



Investigation of MERS-CoV seropositivity among Umrah visitors from the Çorum Region of Turkey

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Abstract

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causes Middle East Respiratory Syndrome (MERS). Since the vast majority of cases (more than 85%) are reported from Saudi Arabia, there is a pandemic potential for pilgrimage due to Hajj or Umrah. It is reported from Turkey that more than 400 thousand people went to Saudi Arabia for umrah and 61 thousand people for Hajj in 2014. In this study it is aimed to investigate the patients who had just returned from Makkah for Umrah and who also applied to the Infectious Disease Clinics at the Hitit University Erol Olcok Training and Research Hospital for having respiratory tract symptoms. Their serologic situations have been determined by ELISA whether there is any risk in terms of performing the Hajj and Umrah, and contracting MERS-CoV. Between January 1st to the 31st of October 2015, 40 people were included in this study, which were admitted to our hospital with upper respiratory tract complaints and had previously been in Saudi Arabia for Umrah within the last 15 days. As a control group, 40 healthy people without any complaints and travel histories to risky areas were selected. Their serum samples were taken and searched by MERS-CoV IgG ELISA (Euroimmun AG, Lübeck, Germany). The results ≤ 0.8 were considered as negative, ≥ 1.1 were as positive, 0.8-1.1 were suspected. All suspected and positive results have been reevaluated and confirmed. Only two (5%) individuals from the patients' group were found as positive for the MERS-CoV IgG antibodies, but individuals from the remaining patients' group and also all control group members were determined as negative. Travels to Saudi Arabia could be a risk for exposure to MERS-CoV. Although there is no evidence, contamination could be realized by anthropologically due to crowds.

Keywords: corona virus, MERS-CoV, respiratory syndrome

1. Introduction

From all over the world 5.5 million Muslims perform Umrah, 1.8 million for the Hajj visit Saudi Arabia. In 2014, 61 thousand people performed the pilgrimage, and more than 400 thousand people from Turkey went to this holy area for Umrah (1). Because of the enormous crowds and close contact, from time-to-time outbreaks of communicable diseases can be seen during the pilgrimage or Umrah.

Middle East Respiratory Syndrome Coronavirus, (MERS-CoV) was identified in a patient from Saudi Arabia in 2012 for the first time and the disease defined as "Middle East Respiratory Syndrome (MERS)", which may progress to death with acute respiratory and multiple organ failure (2, 3). The virus causes high mortality in individuals who especially have underlying diseases. Camels are carriers of this virus and it's mainly transmitted to humans from them. However, the contamination risk from animals to humans is not high, and human-to-human transmission needs prolonged close contact (4, 5).

The World Health Organization (WHO) reported that at

least 356 of 971 MERS-CoV cases confirmed by the reference laboratory have died by February 2015 (6). The vast majority of the MERS was seen in the Arabian Peninsula or the countries close to this area; almost 85% of the cases were from Saudi Arabia with a 35% mortality rate (6, 7).

Although detection of viral nucleic acid is the best method for diagnosis of MERS-CoV, where NAAT is not possible seroconversion in samples taken two weeks apart could be used for confirmation of the infection. Also, serological methods could be useful for the investigation of an ongoing outbreak and retrospectively assess the extent of an epidemics (8-11).

In this study, it is aimed to investigate the patients who had just returned from Makkah for Umrah and who also applied to the Infectious Disease Clinics at the Hitit University Erol Olcok Training and Research Hospital for having respiratory tract symptoms. Their serologic situations have been determined by ELISA whether there is any risk in terms of performing the Hajj and Umrah and contracting

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MERS-CoV.

2. Materials and methods

Forty patients who were admitted to our hospital's Infectious Diseases Clinics with upper respiratory tract complaints, and who had also previously been in Saudi Arabia within 15 days from January 1st to the 31st of October 2015 were included in this study. As a control group, 40 healthy people had been selected who had no history of visiting risky areas and respiratory tract complaints. Both suspicious and control groups have been examined by infectious diseases specialists and, their histories have been evaluated and recorded. From all people included in this study, 10 ml blood samples were collected, centrifuged, and stored at -20 °C.

