http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet

Asian Network for Scientific Information

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.119.125

Research Article
Common Co-morbidities Are Challenging in the Diagnosis of Middle East Respiratory Syndrome (MERS-CoV) in Saudi Arabia

[1,2]Rania Ali El Hadi Mohamed, [3]Fadilah Sfouq Aleanizy, [3]Fulwah Yahya Alqahtani, [4]Marzouqah Sfouq Alanazi and [5]Nahla Mohamed

[1]Department of Biology, College of Science, Princess Nourah Bint Abdulrahman University, 12484 Riyadh, Saudi Arabia
[2]Federal Ministry of Health, 303 Khartoum, Sudan
[3]Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 22452, 11495 Riyadh, Saudi Arabia
[4]Department of Emergency, Prince Mohammed Bin Abdulaziz Hospital, Ministry of Health, 12455 Riyadh, Saudi Arabia
[5]Researcher at University Hospital, Virology, Plan 2, Umea University, SE-901, 85 Umea, Sweden

Abstract
Background and Objective: Middle East respiratory syndrome coronavirus (MERS-CoV) is a relatively recent human disease reported initially in Saudi Arabia in September, 2012. Morbidities investigation includes a process of excluding other possible options until certain suspected cases are confirmed. **Material and Methods:** In this study, we formulated a model under the authorization of the Saudi Ministry of Health to accurately identify cases among admitted suspected cases depending on specific signs and symptoms. Real-time polymerase chain reaction was used for confirmation of the positive cases. **Results:** The results showed that the number of patients with combined symptoms of fever/sore throat and fever/cough/SOB was significantly higher in confirmed cases than in non-confirmed cases ($p<0.05$). Besides, the number of confirmed MERS-CoV cases was significantly higher among cases included in the study than excluded cases. It was also clearly demonstrated that fever combined with other symptoms represents 60% of the confirmed cases, which is significantly higher than for cases with other combined symptoms ($p<0.0001$). **Conclusion:** To the best of our knowledge, there are no appropriate diagnostic models that can differentiate human MERS-CoV infection among other respiratory infections. Therefore, we recommend the adoption of this newly established model of MERS-CoV to short- list corona suspected cases in Saudi Arabia.

Key words: MERS-CoV, coronavirus, combined features, co-morbidities, clinical features

Citation: Rania Ali El Hadi Mohamed, Fadilah Sfouq Aleanizy, Fulwah Yahya Alqahtani, Marzouqah Sfouq Alanazi and Nahla Mohamed, 2020. Common co-morbidities are challenging in the diagnosis of middle east respiratory syndrome (MERS-CoV) in Saudi Arabia. Pak. J. Biol. Sci., 23: 119-125.

Corresponding Author: Rania Ali El Hadi Mohamed, Department of Biology, College of Science, Princess Nourah Bint Abdulrahman University, 12484 Riyadh, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Middle East respiratory syndrome coronavirus (MERS-CoV) is a viral respiratory disease. Most patients of this disease exhibit severe acute respiratory disorders. The disease was firstly reported in Saudi Arabia in 2012 and then disseminated to several other countries[1]. The patients are characterized by severe respiratory illnesses, which occasionally result in mortality[2].

Corona virus is known to be difficult to diagnose because it can mimic other diseases. Therefore, it is essential to determine which patients should be tested. In addition to this, other potential causes of respiratory infections should be excluded out using routine laboratory tests as recommended in local management guidelines for communicable pneumonia, to determine the presence of other potential infectious agents[3]. Examples of these agents include infections with *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Legionella pneumophila*, influenza virus and respiratory syncytial virus. Several human CoVs are known to be responsible of respiratory tract infections; these include the beta-corona viruses HCoV-OC43 and HCoV-HKU1 and the alpha-COV HCoV-229E and[4] HCoV-NL63.

Diagnosis of corona virus depends on: Aspiration of foreign bodies such as, *Avium-intracellulare*, *Mycobacterium* and influenza as well as other atypical mycobacterial diseases, *Mycoplasma* infection, pleural effusion, pneumococcal infection, rhinovirus infection, bacterial sepsis, rickettsial pox, Q fever and upper respiratory infection.

While some serological tests such as ELISA can reveal the presence of corona virus antibodies, it cannot differentiate the type of coronavirus or even whether it is the causative agent of the patient's illness, only that the patient has acquired the virus and has developed antibodies to it. The level of antibodies is not an indicator for the patient's susceptibility to develop the disease. There are also insignificant changes in a biochemistry profile screening. More specific investigations may be used by clinicians, including molecular tests such as polymerase chain reaction (PCR), which may differentiate the unique DNA of the virus, however, this often only useful to show the corona virus but not distinguish its type[2].

