Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Infection and Public Health 15 (2022) 1315–1320

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: www.elsevier.com/locate/jiph

Original article

Differential expression of carcinoembryonic antigen-related cell adhesion molecule-5 (CEACAM5) and dipeptidyl peptidase-4 (DPP4) with detection of Middle East respiratory syndrome-coronavirus in peripheral blood

Abdulkarim Alhetheel [a,b,*], Ahmed Albarrag [a,b], Zahid Shakoor [a,b], Ali Somily [a,b], Mazin Barry [a,c], Hifa Altalhi [a], Muhammed Bakhrebah [d], Majed Nassar [d], Mohamed Alfageeh [d], Ayed Assiri [e], Sarah Alfaraj [f], Ziad A. Memish [g,h,i,1]

[a] King Khalid University Hospital, Riyadh, Saudi Arabia
[b] Department of Pathology, College of Medicine, King Saud University, Riyadh, Saudi Arabia
[c] Department of Infectious Diseases, College of Medicine, King Saud University, Riyadh, Saudi Arabia
[d] King Abdulaziz city for Science and Technology, Riyadh, Saudi Arabia
[e] Critical Care Unit, Prince Mohammed Bin Abdulaziz Hospital, Ministry of Health, Riyadh, Saudi Arabia
[f] Corona Center, Prince Mohammed Bin Abdulaziz Hospital, Ministry of Health, Riyadh, Saudi Arabia
[g] Research and Innovation Center, King Saud Medical City, Ministry of Health, Riyadh, Saudi Arabia
[h] College of Medicine, Alfaisal University, Riyadh, Saudi Arabia
[i] Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA

ARTICLE INFO

Article history:
Received 16 July 2022
Received in revised form 13 September 2022
Accepted 6 October 2022

Keywords:
CD66e
CD26
CEACAM5
DPP4
PBMCs
MERS-CoV
Saudi Arabia

ABSTRACT

Background: Middle East respiratory syndrome-coronavirus (MERS-CoV) utilizes CD26 (dipeptidyl peptidase-4) and CD66e or CEACAM5 (carcinoembryonic antigen-related cell adhesion molecule 5) receptors for cell infection. Peripheral blood mononuclear cells (PBMCs) play a critical role in mounting adaptive immune response against the virus. This study was performed to assess the expression of CD26 and CD66e on PBMCs and their susceptibility to MERS-CoV infection.

Methods: Surface expression of CD26 and CD66e receptors on PBMCs from MERS-CoV patients (n = 20) and healthy controls (n = 20) was assessed by flow cytometry and the soluble forms were determined by enzyme-linked immunosorbent assay (ELISA). MERS-CoV *UpE* and *Orf1a* genes in PBMCs were detected by using Altona diagnostics reverse transcription polymerase chain reaction (RT-PCR) kit.

Results: Mean fluorescent intensity (MFI) of CD66e was significantly higher on CD4 + lymphocytes (462.4 ± 64.35 vs 325.1 ± 19.69; $p < 0.05$) and CD8 + lymphocytes (533.8 ± 55.32 vs 392.4 ± 37.73; $p < 0.04$) from patients with MERS-CoV infection compared to the normal controls. No difference in MFI for CD66e was observed on monocytes (381.8 ± 40.34 vs 266.8 ± 20.6; $p = 0.3$) between the patients and controls. Soluble form of CD66e among MERS-CoV patients was also higher than the normal controls (mean= 338.7 ± 58.75 vs 160.7 ± 29.49 ng/mL; $p < 0.01$). Surface expression of CD26 on PBMCs and its soluble form were no different between the groups. MERS-CoV was detected by RT-PCR in 16/20 (80%) patients from whole blood, among them 8 patients were tested in PBMCs, 4/8 (50%) patients were positive.

Conclusion: Increased expression levels of CD66e (CEACAM5) may contribute to increased susceptibility of PBMCs to MERS-CoV infection and disease progression.

* Correspondence to: Department of Pathology (32), College of Medicine and University Hospitals, King Saud University, P.O.Box: 2925, Riyadh 11461, Saudi Arabia.
E-mail addresses: aalhetheel@ksu.edu.sa, abdulkarimfahad@hotmail.com (A. Alhetheel).
[1] ORCID 0000-0002-50990714

