Original Research Article

Assessing the Detection of Middle East Respiratory Syndrome Coronavirus IgG in Suspected and Proven Cases of Middle East Respiratory Syndrome Coronavirus Infection

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Abstract

Middle East respiratory syndrome coronavirus (MERS-CoV) causes severe respiratory disease with significant mortality. Two testing methods are currently used for MERS-CoV diagnostics: nucleic acid detection (the gold standard) and serological analysis. In this study, we evaluated the detection of MERS-CoV-IgG in suspected and proven cases of MERS-CoV infection. We enrolled 174 patients: 113 had respiratory symptoms/suspected MERS-CoV infection, 31 had confirmed influenza A or B infection, 23 had a recent confirmed MERS-CoV infection, and 7 had confirmed MERS-CoV infection 1 year before. All underwent MERS-CoV RNA and MERS-CoV-IgG testing. Thirty patients were found to be MERS-CoV RNA positive; however, during serological analysis, only 6 (3.4%) patients were positive for MERS-CoV-IgG, 1 (0.6%) patient was equivocal, and 167 (96%) patients were negative. Among the serological positives, four were recently MERS-CoV RNA positive and two were MERS-CoV RNA negative. No cross-reactivity to influenza A or B was detected. Based on the lack of correlation between nucleic acid and serological analysis, we conclude that MERS-CoV-IgG testing may not be suitable for diagnosing acute infection or estimating its prevalence during an outbreak. In addition, our findings show that MERS-CoV-IgG may not have significant value in determining disease severity or prognosis.

Keywords: MERS-CoV-IgG, MERS-CoV RNA, MERS diagnostic testing

Introduction

Coronaviruses are a group of positive-sense, single-stranded RNA-enveloped viruses capable of infecting and causing diseases both in humans and in several animal species, including birds, bats, mice, dogs, pigs, cattle, and camels (15,20). In humans, coronavirus infections mostly result in mild respiratory, enteric, and neurologic diseases (18,19). However, in June 2012, a novel coronavirus—Middle East respiratory syndrome coronavirus (MERS-CoV)—was detected in a Saudi patient who had experienced pneumonia and acute renal failure (1,15,20). Evidence has shown that MERS-CoV is capable of animal-to-human and human-to-human transmission through droplets and close contact (10). Although the efficiency of human-to-human transmission of MERS-CoV is lower com-pared with severe acute respiratory syndrome coronavirus (SARS-CoV) (3,10), it has been proposed that MERS-CoV, after further adaptation and evolution in humans, may acquire increased human-to-human transmissibility and have the potential to cause a global pandemic like the one caused by SARS-CoV in 2003 (14,21). To date, the World Health Organization has reported more than 1,879 confirmed cases of MERS-CoV worldwide; the majority of these patients had evidence of exposure to MERS-CoV in Saudi Arabia (4). Furthermore, it appears that the mortality rate due to MERS-CoV infection is 35%, and most deaths occur in patients with comorbidities such as chronic lung, heart, and kidney diseases, diabetes, hypertension, and cancer (5).

There are no vaccines or effective therapies currently available for clinical cases of MERS-CoV infection. Thus,

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early and precise detection of MERS-CoV infection is critical for preventing spread of the disease. Several methods have been developed to diagnose MERS-CoV infection, including molecular MERS-CoV-RNA testing (the gold standard) and serologic MERS-CoV antibody testing. Serological studies have shown that infection with MERS-CoV induces the production of antibodies within the second or third week of exposure (7,8,13), with no difference in the detection of immunoglobulin (Ig) M or IgG antibodies during MERS-CoV infection (8). A recent report from Saudi Arabia showed that between April 2015 and February 2016, of >57,000 sample tested by molecular assays, only 384 (0.7%) samples produced positive polymerase chain reaction (PCR) results (16). This costs the country a lot of money when compared with serological alternatives. In this study, we aim to assess the detection of MERS-CoV-IgG in suspected and proven cases of MERS-CoV infection.