Since no available data found to distinguish MERS-CoV signs and symptoms from other diseases exhibit similar co-morbidities, the main objective of this study was to create a model that is useful to identify the clinical features of MERS-CoV infections from other infections exhibited similar manifestations in cases reported to the Saudi Ministry of Health from 29th April, 2014, to 7th June, 2016.

MATERIALS AND METHODS

Sample collection: Samples selected from laboratory-suspected MERS-CoV cases reported by the Saudi Ministry of Health (MOH) from 29th April, 2014, to 7th June, 2016, were included in the present study.

Exclusion criteria: Coronavirus infections cause the common cold, which can be accompanied with the infections of chronic bronchitis and asthma and infrequently cause pneumonia and bronchiolitis[5]. Suspected patients with pneumonia and bronchiolitis were first excluded from the present study. After the preliminary clinical and laboratory investigations, patients confirmed with *Mycobacterium* or *Mycoplasma* infection, rickettsia, or infection with any bacterial type were also excluded from the study (Fig. 1).

Inclusion criteria: Most MERS-CoV positive patients exhibited violent intensive aerobic sickness with symptoms of febrile, cough and shortness of breath[6]. Patients suspected to have MERS-CoV with one or more of these symptoms were selected for the present study.

National endorsement

Viruses and clinical specimens: The MERS-CoV strain Saudi Arabia-N3/NCV (2012905864/VeroP1) was kindly provided by the Saudi MOH MERS-CoV regional laboratory in Riyadh with permission from the Saudi MOH. Other high-titer respiratory virus stocks and virus-positive and negative clinical specimens used for assay specificity studies were available from the collections of the Saudi National Reference Laboratory in Riyadh. Extracts from pooled nasal wash specimens forecasted to contain diverse human microbiological flora from consenting healthy new recruits were kindly provided by Saudi MOH MERS-CoV regional laboratory technicians. In total, 897 diverse fresh or frozen clinical specimens were collected from 1763 individuals who had severe acute respiratory illness (SARI) and who were either a resident in or had a history of travel outside Saudi Arabia before the study period.

Samples treating: Swab specimens were collected in viral transport medium (VTM) from patients admitted to different therapeutic institutions in Saudi Arabia. A bronchoalveolar wash liquid specimen and a serum sample collected by Saudi MOH Central Public Health Laboratory staff from two mortal SARI patients from a MERS-CoV pneumonia epidemiological mass at a Saudi hospital in April, 2012 and autonomously assured as positive for MERS-CoV by real-time PCR, were also

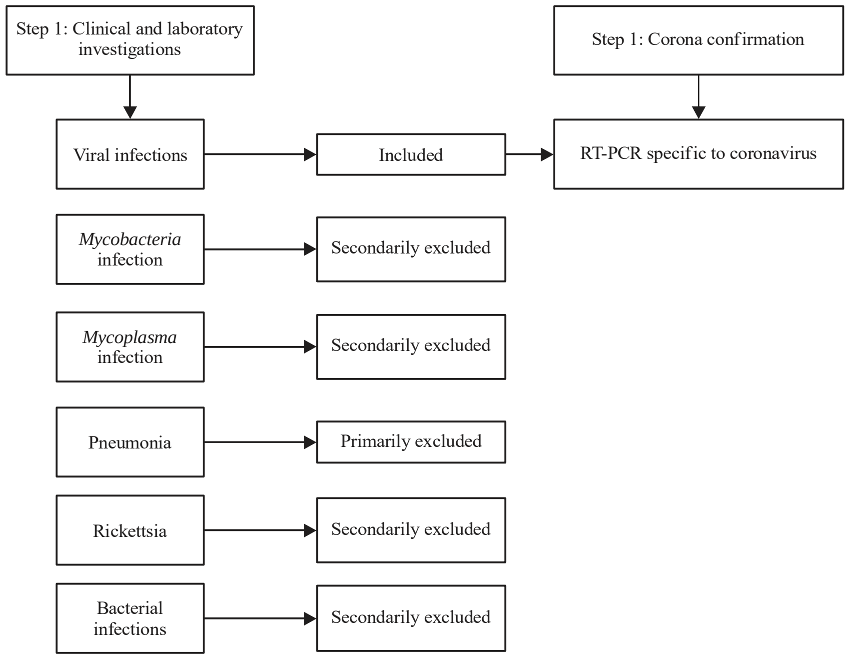

Fig. 1: Steps of the exclusion and inclusion of patients

obtainable for screening. All PCR experiments were conducted in the Saudi MOH MERS-CoV provincial laboratory in Riyadh. Aerobic specimens were gained from all patients and offered to the provincial lab for MERS-CoV contagion testing using primers that increased both the upstream E protein (upE) and ORF1a genes. Specimens that examined affirmative for both upE and ORF1a gene targets were supposed positive cases. Every patient was tested at least two times, on variable days.

