently higher levels of IL-12, IFN- β, RIG-1 and MDA-5 and lower levels of IL-10.   Most of the cytokines were at their peak levels between 4-7 days after infection indicating the crucial role of IFN pathway in initial stages for the successful clearance of the virus [27]. The disease is reported with symptoms of fever, chills, myalgia, shortness of breadth, cough and sometimes diarrhea, vomiting and abdominal pain.   Thrombocytopenia, lymphopenia and lymphocytosis were the most common hematological abnormalities with no change in neutrophils and monocytes being observed. More often, the disease affects patients with comorbid conditions with diabetes, hypertension, chronic renal disease and chronic cardiac disease as the most significant comorbid conditions [28]. A recent report suggests low serum albumin to be an independent risk factor for the infection as it might indicate nutritional status of the patients [29]. Chest radiographs of patients are similar to those with community-acquired pneumonia, with pulmonary consolidation as the major feature. Specimens collected from lower respiratory tract indicate higher viral load compared to upper respiratory tract [30]. Several animal models have been tried and tested to recapitulate disease progression and pathogenesis as in humans. The two models that came very close to achieving this goal albeit with some caveats

are rhesus macaques and marmosets. In macaques a non-lethal, milder version of the disease developed for a short period of time without affecting kidneys, unlike in many human cases. The virus replicated predominantly in lower respiratory tract and innate immune response peaked within 3 days of infection and declined later on [31]. In marmosets however, the disease was of more severity with 1000 fold higher viral loads than in macaques and of longer duration but similarly replication of virus was predominantly in the lower respiratory tract as in macaques and humans [32]. Both these models taken together could be useful in understanding disease pathogenesis and help in future for developing therapeutic strategies.

3.2 Epidemiological analysis of MERS-CoV in Saudi Arabia

Saudi Arabia with its population of nearly 28 million where 83% lives in urban areas dedicates 3.2% of its gross domestic product to healthcare. The average life expectancy at birth is 76 years as compared to the global average of 70 years. The number of cases of malaria reported in 2012 was 0.3 per 100,000 of population and that of tuberculosis were 17 per 100,000 of population, which is much lower than the global average [33]. Vector borne diseases like malaria and cutaneous leishmaniasis are gradually declining due to scaling up of vector control measure [34,35]. All health indicators like high life expectancy, low maternal and child death rate at birth and a high per capita expenditure on health point to the fact that Saudi Arabia has a successful healthcare program, which serves Saudi and a large number of expatriate populations quite effectively. Saudi Arabia also oversees world's largest annual gathering during hajj where nearly 2 million pilgrims descends into the cities of Makkah and Madinah. Managing such mass gatherings without an outbreak of any infectious disease despite the fact that pilgrims come from many countries some of which still have not been able to control infectious disease like malaria and tuberculosis is an achievement.

The emergence of MERS-CoV has produced a challenge for Saudi health officials. Initial panic about its spread due to its similarity with SARS-CoV has now made way for more detailed understanding of its epidemiology. Untill November 2014 a total of 940 cases have been reported worldwide, of which 804 were reported from Saudi Arabia alone, and a total of 376 have died due to the infection [2]. In cases of primary infection the virus seems to affect males (61%) more than females (39%) which could be explained due to higher risk of exposure of male population to the animal reservoir of the virus, camels, while secondary infection show an equal gender distribution indicating no specific gender preference as earlier reported [36]. The basic reproduction number-R_0 of the virus is 0.69, which is lower in comparison to SARS-CoV (R_0-0.80) indicating low epidemic potential and the rate for secondary transmission is 4% only[8,37]. The virus shows no preference for a particular age group though case-fatality increases with advancing age possibly due to a weaker immune response. The reach of the virus has spread to 23 countries covering Asia, Africa, North America and Europe. All of these cases trace their origin back to Middle East, affecting travellers who have a recent

history of travel to this region or have been in contact with someone who has travelled to this region. 85% percent of the cases are reported from Saudi Arabia followed by United Arab Emirates (UAE) and Jordan. Another pattern of spread has been observed in health care and interfamilial settings, which represent a close group of people either taking care of the patients or residing with them and coming in direct and repeated contact with the virus, becoming its unsuspecting victims [38]. However very low levels of virus in stool, urine and blood samples from infected patients indicate an airborne route of spread and infection [39]. In Saudi Arabia, Jeddah and Riyadh are the two major regions form where most cases are being reported. Given a large number of expatriate populations in Saudi Arabia, 35% of the cases reported affect non-Saudis living and working in Saudi Arabia. The disease seems to follow a seasonal pattern with maximum number of cases being reported in the months of April and May.

One of the major concerns regarding the spread of SARS-CoV was international travel and exchange between different countries that exacerbated its spread. Saudi Arabia hosts' nearly 2 million pilgrims every year during hajj and the chances of a potential MERS-CoV epidemic outbreak in such a large gathering are high. In the last two years no such outbreaks were reported neither pilgrims returning to there home countries carried the infection. Apart from MERS-CoV surveillance, the Saudi health ministry prescribed preventive measure for the 2014 Hajj season included vaccination for Yellow Fever, Polio, Meningitis and seasonal influenza virus [40]. Additionally, due to current Ebola outbreak no Hajj visas were issues for Guinea, Liberia and Sierra Leone.