https://doi.org/10.1016/j.jiph.2022.10.008

1. Introduction

The Middle East Respiratory Syndrome coronavirus (MERS-CoV) belongs to a group of Beta-coronaviruses that first emerged in the Middle East, especially Saudi Arabia in 2012 [1]. The virus causes severe lower respiratory tract infection with up to 30% mortality, particularly in patients with co-morbidities [2], and has led to numerous hospital outbreaks [3–10]. MERS-CoV is a zoonotic infection that originated from bats [11]. Later, the virus was transmitted to humans through dromedary camels, that are believed be the intermediate hosts [12]. To date, the precise mechanism of animal to human and/or human to human transmission is not clear. With MERS-CoV circulating around the Arabian Peninsula and from there spread worldwide to 27 countries, there are currently no authorized vaccines or therapeutics presently available for clinical use [13]. The clinical presentation of MERS-CoV infection ranges from asymptomatic or mild disease, to critical illness resulting in acute respiratory distress syndrome (ARDS) and multiorgan failure, the mechanisms involved in disease pathogenesis is still not fully understood [14]. A recent in-vitro study has shown that the spike protein of MERS-CoV mediates infection by binding to CD26 (dipeptidyl peptidase-4 (DPP4) as well as CD66e (carcinoembryonic antigen-related cell adhesion molecule-5 (CEACAM-5)) receptors on host cells surfaces [15–17]. Additionally, blocking the interaction between the viral spike protein and the cell surface CD26 or CD66e receptors with specific antibodies, recombinant proteins, or small interfering RNA (siRNA) could effectively block viral cell entry [18,19]. CD26 is a co-stimulatory molecule involved in T cell activation, it is expressed on many cell types including epithelial cells of many organs including lungs, kidneys, thymus, intestine, liver, and activated T lymphocytes in bone marrow [16,17,20]. On contrary, CD66e is a member of CD66 adhesion molecules family which has been shown to be involved in cell differentiation, cell survival and apoptosis, and expressed on epithelial cells as well as on leukocytes including T cells and monocytes [17,21–23]. peripheral blood mononuclear cells (PBMCs) including lymphocytes (CD4 T cells, CD8 T cells) and monocytes play a vital role in controlling and clearing pathogens by their anti-microbial properties. They act as phagocytic and cytolytic cells. In addition, upon activation they produce various cytokines such as IL-1-α and β, IL-6, IL-2, IL-12, IL-10, and IL-18, which serve to propagate the adaptive immune responses. Moreover, they function as antigen presenting cells [24–26]. The role of PBMCs in MERS-CoV pathogenesis and subsequent spread to the internal organs remain unclear. Thus, we aimed to evaluate the expression levels of soluble and surface CD26 and CD66e receptors on PBMCs from MERS-CoV infected patients and compared them to that of healthy controls and correlated it with the detection of MERS-CoV.

2. Material and methods

A cross-sectional observational study conducted between December 2018 and December 2019 to assess the expression levels of soluble and surface CD26 and CD66e receptors on PBMCs from MERS-CoV infected patients and compared them to healthy controls. Detection of MERS-CoV infection within whole blood and PBMCs was also measured. A total of 20 (17 males and 3 females) MERS-CoV infected patients were included. The mean age of patients was 50.6 ± 17 years (range 25–93 years). Diagnosis of MERS-CoV infection was confirmed by detecting MERS-CoV *UpE* and *Orf1a* genes using RT-PCR. The most frequent symptom among the patients was fever in 17 (85%) patients followed by cough in 13 (65%) (Table 1). None of the patients received any antiviral, monoclonal antibodies or immunosuppressive medications. A group of 20 healthy volunteers were included in the study to act as controls. They were all males, mean age was 36 ± 11 years (range 26–68 years). All the controls were screened for hepatitis B or C viruses (HBV, HCV), human immunodeficiency virus (HIV) and human T-lymphotropic leukaemia virus (HTLV) and were all negative. This study was approved by the Institutional Review Board (IRB) Committee, research project # E15–1625, and all patients and controls signed an informed consent.

Table 1
Details of MERS-CoV infected patients.

Patient #	Age	Gender	Clinical signs and symptoms	Outcome
P1	67	M	Asymptomatic	Survived
P2	45	M	Fever, Nausea	Survived
P3	83	M	Fever, Cough, fatigue, chest pain	Survived
P4	56	M	Fever	Survived
P5	44	M	Fever & cough	Survived
P6	61	M	Fever & Rigors	Died
P7	75	M	Shortness of breath & Productive cough	Survived
P8	66	F	Fever & cough	Survived
P9	35	M	Fever & cough	Survived
P10	41	M	Fever	Survived
P11	93	M	Vomiting, Diarrhea, Fever, Fatigue, Malaise	Survived
P12	40	M	Fever, Shortness of breath, Cough	Survived
P13	30	M	Altered consciousness/confusion	Survived
P14	71	F	Fever, Cough, Diarrhea	Died
P15	25	M	Fever, Shortness of breath, Cough	Survived
P16	43	M	Fever & cough	Survived
P17	42	M	Fever, Shortness of breath, Cough	Survived
P18	70	F	Fever & cough	Survived
P19	57	M	Fever, Shortness of breath, Cough	Survived
P20	73	M	Fever, Fatigue, Productive cough, Vomiting, Disorientation, Dysuria, Hematuria	Died

2.1. Soluble CD26 and CD66e using enzyme-linked immunosorbent assay (ELISA)

Plasma samples were assayed for soluble CD26 and CD66e using separate ELISA kits for CD26 (ab222872, Abcam, USA) and CD66e (ab99992, Abcam, USA) in accordance with the instructions of manufacturers. Briefly, 50 μL of each sample and serially diluted standards provided were loaded into a 96-well CD26 or CD66e antibody precoated plates. This was followed by incubation for 2 hours (hrs) at room temperature. The plates were then washed 5 times with the buffer and 50 μL of biotinylated anti-human monoclonal antibody was added and incubated for 1 hr. The plates were washed again 5 times with the buffer and 50 μL of streptavidin-horseradish peroxidase conjugate was dispensed in each well and incubated at room temperature for 30 minutes (min). After washing, 50 μL of substrate solution (TMB) was added and finally the reaction was stopped using 50 μL of H_2SO_4 after 30 min. The colorimetric signal was measured by absorbance at 450 nm using the Anthos Zenyth 200rt microplate reader (biochrom). The limit of detection for CD26 is 50 ng/mL whereas the detection limits for CD66e is 0.2 ng/mL.