MERS-CoV IgG antibodies in serum samples have been searched by a recombinant enzyme-linked immunosorbent assay (ELISA; Euroimmun, Lübeck, Germany) according to the manufacturer's recommendation as mentioned by Corman et al. (12). Briefly, serum samples were diluted as 1: 101 then incubated in the microplates coated with purified soluble MERS-CoV spike protein S1 domain expressed in HEK-293T cells, and the plates were read at 450 nm in a spectrophotometer. All serum samples were searched twice, and the arithmetic mean of the two measurements was considered. The results ≤ 0.8 were considered as negative, ≥ 1.1 were as positive, 0.8-1.1 were suspected. This study's ethical approval was taken from the Istanbul University School of Medicine Ethical Board (2015- 107113).

3. Results

The average age of the suspicious group with upper respiratory tract symptoms and who had a history of performing Umrah included in this study was 59.5 (29-89), the average age of the control group was 52.4 (36-93). In the suspicious group, 25 were female and 15 were male, while in the control group 26 were female and 14 were male. Only two (5%) individuals from the suspicious group were found as positive with the MERS-CoV IgG antibodies, but other individuals including the control group were determined as negative.

From the Hospital Database System, data of positive cases who had the IgG antibodies against the MERS-CoV were reviewed. There was no underlying disease history among the two seropositive patients. One of the cases was a 60-year-old female patient with positive antibody results determined as 1.15. Because she had a cough, her results were re-evaluated retrospectively, and it was found that the blood count results were normal, but her C-reactive protein (CRP) levels were high (24.5 mg/l, reference value: 0-5 mg/l). This patient and also her relatives were called to obtain control blood samples but could not be reached.

The other probable patient was a 40-year-old male who had a cough and a high fever history, whose antibody result was found to be 1.25. After evaluating retrospectively, his

blood count results were found as normal while his CRP levels were high (22.6 mg/l). He and his relatives were invited to check for their blood samples. Samples were taken from this probable patient for a second time and the result was again positive (1.15) for MERS CoV IgG. Although his mother and his wife who had traveled with him at the same time had respiratory tract complaints, were found to be negative.

4. Discussion

MERS is a viral disease that can have pandemic potential. By the end of June 2018, 2,229 laboratory-confirmed MERS-CoV cases and 791 deaths (case-fatality rate 35.5%) were reported worldwide (13). Most of the cases were reported from Saudi Arabia (1,853 cases/717 deaths, case-fatality rate 38.7%). While most of the primary cases had a history of camel contact or consuming camel milk, most of the secondary cases were seen as nosocomial contamination in healthcare facilities (13).

Epidemiologic and genomic studies especially have taken place in home and hospital settings confirmed that the disease may be transmitted from human to human. (14, 15). In April and May 2013, hospital-borne outbreaks have occurred in Saudi Arabia and 23 patients from the intensive care unit and the hemodialysis unit were found to have the same genotype of the virus (14). It is thought that a large droplet and contact transmission were the main sources for human-to-human contamination, but also spread through the air or fomite should not be ignored (16). The average incubation period of the virus ranges 2-16 days, after contracting the infection, a healthy human can contaminate other people after 13-14 days (17, 18).

The disease is mainly caused by the contact of MERS-CoV-infected pilgrims who are hospitalized for any reason because camel contact is not expected during the Hajj/Umrah visit. But the risk of contamination should not be ignored, Since the Hajj period is almost a month (13). In our country, the first imported case of MERS-CoV was reported from Hatay in 2014 (19). This case was a 42-year-old male worker in Jeddah, who applied to the hospital with complaints of fever, weakness, respiratory failure, cough, and sweating but unfortunately, the patient lost his life in the intensive care unit. For this patient, the diagnosis was achieved by molecular methods based on the detection of the virus in the tracheal aspirate (19).

There is no vaccines or clinically applicable treatments are currently available for MERS-CoV, so rapid diagnosis is crucial for prompt treatment and protection against viral outbreaks. In the diagnosis of MERS-CoV, LLC-MK2 and Vero cell culture and in-house RT-rtPCR methods are considered as the gold standards (3, 20, 21). Cell culture techniques are slow and laborious, so PCR-based techniques are rather preferred (22, 23). But in any of the surveys conducted for infection risk of MERS-CoV and Hajj, the