Materials and Methods

Patient groups

This cross-sectional study was conducted at King Khalid University Hospital (KKUH), Riyadh, between January 2016 and December 2016, which was inclusive of a MERS-CoV outbreak episode. A total of 174 patients were enrolled. These included 113 patients with respiratory symptoms or who were suspected of having MERS-CoV infection, 31 who tested positive for influenza A and B infection, 23 who were known to have had a recent MERS-CoV infection, and 7 who were known to have had MERS-CoV infection 1 year before. The inclusion criteria for MERS-CoV molecular and serology screening included any patient presenting with fever and signs of respiratory symptoms and/or history of camel contact and/or contact with a MERS-CoV positive case. All patients were tested for the presence of MERS-CoV RNA and MERS-CoV-IgG, either simultaneously or at different time points. None of the MERS-CoV positive patients was under immunosuppressive therapy or steroid treatment, except one asthmatic patient who was on a nasal steroid spray (budesonide) at the time of testing. The study has been performed according to the ethical standard expressed by Declaration of Helsinki. Patients' samples were collected after obtaining written informed consent. The study was approved by and performed according to the guidelines of the KKUH and College of Medicine Institutional Review Board committee (research project No. E15-1625).

Detection of MERS-CoV RNA by real-time reverse transcription PCR

MERS-CoV RNA was detected from nasopharyngeal swab specimens in viral transport medium (NPS-VTM; Copan, Brescia, Italy), as previously described (11). Briefly, viral RNA was extracted from $300 \mu L$ NPS-VTM using the MagNA pure compact Nucleic Acid isolation kit I (Roche Diagnostics, Indianapolis, IN) on the MagNA Pure Compact System (Roche Applied Science, Indianapolis, IN). The extracted viral RNA was eluted into $50 \mu L$ with an elution buffer. Using random primers, $10 \mu L$ of RNA was reverse transcribed to cDNA. The resultant cDNA was then amplified and screened for the detection of MERS-CoV *upE* and *orf1a* genes using the specific primers and probes of the

RealStar® MERS-CoV RT-PCR kit (Altona Diagnostics, Hamburg, Germany) on the Rotorgene Q instrument (Qiagen, Santa Clarita, CA).

Detection of influenza A, influenza H1N1, and influenza B RNA using PCR

Detection of influenza A, influenza H1N1, and influenza B was performed directly from NPS-VTM samples using the Xpert Flu kit on the GeneXpert PCR system (Cepheid, Sunnyvale, CA), following the manufacturer's instructions.

Detection of MERS-CoV-IgG antibodies using enzyme-linked immunosorbent assay

Serum samples from patients suspected of, or known to have, MERS-CoV infection were assayed using the MERS-CoV-IgG enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun, Luebeck, Germany) in accordance with the manufacturer's instructions. The test is designed to detect IgG antibodies specific to the S1 antigen of MERS-CoV in human serum. Briefly, diluted samples or controls were loaded into a 96-well plate precoated with recombinant MERS-CoV-specific spike antigen. The plate was then incubated for 30 min at 25°C to allow the formation of antigen–antibody complexes. The plate was washed five times, and horseradish-peroxidase conjugate was added and incubated for a further 30 min at 25°C. Thereafter, a washing step was performed and chromogen/substrate solution (3,3′, 5,5;-tetramethylbenzidine [TMB]/H_2O_2) was added for detection. Finally, the reaction was stopped using sulfuric acid (H_2SO_4), and the colorimetric signal was measured by absorbance at 630 nm using a spectrophotometer. The limit of detection of this kit, as indicated by the manufacturer, is ratio 0.04. A sample is considered negative if the ratio <0.80, positive if the ratio >1.10, or equivocal if the ratio >0.80 and <1.10.

Results

Of the 174 study patients (including 30 known MERS-CoV cases), 6 (3.4%) patients tested positive, 1 (0.6%) patient had an equivocal test result, and 167 (96%) patients tested negative for MERS-CoV-IgG (Table 1). Among the MERS-CoV IgG positives, 4 (2.3%) patients were recent MERS-CoV RNA-positive cases and 2 (1.1%) patients tested MERS-CoV RNA negative (Table 2). The MERS-CoV-IgG-positive group comprised two primary cases and two

TABLE 1. PATIENTS ENROLLED IN THE STUDY
AND MERS-CoV-IgG+ ELISA RESULTS

Patients	Samples tested, n	MERS-CoV-IgG+ ELISA, n (%)
Suspected cases	113	2 (1.8)
MERS-CoV+ PCR	30	4 (13.3)
Influenza A+	10	0 (0)
Influenza A+ (H1N1+)	5	0 (0)
Influenza B+	16	0 (0)
Total	174	6 (3.4)

ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; MERS-CoV, Middle East respiratory syndrome coronavirus; PCR, polymerase chain reaction.