2.2. PBMC isolation and cell surface staining

Whole blood was collected in 10 mL purple top vacutainer tubes containing EDTA (BD Biosciences, Franklin Lakes, NJ, USA). PBMCs were isolated from whole blood by density gradient centrifugation. The blood was layered on top of 4 mL of Ficoll-Paque™ Plus (GE Healthcare, Piscataway, NJ, USA) in 15 mL tubes and centrifuged for 30 min at 400g with the brake set to off. PBMCs were collected, washed twice with sterile phosphate-buffered saline (PBS), re-suspended in freezing medium (fetal bovine serum (GIBCO) with 10% dimethylsulfoxide (DMSO), and store at − 80 °C until further use.

For cell surface staining, PBMCs were thawed and washed three times with PBS. Cells were counted and distributed equally (4×10^5 cells / FACS tubes). Cells were then stained with CD66e-FITC

(Abcam), CD26-PE (BD biosciences, USA), CD4-PE-Cy7 (Abcam), CD8-APC (Abcam), CD14-PerCp (Meltini biotec, Germany) antibodies and incubated in the dark for 20 min at 25 °C. The cells were then washed once and run using the BD Biosciences LSRII flow cytometry (BD Biosciences, USA). Data were analyzed using FACS diva software (BD Biosciences, USA). For CD66e and CD26 expression on monocytes, the gating was performed based on CD14 positive within monocytes gate. For CD66e and CD26 expression on lymphocytes, the gating was performed based on CD4 positive or CD8 positive within lymphocytes gate.

2.3. RNA extraction and MERS-CoV detection using RT-PCR

Total nucleic acid extractions from PBMCs performed using the Nucleic Acid Isolation Kit I and the MagNA Pure Compact system (Roche Applied Science) at default settings. Extractions were performed on 1 × 10^6 / 200 μL of each specimen, with a final elution volume of 50 μL. This was followed by reverse transcription of a 10 μL of the extracted RNA into cDNA using random primer. The synthesized cDNA was then amplified and screened for detection MERS-CoV genes (*UpE* and *Orf1a*) using specific primers and probes of the RealStar® altona diagnostics kit (Humburg, Germany), and Rotor-Gene Q (Qiagen, Santa Clarita, CA). All patients were confirmed to have MERS-CoV infection by detecting both *UpE* and *Orf1a* genes from nasopharyngeal swab (NPS). For detection MERS-CoV from whole blood and isolated PBMCs samples, a patient was considered positive if at least one gene (*UpE* or/and *Orf1a*) was detected.

2.4. Statistical analysis

Data were collected and statistically analyzed using GraphPad Prism 5 software. Non-paired two tails t-test was used for determination of the statistical significance between the study groups. A $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Evaluation of soluble and surface CD26 and CD66e receptors expression levels

We hypothesized that increased expression of CD26 and/or CD66e receptors may contribute to MERS-CoV infection and associated complications frequently observed in MERS-CoV patients such as renal failure [27–30]. Thus, the surface CD26 and CD66e receptors expression levels on PBMCs including CD4 + T cells, CD8 + T cells, and CD14 + monocytes from the study groups were assayed using flow cytometry (Figs. 1 and 2). Data for expression of CD66e are presented in Fig. 1. There was a significant increase in the expression level of surface CD66e receptors on CD4 + T cells (mean florescence intensity (MFI) = 462.4 ± 64.35, P < 0.05) and CD8 + T cells (MFI = 533.8 ± 55.32, P < 0.04) but not on CD14 + monocytes (MFI = 381.8 ± 40.34, P = 0.3) from MERS-CoV patients compared to healthy controls (CD4 + T cells; MFI = 325.1 ± 19.69, CD8 +T cells; MFI= 392.4 ± 37.73, CD14 + monocytes; MFI= 266.8 ± 20.6). Fig. 2 shows data for CD26 expression. There was no significant difference between surface CD26 expression of CD4 + T cells, CD8 + T cells, and CD14 + monocytes between MERS-COV infected patients (CD4 + T cells; MFI = 931.9 ± 132.9, CD8 + T cells; MFI = 1134 ± 122.6, CD14 + monocytes; MFI = 883.1 ± 121.1) and healthy controls (CD4 +T cells; MFI = 819.4 ± 106.2, CD8 +T cells; MFI = 902.1 ± 144.4, CD14 + monocytes; MFI= 643.5 ± 72.23).