Real-time PCR examination: The rRT-PCR examination was conducted via the Invitrogen SuperScript III Platinum One-Step quantitative RT-PCR system (Life Technologies) according to the instructions of Ma *et al*.[2]. Each reaction mixture of 25 µL contained 12.5 µL of 2× Master Mix, 0.5 µL of SuperScript III reverse transcriptase/Platinum Taq DNA polymerase, 0.5 µL of probe, 0.5 µL each of the forward and reverse primers, 5.5 µL of nuclease-free water and 5 µL of nucleic acid extract. Expansion was conducted in 96-well plates on an Applied Biosystems 7500 Fast Dx real-time PCR machine (Life Technologies). Thermocycling conditions included 30 min at 50°C for reverse transcription, 2 min at 95°C for stimulation of the Platinum Taq DNA polymerase and 45 cycles of 15 s at 95°C and 1 min at 55°C. Each run comprised one viral template control and at least two null-template controls (NTCs) for the sample extraction and reaction set-up proceedings. An affirmative examination finding was specified as a well-manifested Accelerated fluorescence curve that exceeded the threshold within 45 cycles. Positive viral template control and NTC samples were contained in all runs to monitor assay performance. All specimens were tested for the human RNase P (RP) gene using rRT-PCR to observe nucleic acid extraction competence and the existence of PCR repressors (if any).

Statistical analysis: Significance of results was determined using chi-squared and non-parametric binomial tests. Statistical analysis were performed using SPSS version 21 software (IBM Corp., Armonk, NY, USA). A value of $p<0.05$ was considered statistically significant.

Ethical approval: This study was approved by the research committee, King Saud University (Permit Number: RGP-1438-003.). All participants have signed informed consent forms upon their admission, According to their agreement they approved their coded data for research purposes.

RESULTS

Co-morbidities of positive MERS- CoV cases: Of the 1763 patients included in the present study, 133 had combined fever/cough, 25 combined fever/diarrhea, 26 combined fever/SOB, 318 combined cough/SOB, 30 combined cough/diarrhea, 208 combined fever/cough/SOB, 3 combined fever/SOB/diarrhea, 3 combined cough/fever/diarrhea, 35 combined fever/cough/sore throat, 1 combined cold/cough/SOB, 13 combined fever/sore throat, 3 combined fever/SOB/vomiting and 3 combined fever/cough/SOB/diarrhea/vomiting. Seventy-six patients were asymptomatic. The remaining patients exhibited only one of the above symptoms (Table 1).

Confirmed cases among the included sample stratum: As presented in Table 2, samples included in this study were significantly positive for MERS-CoV (52.4% positive versus 47.6% negative sample respectively) compared to the excluded samples (11.9% positive versus 88.9% negative sample, respectively).

Determination of the most frequent sign manifested between MERS-CoV confirmed cases: Results clearly demonstrated that fever combined with other symptoms (pool 1) represents 60% of the confirmed cases, which is significantly higher than for cases with other combined symptoms (33%, pool 2) (Table 3).

Fatality of MERS-CoV infection among confirmed cases: Among confirmed MERS-CoV cases, the number of live patients was significantly higher than the number of deceased ones (Table 4).

Statistical analysis: The number of patients with combined symptoms of fever/sore throat and fever/cough/SOB was significantly higher in confirmed cases than in non-confirmed cases ($p< 0.05$). Statistical analysis also exhibited that number of confirmed MERS-CoV cases among the included samples strata significantly exceeded the number of positive patients among the excluded samples strata ($p<0.0001$). Results showed that; fever was significantly frequent in the combined signs and symptoms ($p<0.0001$). Statistical analysis also revealed that MERS-CoV infection is rarely life threatening ($p<0.0001$).