Table 2. Clinical Details of the Polymerase Chain Reaction-Positive Middle East Respiratory Syndrome Coronavirus Patients

MERS+ patients	Signs and symptoms	Co-morbidities	Time[a]	MERS-IgG (ratio)	Outcome
Pri case	Pneumonia	DM	7 days	2.84 pos	Died
Pri case	Pneumonia	No history	7 days	0.12 neg	Survived
Pri case	Fever, cough, runny nose	DM, HT	7 days	1.48 pos	Survived
Pri case	Fever, abdominal pain, dysuria	DM, CLD	1 day	0.20 neg	Survived
Pri case[b]	Fever, pneumonia	DM, HT, PVD	7 days	0.17 neg	Died
Cont (HW)[b,c]	Cough, cold	A, HT, GERD	28 days	0.19 neg	Survived
Cont (HW)[b]	Cough, cold	No history	28 days	0.24 neg	Survived
Cont (HW)[b]	Sore throat	No history	28 days	0.25 neg	Survived
Cont (HW)[b]	Asymptomatic	HT	28 days	0.20 neg	Survived
Cont (HW)[b]	Asymptomatic	DM	28 days	1.05 equ	Survived
Cont (HW)[b]	Body pain, cough, cold	No history	28 days	1.28 pos	Survived
Cont (HW)[b]	Asymptomatic	DM,HT	28 days	3.14 pos	Survived
Cont (HW)[b]	Asymptomatic	No history	28 days	0.27 neg	Survived
Cont (HW)[b]	Asymptomatic	No history	28 days	0.18 neg	Survived
Cont (HW)[b]	Asymptomatic	No history	28 days	0.60 neg	Survived
Cont (HW)[b]	Asymptomatic	No history	28 days	0.32 neg	Survived
Cont (HW)[b]	Asymptomatic	No history	28 days	0.56 neg	Survived
Cont (P)[b]	Asymptomatic	sigmoid CC	2 days	0.31 neg	Survived
Cont (P)[b]	Pneumonia	DM, DVT, CC	7 days	0.08 neg	Survived
Cont (P)[b]	Asymptomatic	No history	7 days	0.11 neg	Survived
Cont (P)[b]	Asymptomatic	DM, HT, A	4 days	0.10 neg	Survived
Cont (P)[b]	Pneumonia	GT	7 days	0.10 neg	Died
Cont (HW)	Mild cough, runny nose	No history	14 days	0.05 neg	Survived
Cont (HW)	Sore throat, headache	DM	12 months	0.21 neg	Survived
Cont (HW)	Mild cough, headache	No history	12 months	0.11 neg	Survived
Cont (HW)	Sore throat, headache	BA	12 months	0.17 neg	Survived
Cont (HW)	Asymptomatic	No history	12 months	0.11 neg	Survived
Cont (HW)	Sore throat, body ache	No history	12 months	0.08 neg	Survived
Cont (HW)	Sore throat, headache	No history	12 months	0.06 neg	Survived
Cont (HW)	Cough, sore throat	No history	12 months	0.12 neg	Survived

The limit of detection of this kit is ratio 0.04 as indicated by the manufacturer. A sample is considered negative if the ratio <0.80, positive if the ratio >1.10, or equivocal if the ratio >0.80 and <1.10.

[a]Time of blood collection and testing IgG after initial PCR diagnosis.

[b]Contacts acquired the infection from one source (the primary case labeled with ''b'').

[c]Patient treated with steroid nasal spray.

A, asthma; BA, bronchial asthma; CC, colon cancer; CLD, chronic liver disease; Cont, contact; DM, diabetes mellitus; DVT, deep vein thrombosis; equ, equivocal; GERD, gastroesophageal reflux disease; GT, gastric tumor; HT, hypertension; HW, healthcare worker; MERS-CoV, Middle East respiratory syndrome coronavirus; neg, negative; P, patient; pos, positive; Pri case, primary case; PVD, peripheral vascular disease.

contacts. The clinical findings of the primary cases showed that one patient had pneumonia and diabetes mellitus and died; the other case had fever, cough, runny nose, diabetes mellitus, and hypertension and survived. Both contacts survived: One experienced body pain, a cough, and cold; the other was asymptomatic with diabetes mellitus and hypertension as comorbidities (Table 2). No MERS-CoV-IgG cross-reactivity to influenza A or B was observed for any of the 31 influenza-positive specimens tested (Table 1).