Fig. 3 shows data for assessment of soluble forms of CD26 and CD66e. Significant elevation of soluble CD66e among MERS-CoV patients (mean= 338.7 ± 58.75 ng/mL, P < 0.01) compared with healthy controls (mean= 160.7 ± 29.49 ng/mL) was observed (Fig. 3A). The levels of soluble CD26 receptors between MERS-CoV patients (mean= 98.22 ± 12.29 ng/mL) and the healthy controls (mean= 81.56 ± 7.525 ng/mL) were however no different (Fig. 3B).

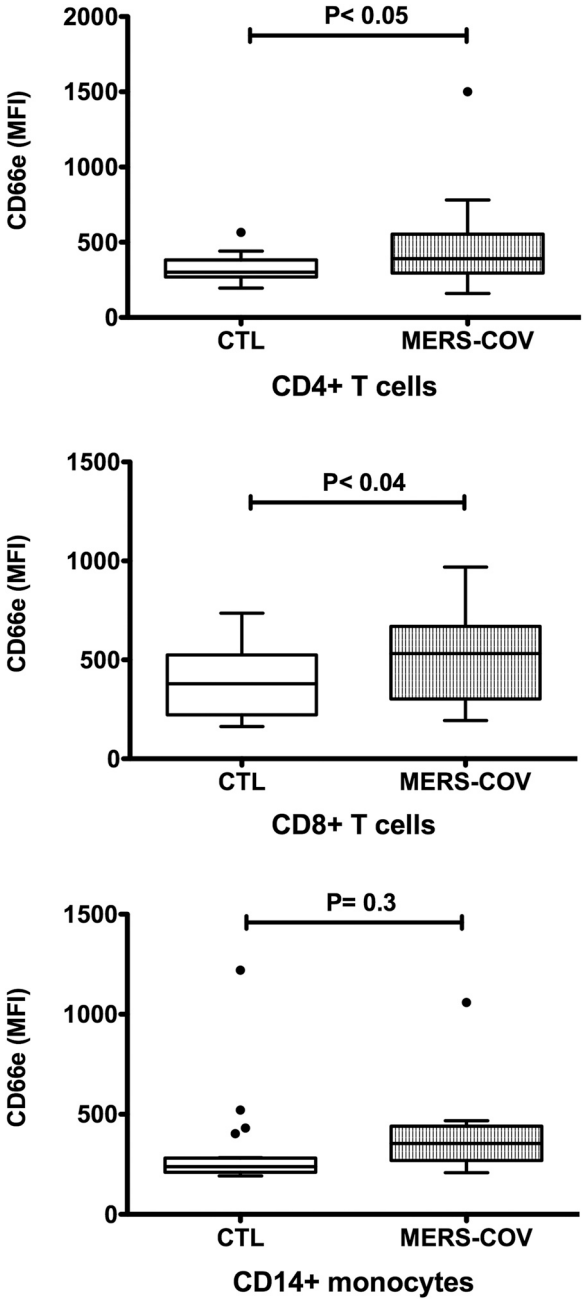

Fig. 1. Surface CD66e receptor expression levels on PBMCs of patient study groups. Isolated PBMCs of MERS-CoV patients (n = 20) and healthy controls (CTL) (n = 20) were collected. The cells (4 ×10^5) were distributed in each FACS tubes and stained with CD66e-FITC, CD4-PE-Cy7, CD8-APC, CD14-PerCp antibodies. The mean fluorescents intensity levels of CD66e on CD4 + T cells, CD8 + T cells, CD14 + monocytes from patients study groups was graphed.

3.2. Detection of MERS-CoV in PBMCs among MERS-CoV patients

In order to determine if PBMCs are susceptible to MERS-CoV infection, we utilized RT-PCR for detection of MERS-CoV *UpE* and *OrfA1* genes in patient whole blood and isolated PBMCs using Altona kit. Out of 20 MERS-CoV patients tested 16 patients (80%) were positive for at least one MERS-CoV gene from whole blood, and among them 8 patients were further tested from isolated PBMCs, 4