Table 1: Sorting of suspected cases according to symptom combination and inclusion and exclusion criteria

	Number of suspected cases (n = 897)		Confirmed cases (n = 427)		Not confirmed cases (n = 470)	
Symptoms	Number	Percentage	Number	Percentage	Number	Percentage
Fever/cough	133	14.80	60	14.05	73	15.53
Fever/diarrhea	25	2.80	12	2.81	13	2.77
Fever/SOB	26	2.90	14	3.28	12	2.55
Fever/sore throat*	13	1.45	12	2.81	1	0.21
Cough/SOB*	318	35.45	128	29.98	190	40.43
Cough/diarrhea	30	3.34	13	3.04	17	3.62
Fever/cough/SOB*	208	23.20	142	33.26	66	14.04
Fever/SOB/diarrhea	3	0.33	1	0.23	2	0.43
Cough/fever/diarrhea	3	0.33	0	0.00	3	0.64
Fever/cough/sore throat	35	3.90	11	2.58	24	5.11
Cold/cough/SOB	1	0.11	0	0.00	1	0.21
Combined fever/SOB/vomiting	3	0.33	0	0.00	3	0.64
Fever/cough/SOB/diarrhea/vomiting	3	0.33	0	0.00	3	0.64
Asymptomatic	76	8.47	30	7.03	46	9.79
Non-combined symptoms*	20	2.23	4	0.94	16	3.40

*Results were significant ($p<0.01$)

Table 2: Number of confirmed cases in the included sample stratum using real-time PCR as a diagnostic tool

		Confirmed cases (n = 427)		Not confirmed cases (n = 470)		
Status in CFI	Number of suspected cases (n = 897)	Number	Percentage	Number	Percentage	p-value
Included	897	427	47.6	470	52.4	p<0.0001
Excluded	866	103	11.9	763	88.1	

CFI: Clinical Features Investigation

Table 3: Confirmed MERS cases correlated with symptom frequency (pools)

Symptoms	Pool 1	Pool 2	Asymptomatic	p-value*
Confirmed cases	252 (60%)	141 (33.33%)	30 (7.09%)	p<0.0001

*By chi-squared test, Pool 1: Fever combined with other symptoms, Pool 2: Combined symptoms except fever

Table 4: Alive: dead ratio among confirmed cases (total number = 1763)

Number of alive cases	Number of dead cases	Alive: dead ratio	*p-value
349 (65.85%)	181 (34.15%)	1:0.52	p<0.0001

*By binomial test

DISCUSSION

There is no diagnosis available that can differentiate between features of many respiratory infections and therefore point decisively to coronavirus. Instead to this, most cases can assumable be diagnosed based on laboratory findings. A complete blood count may show changes in the number of white blood cells that indicates an infection is present, although the infection type may not be distinguished.

The MERS; the newly emerged human disease[7] reported for the first time in Saudi Arabia in September, 2012. It was established after the identification of a novel CoV from a male Saudi Arabian patient whose reason of death was reported as severe respiratory[7,8] illness in June 2012.

Identification of the clinical features involves a process of exclusion. This starts with a list of possible causes of the exhibited symptom(s), then, by exclude them one-by-one, this list is narrowed down to a single cause, providing the exact diagnosis[9]. Physicians usually use this process to determine their patient's diagnosis, unless they consider the cause of symptoms is obvious. Clear and easy diagnoses might be flu symptoms[9]. Then MERS-CoV symptoms are often common during winter, therefore, such cases may be misdiagnosed as winter infections, such as influenza[10]. This gave the rationale of this study to formulate a simple model to distinguish signs and symptoms of MERS-CoV among other infections that exhibit similar co-morbidities.

The CDC has reported that MERS-CoV as a flu-like non-fatal and self healing infection, including fever with feeling chills, cough, sore throat, runny nose, jaundice pain, headaches and fatigue[10]. This matches with the findings of this research where proportion of live cases significantly exceeded that of the deceased ones (p<0.0001) (Table 4).

Some studies revealed that cough is frequently misdiagnosed as allergic rhinitis, asthma, sinusitis, bacterial pneumonia, tuberculosis, *Mycobacterium* or *Cytomegalovirus* infection, hypoxia, hypercarbia, histoplasmosis, coccidioidom, or exposure to virally infected animals[9].

In a review, it was mentioned that infections are the most common cause of fever, but various conditions, illnesses and medicines can raise the body temperature. These include infectious diseases such as influenza, common cold, HIV, malaria, infectious mononucleosis, gastroenteritis and other viral infections[11].