virus has not been determined by molecular techniques. Memish et al. have taken nasopharyngeal samples from 3,210 people before and 2,025 people after Hajj; they searched these samples by RT-PCR but did not find the MERS-CoV genome (24). The same year, nasopharyngeal samples were taken from 839 African pilgrims just after returning to Ghana, the samples taken showed that there was no MERS-CoV genome by RT-PCR (25). The same results were also found in 129 French pilgrims just before returning to the country (26). Therefore, it is suggested that the PCR screening should be performed during the Hajj substantially (27). In this context, Barasheed et al. conducted a study during the Hajj in 2013 and nasal swab samples were taken from 1,038 pilgrims who had an influenza-like illness but none of them were found to have the MERS-CoV by molecular method (28). Besides the detection of the MERS-CoV RNA by real-time PCR, a humoral immune response against the infection is also the method used for diagnosis (29). Serological tests could be useful in the determination of asymptomatic cases because they are easy to use and inexpensive and can also be applied to investigate mass screening and epidemiologic researches (29). ELISA, antibody array, immunofluorescence, microneutralization plaque reduction neutralization, and MERS spike pseudoparticulate neutralization tests are used for detecting antibodies against MERS (30-32).

In our study, two (5%) of 40 people returning from Umrah were positive for the MERS-CoV IgG antibodies. In Saudi Arabia, serosurvey studies of the general population were conducted by using similar serological methods that we used, and the MERS-CoV antibodies were determined as 0.15% (15 of 10 009) of the population (33). Our higher rate could be explained that we searched symptomatic patients who visited the epidemic region. On the other hand, our study has some limitations since lower respiratory tract specimens had not been searched, either the budget restriction or the absence of molecular analyses infrastructure at that time. Even we realized probably we would not determine the virus molecularly since asymptomatic and mildly symptomatic patients' lower respiratory tract specimens could not be found PCR positive for only two weeks (23, 24). The Hajj period is almost a month, but the incubation period of the MERS-CoV infection is around 14 days (34). It is stated that even if individuals became infected, they probably would have recovered already by the time they returned to their homes. In several studies, none of the pilgrims were found as positive for the MERS-CoV when they returned to home and even during Hajj (23-28).

Fever, chills, sore throat, dry cough, shortness of breath, muscle pain, headaches, dizziness, and gastrointestinal symptoms are prodromal symptoms, but later serious systemic and respiratory symptoms could be developed (35, 36). MERS-CoV may progress to death with acute respiratory and multiple organ failure in individuals who especially have underlying diseases (2, 3). Our probable two patients who

applied to our clinic with upper respiratory tract complaints healed completely that the reason might be explained due to the lack of underlying disease. These findings remain unclear because they were searched molecularly neither for MERS nor for other respiratory pathogens during their application.

In the Netherlands, two people having gastrointestinal complaints and returning from the Hajj's were determined as having MERS-CoV by RT-PCR method from throat swab samples but none of 78 people were positive although they had contact with these patients (35). In our study, only one of the patient's relatives could be achieved. This patient's wife and mother were found seronegative although they were together during Umrah.

For understanding of infection statistics at a population level, serology is the key element. The presence of MERS-CoV antibodies in a mildly symptomatic patients can last more than a year, after the first positive PCR results. In a study conducted in Abu Dhabi, it is found that 13 of 24 PCR-positive MERS patients' sera had detectable MERS-CoV antibodies for 45-348 days (34). Recent publications indicated that ELISA is 10-fold more sensitive than IFA, and appropriate as a screening tool for MERS (33). Both rS1- and rS-ELISAs maintained high sensitivity and specificity ($\geq 90\%$) moreover showed better agreement and correlation with microneutralization assay which is the gold standard method for detecting antiviral antibodies, especially exhibiting virus-killing function (37, 38). On the other hand, cross-reaction with seasonal human coronavirus antibodies could be seen by rELISA, because of the higher sensitivity of the test (33, 39). In our study, none of the control group's members found as positive shows that there might be no cross-reactivity because we preferred to use ELISA based on purified soluble MERS-CoV spike protein S1 domain.

As a result, individuals traveling to Saudi Arabia for Hajj or Umrah have a risk of exposure to MERS-CoV. In our study, we defined two MERS CoV IgG positive cases in which the source was probably related to Umrah. Although there is no evidence for the source of the virus, if it is zoonotic or anthroponotic in origin, human-to-human contamination is more probable to the crowd and travel history to the risky area. Therefore, the people who are planning to visit this holy area should be informed about the risk of MERS-CoV and personal security measures should be taken.

Conflict of interest

None to declare.

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