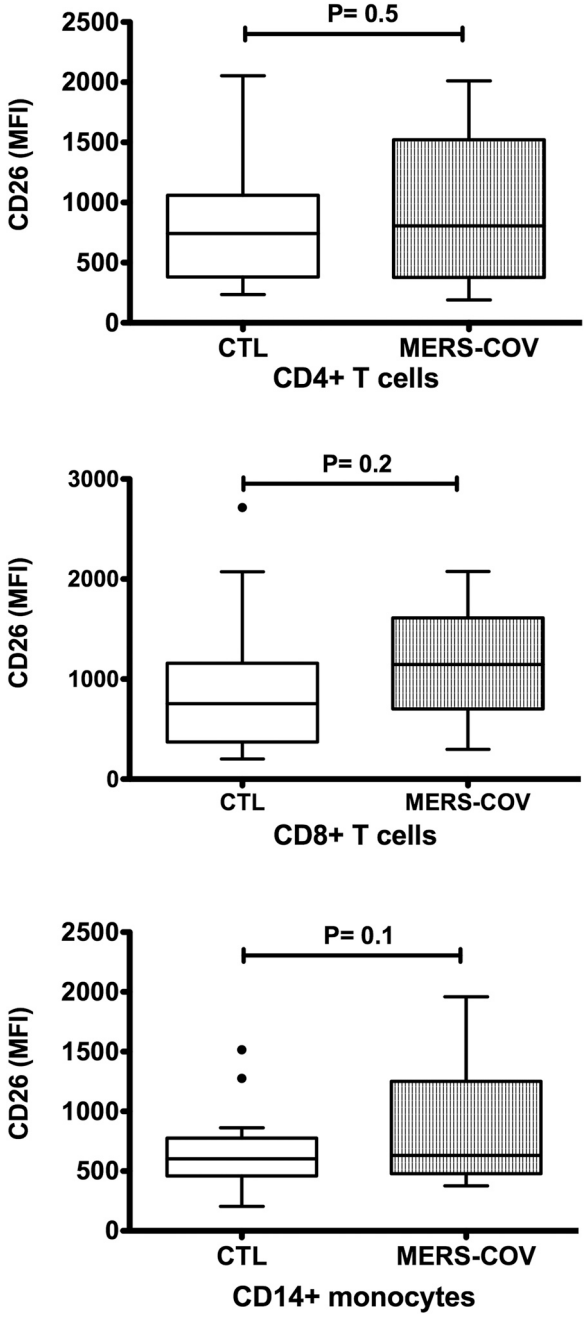

Fig. 2. Surface CD26 receptor expression levels on PBMCs of patient study groups. Isolated PBMCs of MERS-CoV patients (n = 20) and healthy controls (CTL) (n = 20) were collected. The cells (4×10^5) were distributed in each FACS tubes and stained with CD26-PE, CD4-PE-Cy7, CD8-APC, CD14-PerCp antibodies. The mean fluorescents intensity levels of CD26 on CD4+ T cells, CD8+ T cells, CD14+ monocytes from patients study groups was graphed.

(50%) patients were positive for at least one MERS-CoV gene (Table 2).

4. Discussion

This study for the first time ever demonstrated that patients with MERS-CoV infection had increased expression of CD66e receptors on CD4 and CD8 lymphocytes compared to the normal healthy controls. Elevated expression CD66e on CD4 + and CD8 + lymphocytes was associated with increased levels of plasma soluble forms of CD66e in MERS-CoV patients as well. The increased expression of CD66e could be due to direct effect of viral antigens on different cell types

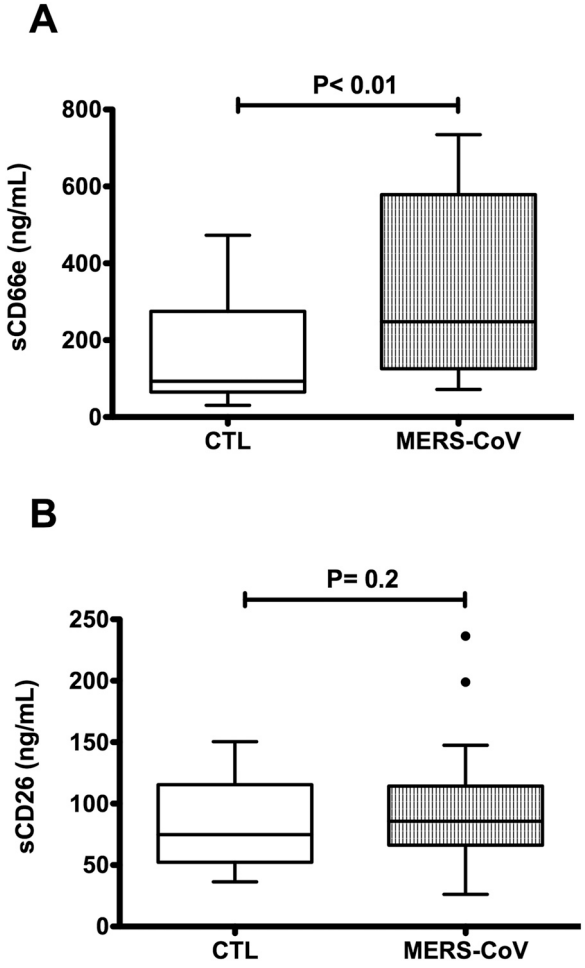

Fig. 3. Soluble CD66e and CD26 receptor expression levels in plasma of patient study groups. Plasma of MERS-CoV patients (n = 20) and healthy controls (CTL) (n = 20) were collected and soluble CD66e and CD26 receptors levels were analyzed using ELISA kits. (A) The mean levels of soluble CD66e receptor and CD26 receptor (B) and from patients study groups were graphed.

including PBMCs or indirectly by the induction of cytokines. Studies have shown that the expression CD66e could be increased on the epithelial cells of the lung by IFN and bacterial respiratory infections

Table 2
Detection of MERS-CoV in nasopharyngeal swab (NPS), whole blood (WB) and PBMCs.