Shortness of breath or dyspnea is the subjective sensation of difficult, labored or uncomfortable breathing. A patient may complain of dyspnea but lack objective findings. Most of the causes of dyspnea are cardiac or pulmonary (two-thirds)[12]. Dyspnea commonly accompanies chest pain with coronary artery disease or it may be the only presentation of an acute coronary syndrome as an 'anginal equivalent.' Likewise, dyspnea may accompany many other cardiac disease states, like pericarditis or pericardial effusion, cardiomyopathies and left-sided congestive heart failure. Dyspnea may also be the only feature of a pulmonary embolus. Other pulmonary causes induce several chronic lung disorders, like asthma, emphysema, cystic fibrosis, or pulmonary hypertension. Acute pulmonary causes include pneumothorax, airway foreign body, allergy and respiratory infections[12]. Some researches mentioned that diarrhea can be associated with many viral infections[13].

In view of this background, in this study, a detailed analysis was performed of 427 confirmed positive cases of MERS-CoV with certain signs and symptoms of the disease. The relationship between the appearance of certain clinical manifestations with disease confirmation was investigated and whether these manifestations are sufficient to select the short-listed patients for further investigations leading to disease confirmation.

Nine combined symptoms were selected as independent variables and correlated them, with disease confirmation as the dependent variable. To ensure the quality of our results, two extra independent variables were included, namely, asymptomatic patients and patients who exhibited non-combined signs and symptoms. Suspected patients undergo pneumonia, bronchiolitis, *Mycobacterium* or *Mycoplasma* infection, rickettsia, or infection with any type of bacteria were initially excluded from the present study (Fig. 2). This study focused on patients with SARI who had symptoms of fever, cough and shortness of breath.

Most patients had a combination of two or more of these symptoms, statistical analysis also showed that patients with non-combined symptoms as well as asymptomatic patients represented 13.15% of the total confirmed cases, whereas, the remaining 86.85% exhibited the above-mentioned co-morbidities (p<0.01) (Table 2).

The results of the present study also showed that the first pool, which represented confirmed cases with fever combined with other symptoms, significantly dominated the second pool, which represented patients with combined symptoms without fever (p<0.0001) (Table 3).

This coincides with the findings of a study that investigated the latest MERS-CoV outbreak in Saudi Arabia and revealed that the common signs and symptoms of patients with CoV include the following: Fever (46 (98%)), fever with chills or rigors (41 (87%)), cough (39 (83%)), shortness of breath (34 (72%)) and myalgia (15 (32%)). Gastrointestinal symptoms were also common, including diarrhea (12 (26%)), vomiting (10 (21%)) and abdominal pain (8 (17%))[14]. It was also mentioned that all patients had abnormal findings on chest

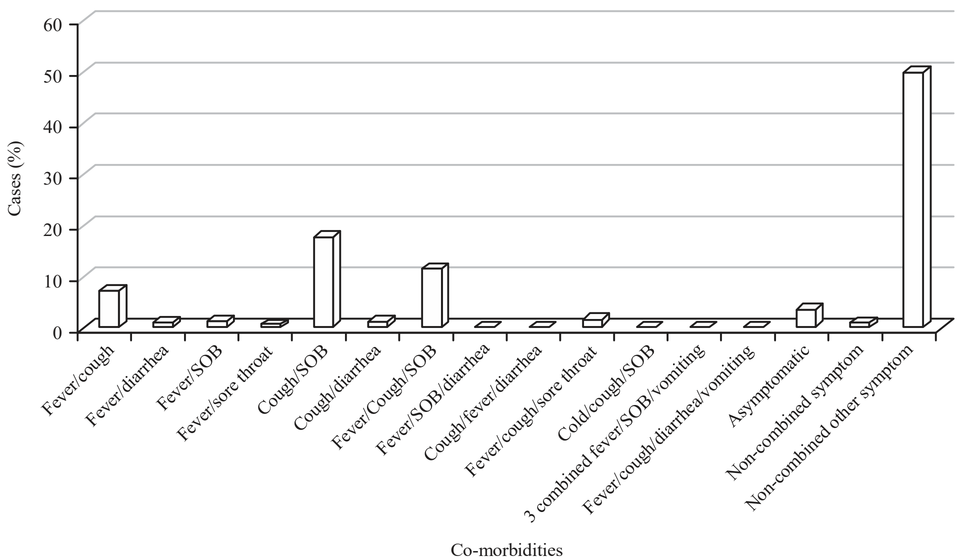

Fig. 2: Proportion of MERS-COV cases exhibited Co-morbidities (n = 1763)

radiography, ranging from subtle to extensive unilateral and bilateral abnormalities[14]. Data found in the literature also revealed that coronavirus infections regularly cause common cold, while infrequently causing pneumonia and bronchiolitis[6].