For this review we analyzed the cases reported in the 2014 Hajj season by the Saudi ministry of health, primarily in the months from August to November 2014 (Table 1). In this period of four months a total of 82 cases were reported to the Saudi ministry of health [41]. Most of the cases were reported in the month of October, primarily from the city of Taif and Riyadh. Among the total reported cases, 37 patients died which shows a 45% mortality rate in these past four months. A total of 61 males and 21 females reported the symptoms of the disease which corroborate earlier published reports indicating a three times higher predisposition among males for developing the disease as compared to the females. Around 80% of the patients have some preexisting comorbid conditions. A total of 13 cases were health care personals and 10 patients were confirmed to have been exposed to camels with one of them ingesting camel milk indicating that camels may be the source of infection but the spread might take place from human to human contact. The median age of those infected was 53 years and case-fatality ratio rose with advancing age (Table 2). For patients above 60 years it was reported to be 65%. Out of those affected with the disease 57 were Saudis and 26 were expatriates. Overall, a lack of increase in the number of reported cases indicate an absence of increased threat for the spread of infection due to Hajj. Another significant fact was that no cases were reported from the city of Makkah, which is the epicenter for Hajj pilgrims.

4 Tables

Table 1. Epidemiological profile of MERS-CoV cases reported from Saudi Arabia from the months of August-November 2014.

Characteristics	Values
Total cases	82 (100%)
Mortality	37 (45%)
Median age	53.5
Gender	
Male	61 (74%)
Female	21 (25.6%)
Nationality	
Saudi Arabia	57 (69.5%)
Expatriates	26 (31.7%)
Month of reporting	
August	6 (7%)
September	11 (13%)
October	37 (45%)
November	28 (34%)
City of residence	
Taif	30 (36.5%)
Riyadh	26 (31.7%)
Al-Kharj	6 (7%)
Others	20 (24%)
Occupation	
Health care personal	13 (15.8%)
Non-health care personal	69 (84%)
Preexisting disease	66 (80%)
*Animal Exposure	10 (14.2%)

*Information available for only 72 patients

Table 2. Demographic profile of MERS-CoV cases reported from Saudi Arabia
from the months of August to November 2014.

Age group	Total No. Of Cases	Total No. Of Deaths	Male patients		Female patients	
			Cases	Deaths	Cases	Deaths
10-20	3	0	2	0	1	0
20-30	5	4	3	2	2	2
30-40	14	2	10	1	4	1
40-50	14	3	10	3	4	0
50-60	18	10	16	9	2	1
60-70	15	9	12	6	3	3
70-80	8	5	5	4	3	1
80-90	4	3	2	1	2	2
90-100	1	1	1	1	0	0

5   Conclusion

MERS-CoV is a recently emerged virus, the earliest case reported was in September 2012, and consequently the information about the virus is scarce. A major concern has been the high fatality rate of the virus and in the absence of clear therapeutic agents; supportive care is the only option. Ribavirin and IFN-α2b therapy has shown promise in macaque models but their efficacy in humans is questionable [42, 43]. A lack of small animal model as an experimental host is a hindrance in the path to understand the potential pathology of the virus. Identification of DPP4 as the receptor for the virus might help in designing inhibitors that can block the spread of virus. Mostly reported data indicate that the virus seems to have evolve mechanisms to suppress innate immune pathways resulting ultimately in the blocking of secretion of type I Interferons, though a recent report show that human plasmacytoid DCs, when stimulated with live virus results in the secretion of type I interferons [44]. Additionally the virus can infect other immune cell types like dendritic cells and macrophages and downregulates immune response from them as well. However, most reports published about immune response to the virus are based upon its behavior in human cell lines, which does not portray accurate picture of the immunopathogenesis. Lack of autopsies from infected patients, is another factor impeding the understanding of the pathogenesis. The only animal models that develop the disease are rhesus macaques and common marmosets, and might offer some insight into pathogenesis and virus behavior.

A high fatality combined with high pandemic potential could have been devastating, however the virus has shown no signs of high human-to-human transmission indicating originally calculated low reproduction rate of the virus to be still relevant. The virus has also shown no signs of evolving into more infectious strain unlike SARS-CoV, which could have impacted its transmissibility. Identifying a clear pattern of transmission is crucial to limit its spread. There is no direct evidence for camel to human spread, however certain factors like the genetic similarity between virus isolated from camels and humans, high incidence of cases in April and May which coincide with camel parturition season and a majority of male patients in primary cases as compared to secondary cases where there is an equal distribution among male and female point to camels as the source of infection. The virus has affected mostly Arabian Peninsula with most cases reported from Jeddah and Riyadh. The disease affects mostly male and the median age of those infected are around 53 years. Around 40% of those infected die making this a highly fatal virus. The disease affects Saudis and expatriates alike, showing no genetic predisposition for infection. Around 27% of those infected were healthcare workers. The large influx of pilgrims during hajj season doesn't seem to exacerbate the spread of disease that might change in future as the hajj season moves towards April and May, which are the peak time for MERS-CoV infection. During the months from August- November which also constitutes the Hajj season, a total of 82 cases were reported form the cities of Taif and Riyadh. A 45 % mortality rate was observed with case-fatality rising with advancing age. A concerted effort in identifying the source of infection and mode of transmission holds the key to prevent its spread. Since many of the viruses in the past few years have emerged from animals, therefore animal disease surveillance is another aspect that needs to be carefully considered.

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