Patient #	NPS MERS-COV UpE/Orf1a	WB MERS-COV UpE/Orf1a	PBMCs MERS-COV UpE/Orf1a
P1	Positive/Positive	Negative/Negative	Not done
P2	Positive/Positive	Positive/positive	Not done
P3	Positive/Positive	Positive/Negative	Positive/Negative
P4	Positive/Positive	Positive/Negative	Not done
P5	Positive/Positive	Positive/Positive	Positive/Negative
P6	Positive/Positive	Positive/Negative	Negative/Negative
P7	Positive/Positive	Positive/Negative	Not done
P8	Positive/Positive	Positive/Positive	Negative/Negative
P9	Positive/Positive	Positive/Positive	Positive/Positive
P10	Positive/Positive	Positive/Negative	Negative/Negative
P11	Positive/Positive	Negative/Positive	Positive/Positive
P12	Positive/Positive	Positive/Positive	Not done
P13	Positive/Positive	Positive/Positive	Not done
P14	Positive/Positive	Positive/Positive	Not done
P15	Positive/Positive	Negative/Negative	Negative/Negative
P16	Positive/Positive	Negative/Negative	Not done
P17	Positive/Positive	Positive/Positive	Not done
P18	Positive/Positive	Positive/Negative	Not done
P19	Positive/Positive	Negative/Negative	Not done
P20	Positive/Positive	Positive/Positive	Not done

[31] or by cytokines such as IL-6 [32]. We and others have previously reported increased levels of several cytokines including IL-10, IL-13, IL-4, IL-5, IL-6, and IL-1 and Th-2 differentiation [33,34]. Moreover, overexpression of CD66e in permissive cells has clearly been shown to enhance MERS-CoV attachment and entry in BHK21 cells [17]. It is possible that CD66e may facilitate MERS-CoV entry in cells in conjunction with CD26.

No difference in CD26 expression on PBMCs or the soluble forms of CD26 was observed between the patients and controls. A recent study has shown decreased levels of soluble CD26 receptors in severe MERS-CoV patients [35]. Another study has reported that persistent infection with MERS-CoV was associated with down-regulation of CD26 expression in bat cells [20]. Following influenza vaccination as an example of resolved successful immune response high level of CD26 expression was induced on memory CD8 + lymphocytes whereas chronic infection with persistent antigen such as cytomegalovirus (CMV), Epstein–Barr virus (EBV) or human immunodeficiency virus (HIV) lead to defective T-cell memory with low expression of CD26 [36]. High expression of CD26 is considered as a characteristic feature of memory cells. The low expression of CD26 observed in the present study could possibly be due to absence of MERS-CoV specific memory lymphocytes due to lack of previous exposure to the virus.

In the current study, we detected MERS-CoV genes in patients whole blood and isolated PBMCs. Although, MERS-CoV is a respiratory virus which targets primarily epithelial cells lining the respiratory system and alveoli, it was detected in 50% of the patients tested for PBMCs confirming its capability of infecting PBMCs through, at least in part, CD66e receptors. Recent studies have suggested that MERS-CoV could also utilize other cell surface proteins such as 78-kDa glucose–regulated protein (GRP78) and the cell surface glycoprotein CD9 to infect human cells [37,38]. These molecules however, were not invested in the present study. In agreement with our result, it has been reported that MERS-CoV infection could be detected and diagnosed from patient serum [39,40]. In an in-vitro study, it has been shown that MERS-CoV could infect macrophages and dendritic cells [41]. Also, it has been found in an *ex-vivo* study that MERS-CoV is capable of infecting T cells and inducing apoptosis [42]. Infection of PBMCs with MERS-CoV could be a similar phenomenon to many viral infections such as HBV, HCV, HIV which have been elucidated in many studies to be involved in establishing the infection and disease progression [43–45]. Thus, infection of PBMCs with MERS-CoV may play a role in disease severity and complications. In the present study, infection of PBMCs with MERS-CoV could only be tested in 8 patients due to limited availability of blood samples. Moreover, the number of patients could not be increased due to the fact that the prevalence of MERS-CoV infection globally is low and this issue is among one of the limitations of the study.

In conclusion, the increased expression levels of CD66e (CEACAM5) may contribute to increased susceptibility of PBMCs to MERS-CoV infection resulting in establishing disease severity and progression. Further investigation during different phases of the disease and the associated co-morbidities may provide a better insight into the pathogenesis of MERS CoV infection, and may help to guide targeted future therapeutics.

Declarations of interest

None.

References

[1] Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012;367(19):1814–20. https://doi.org/10.1056/NEJMoa1211721

[2] Mackay IM, Arden KE. MERS coronavirus: diagnostics, epidemiology and transmission. Virol J 2015;12:222. https://doi.org/10.1186/s12985-015-0439-5

[3] Alenazi TH, Al Arbash H, El-Saed A, Alshamrani MM, Baffoe-Bonnie H, Arabi YM, et al. Identified transmission dynamics of middle east respiratory syndrome coronavirus infection during an outbreak: implications of an overcrowded emergency department. Clin Infect Dis 2017;65(4):675–9. https://doi.org/10.1093/cid/cix352. PubMed PMID: 28575307; PubMed Central PMCID: PMCPMC7108118.