Statistical analysis also showed that the proportion of cases short-listed as a result of this differential diagnosis (representing 52.4% of the confirmed cases) significantly exceeded the proportion of excluded cases (p<0.0001). This indicates that the adoption of this short-listed signs and symptoms of coronavirus infections can save time and resources for containment and diagnosis during MERS-CoV outbreaks in Saudi Arabia.

To the best of our knowledge, there are no appropriate diagnostic models that can differentiate human MERS-CoV infection among other respiratory infections. Therefore, it's recommend the adoption this newly established model of MERS-CoV to short-list corona suspected cases in Saudi Arabia.

CONCLUSION

According to the results of this study a model of MERS-CoV can be established to short-list corona suspected cases in Saudi Arabia. Combined symptoms of: Fever/sore-throat pain and fever/cough/SOB was significantly higher in confirmed cases than in non-confirmed cases.

ACKNOWLEDGMENT

This research was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University through the Fast-track Research Funding Program.

REFERENCES

1. Alserehi, H., G. Wali, A. Alshukairi and B. Alraddadi, 2016. Impact of Middle East Respiratory Syndrome coronavirus (MERS CoV) on pregnancy and perinatal outcome. BMC Infect. Dis., Vol. 16, No. 1. 10.1186/s12879-016-1437-y.
2. Ma, L., F. Zeng, B. Huang, F. Cong, R. Huang, J. Ma and P. Guo, 2018. Development of a conventional RT-PCR assay for rapid detection of porcine deltacoronavirus with the same detection limit as a SYBR green-based real-time RT-PCR assay. BioMed Res. Int., Vol. 2018. 10.1155/2018/5035139.
3. Huang, P., H. Wang, Z. Cao, H. Jin and H. Chi *et al*., 2018. A rapid and specific assay for the detection of MERS-CoV. Front. Microbiol., Vol. 9. 10.3389/fmicb.2018.01101.
4. Bruning, A.H.L., H. Aatola, H. Toivola, N. Ikonen and C. Savolainen-Kopra *et al*., 2018. Rapid detection and monitoring of human coronavirus infections. New Microbes New Infect., 24: 52-55.
5. Hemida, M.G., 2019. Middle East respiratory syndrome coronavirus and the one health concept. PeerJ., Vol. 7. 10.7717/peerj.7556.
6. Center of Disease Control and Prevention, 2016. Middle East Respiratory Syndrome (MERS). National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases. https://www.cdc.gov/ncird/index.html.
7. Centers for Disease Control and Prevention (CDC), 2012. Severe respiratory illness associated with a novel coronavirus-Saudi Arabia and Qatar. MMWR Morb. Mortal. Wkly. Rep., 61: 820-820.
8. Al Mutair, A. and Z. Ambani, 2019. Narrative review of Middle East respiratory syndrome coronavirus (MERS-CoV) infection: Updates and implications for practice. J. Int. Med. Res. 10.1177/0300060519858030.

9. Raftery, A.T., E. Lim and A. Östör, 2014. Churchill's Pocketbook of Differential Diagnosis. 4th Edn., Elsevier, Philadelphia.
10. Centers of Diseases Control and Prevention (CDC), 2017. Key facts about influenza (Flu). National Center for Immunization and Respiratory Diseases (NCIRD). October, 2017. https://www.cdc.gov/flu/keyfacts.htm
11. High, K.P., S.F. Bradley, S. Gravenstein, D.R. Mehr, V.J. Quagliarello, C. Richards and T.T. Yoshikawa, 2009. Clinical practice guideline for the evaluation of fever and infection in older adult residents of long-term care facilities: 2008 update by the infectious diseases society of America. Clin. Infect. Dis., 48: 149-171.
12. Rushton, C.A. and U.T. Kadam, 2014. Impact of non-cardiovascular disease comorbidity on cardiovascular disease symptom severity: A population-based study. Int. J. Cardiol., 175: 154-161.
13. World Health Organization, 2009. Disease handbook for childcare providers. Division of Public Health Services, Communicable Disease Control Section, Revised–April, 2009.
14. Assiri, A., J.A. Al-Tawfiq, A.A. Al-Rabeeah, F.A. Al-Rabiah and S. Al-Hajjar *et al*., 2013. Epidemiological, demographic and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: A descriptive study. Lancet Infect. Dis., 13: 752-761.