Discussion

In this study, we found that the majority of the MERS-CoV PCR-confirmed cases did not develop detectable levels of MERS-CoV-IgG and yet they cleared the infection and survived. Of the 12 contacts with recent MERS-CoV infection (acquired from one primary case) who had enough time to produce antibodies, 9 (75%) did not develop detectable IgG antibodies, 2 (16.6%) produced IgG antibodies, and 1 (8.3%) had an equivocal result; however, all cleared the virus (Table 2). In agreement with our results, two re-

ports have demonstrated clearance of MERS-CoV infection from mild symptomatic patients without antibody production (2,9). Assuming that the virus did not mutate significantly within a short period of time, these results suggest that the immune system of each individual reacts differently to the virus and not all individuals infected with MERS-CoV will develop a humoral immune response. In addition, it appears that cellular immunity may be more important than humoral immunity in clearing the virus. In support of this hypothesis, using sequencing analysis, two outbreak reports demonstrated that MERS-CoV did not change among infected close contacts and healthcare workers (6,12).

In contrast to our results, other studies have demonstrated detection of MERS-CoV-IgG in 50% or more of the cohort patients (2,13). However, this discrepancy of the results could be related to the differences in the patient sample size (2,13). One limitation of most MERS-CoV studies is the small number of MERS-CoV-positive specimens available for analysis. Park et al. (13) investigated serological responses in 17 MERS-CoV-positive patients, with only 12 patients tested beyond day 18 of illness onset. Similarly,

Alshukairi *et al.* (2) investigated serological responses only in nine patients.

In this study, the clinical symptoms among the primary cases and contacts were variable and inconsistent. For instance, two primary cases with pneumonia did not develop MERS-CoV-IgG antibodies, yet another three patients (one primary and two contacts) with no or only mild symptoms developed an immune response (Table 2). In addition, of the most recent infections, 16 out of 18 patients (89%) who did not develop IgG antibodies survived, whereas, 1 out of 4 patients (25%) who developed IgG antibodies died (Table 2). These findings suggest that there are no correlations between clinical manifestations or disease progression, and antibody production. Consistent with these results, it has been shown that MERS-CoV humoral immune responses are induced in symptomatic patients as well as in patients with subclinical disease (9). In this study, we did not detect MERS-CoV-IgG antibodies in the seven healthcare contacts who had been infected one year before. Some of these patients might have developed a humoral immune response at the time of infection, but the antibodies vanished over time. In a recent study of nine surviving healthcare workers who were followed up for serologic testing for 3–18 months after exposure to MERS-CoV, five developed a MERS-CoV-IgG humoral immune response within 3 months; all five presented with pneumonia. After 15 months, however, antibodies persisted in only two patients (2). The phenomenon of declining humoral immune responses has been observed not only with MERS-CoV infections but also with SARS-CoV infections (17). In a report of 23 survivors of SARS-CoV infection who had follow-up serologic testing performed for up to 6 years postinfection, declining SARS-CoV-IgG antibody titers were observed in all patients from the first month postinfection, reaching undetectable levels by 4.5 years postinfection in 21 patients (17). Thus, it appears that the nature of infection with beta-coronaviruses is to induce relatively weak and short-lived antibody responses. Hence, survivors of MERS-CoV infection could remain at risk of subsequent reinfection after a very short period of time.

A limitation of this study was that most of the patients (primary cases or contacts) were tested only once within the acute phase of infection (within 7 days of PCR diagnosis). This was because they were discharged from the hospital and could not be reached to collect subsequent specimens. In addition, in this study, two cases tested positive for MERS-CoV-IgG antibodies, but tested negative for MERS-CoV-RNA, as well as negative for influenza A, H1N1, and influenza B RNA. This result does not exclude cross-reactivity with other respiratory viruses, which was not explored in this study. Furthermore, neutralization assays were not performed because all patients tested for MERS-CoV-IgG, including the suspected cases and influenza A- and B-positive patients were also tested for MERS-CoV-RNA by PCR.

In conclusion, based on the lack of correlation between nucleic acid and serological analysis, our results suggest that detection of MERS-CoV-IgG may not be suitable for diagnosing acute infection or for estimating the prevalence of MERS-CoV during an outbreak. In addition, our finding shows that MERS-CoV-IgG antibody testing may have no significant value in determining the severity of the infection or its prognosis.

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