[4] Amer H, Alqahtani AS, Alzoman H, Aljerian N, Memish ZA. Unusual presentation of Middle East respiratory syndrome coronavirus leading to a large outbreak in Riyadh during 2017. Am J Infect Control 2018;46(9):1022–5. https://doi.org/10.1016/j.ajic.2018.02.023

[5] Assiri A, McGeer A, Perl TM, Price CS, Al Rabeeah AA, Cummings DA, et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. N Engl J Med 2013;369(5):407–16. https://doi.org/10.1056/NEJMoa1306742

[6] Balkhy HH, Alenazi TH, Alshamrani MM, Baffoe-Bonnie H, Arabi Y, Hijazi R, et al. Description of a Hospital Outbreak of Middle East Respiratory Syndrome in a Large Tertiary Care Hospital in Saudi Arabia. Infect Control Hosp Epidemiol 2016;37(10):1147–55. https://doi.org/10.1017/ice.2016.132

[7] Barry M, Phan MV, Akkielah L, Al-Majed F, Alhetheel A, Somily A, et al. Nosocomial outbreak of the Middle East Respiratory Syndrome coronavirus: A phylogenetic, epidemiological, clinical and infection control analysis. Travel Med Infect Dis 2020;37:101807https://doi.org/10.1016/j.tmaid.2020.101807

[8] Drosten C, Muth D, Corman VM, Hussain R, Al Masri M, HajOmar W, et al. An observational, laboratory-based study of outbreaks of middle East respiratory syndrome coronavirus in Jeddah and Riyadh, kingdom of Saudi Arabia, 2014. Clin Infect Dis 2015;60(3):369–77. https://doi.org/10.1093/cid/ciu812

[9] Fagbo SF, Skakni L, Chu DK, Garbati MA, Joseph M, Peiris M, et al. Molecular Epidemiology of Hospital Outbreak of Middle East Respiratory Syndrome, Riyadh, Saudi Arabia, 2014. Emerg Infect Dis 2015;21(11):1981–8. https://doi.org/10.3201/eid2111.150944

[10] Oboho IK, Tomczyk SM, Al-Asmari AM, Banjar AA, Al-Mugti H, Aloraini MS, et al. 2014 MERS-CoV outbreak in Jeddah–a link to health care facilities. N Engl J Med 2015;372(9):846–54. https://doi.org/10.1056/NEJMoa1408636

[11] Mohd HA, Al-Tawfiq JA, Memish ZA. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) origin and animal reservoir. Virol J 2016;13:87. https://doi.org/10.1186/s12985-016-0544-0

[12] Dudas G, Carvalho LM, Rambaut A, Bedford T. MERS-CoV spillover at the camel-human interface. Elife 2018:7. https://doi.org/10.7554/eLife.31257

[13] Memish ZA, Perlman S, Van Kerkhove MD, Zumla A. Middle East respiratory syndrome. Lancet 2020;395(10229):1063–77. https://doi.org/10.1016/S0140-6736(19)33221-0. PubMed PMID: 32145185; PubMed Central PMCID: PMCPMC7155742.

[14] Azhar EI, Hui DSC, Memish ZA, Drosten C, Zumla A. The Middle East Respiratory Syndrome (MERS). Infect Dis Clin North Am 2019;33(4):891–905. https://doi.org/10.1016/j.idc.2019.08.001

[15] Mou H, Raj VS, van Kuppeveld FJ, Rottier PJ, Haagmans BL, Bosch BJ. The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies. J Virol 2013;87(16):9379–83. https://doi.org/10.1128/JVI.01277-13

[16] Raj VS, Mou H, Smits SL, Dekkers DH, Muller MA, Dijkman R, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 2013;495(7440):251–4. https://doi.org/10.1038/nature12005

[17] Chan CM, Chu H, Wang Y, Wong BH, Zhao X, Zhou J, et al. Carcinoembryonic Antigen-Related Cell Adhesion Molecule 5 Is an Important Surface Attachment Factor That Facilitates Entry of Middle East Respiratory Syndrome Coronavirus. J Virol 2016;90(20):9114–27. https://doi.org/10.1128/JVI.01133-16

[18] Ohnuma K, Haagmans BL, Hatano R, Raj VS, Mou H, Iwata S, et al. Inhibition of Middle East respiratory syndrome coronavirus infection by anti-CD26 monoclonal antibody. J Virol 2013;87(24):13892–9. https://doi.org/10.1128/JVI.02448-13

[19] Tanaka T, Camerini D, Seed B, Torimoto Y, Dang NH, Kameoka J, et al. Cloning and functional expression of the T cell activation antigen CD26. J Immunol 1992;149(2):481–6. Epub 1992/07/15. PubMed PMID: 1352530.

[20] Cai Y, Yu SQ, Postnikova EN, Mazur S, Bernbaum JG, Burk R, et al. CD26/DPP4 cell-surface expression in bat cells correlates with bat cell susceptibility to Middle East respiratory syndrome coronavirus (MERS-CoV) infection and evolution of persistent infection. PLoS One 2014;9(11):e112060https://doi.org/10.1371/journal.pone.0112060

[21] Singer BB, Scheffrahn I, Heymann R, Sigmundsson K, Kammerer R, Obrink B. Carcinoembryonic antigen-related cell adhesion molecule 1 expression and signaling in human, mouse, and rat leukocytes: evidence for replacement of the short cytoplasmic domain isoform by glycosylphosphatidylinositol-linked proteins in human leukocytes. J Immunol 2002;168(10):5139–46. https://doi.org/10.4049/jimmunol.168.10.5139

[22] Thistlethwaite FC, Gilham DE, Guest RD, Rothwell DG, Pillai M, Burt DJ, et al. The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. Cancer Immunol Immunother 2017;66(11):1425–36. https://doi.org/10.1007/s00262-017-2034-7

[23] Yu Q, Chow EM, Wong H, Gu J, Mandelboim O, Gray-Owen SD, et al. CEACAM1 (CD66a) promotes human monocyte survival via a phosphatidylinositol 3-kinase- and AKT-dependent pathway. J Biol Chem 2006;281(51):39179–93. https://doi.org/10.1074/jbc.M608864200

[24] Chaplin DD. 1. Overview of the immune response. J Allergy Clin Immunol 2003;111(2 Suppl):S442–59. https://doi.org/10.1067/mai.2003.125

[25] Delves PJ, Roitt IM. The immune system. Second of two parts. N Engl J Med 2000;343(2):108–17. https://doi.org/10.1056/NEJM200007133430207
[26] Medzhitov R, Janeway Jr. C. Innate immunity. N Engl J Med 2000;343(5):338–44. https://doi.org/10.1056/NEJM200008033430506
[27] Alsaad KO, Hajeer AH, Al Balwi M, Al Moaiqel M, Al Oudah N, Al, Ajlan A, et al. Histopathology of Middle East respiratory syndrome coronovirus (MERS-CoV) infection - clinicopathological and ultrastructural study. Histopathology 2018;72(3):516–24. https://doi.org/10.1111/his.13379
[28] Arabi YM, Arifi AA, Balkhy HH, Najm H, Aldawood AS, Ghabashi A, et al. Clinical course and outcomes of critically ill patients with Middle East respiratory syndrome coronavirus infection. Ann Intern Med 2014;160(6):389–97. https://doi.org/10.7326/M13-2486
[29] Eckerle I, Muller MA, Kallies S, Gotthardt DN, Drosten C. In-vitro renal epithelial cell infection reveals a viral kidney tropism as a potential mechanism for acute renal failure during Middle East Respiratory Syndrome (MERS) Coronavirus infection. Virol J 2013;10:359. https://doi.org/10.1186/1743-422X-10-359
[30] Wise J. Patient with new strain of coronavirus is treated in intensive care at London hospital. BMJ 2012;345:e6455https://doi.org/10.1136/bmj.e6455
[31] Klaile E, Klassert TE, Scheffrahn I, Muller MM, Heinrich A, Heyl KA, et al. Carcinoembryonic antigen (CEA)-related cell adhesion molecules are co-expressed in the human lung and their expression can be modulated in bronchial epithelial cells by non-typable Haemophilus influenzae, Moraxella catarrhalis, TLR3, and type I and II interferons. Respir Res 2013;14:85. https://doi.org/10.1186/1465-9921-14-85
[32] Holmer R, Watzig GH, Tiwari S, Rose-John S, Kalthoff H. Interleukin-6 trans-signaling increases the expression of carcinoembryonic antigen-related cell adhesion molecules 5 and 6 in colorectal cancer cells. BMC Cancer 2015;15:975. https://doi.org/10.1186/s12885-015-1950-1
[33] Alhetheel A, Albarrag A, Shakoor Z, Somily A, Barry M, Altalhi H, et al. Assessment of Th1/Th2 cytokines among patients with Middle East respiratory syndrome coronavirus infection. Int Immunol 2020;32(12):799–804. https://doi.org/10.1093/intimm/dxaa047
[34] Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. Cytokine 2018;104:8–13. https://doi.org/10.1016/j.cyto.2018.01.025
[35] Inn KS, Kim Y, Aigerim A, Park U, Hwang ES, Choi MS, et al. Reduction of soluble dipeptidyl peptidase 4 levels in plasma of patients infected with Middle East respiratory syndrome coronavirus. Virology 2018;518:324–7. https://doi.org/10.1016/j.virol.2018.03.015
[36] Ibegbu CC, Xu YX, Fillos D, Radziewicz H, Grakoui A, Kourtis AP. Differential expression of CD26 on virus-specific CD8(+) T cells during active, latent and resolved infection. Immunology 2009;126(3):346–53. https://doi.org/10.1111/j.1365-2567.2008.02899.x
[37] Chu H, Chan CM, Zhang X, Wang Y, Yuan S, Zhou J, et al. Middle East respiratory syndrome coronavirus and bat coronavirus HKU9 both can utilize GRP78 for attachment onto host cells. J Biol Chem 2018;293(30):11709–26. https://doi.org/10.1074/jbc.RA118.001897
[38] Earnest JT, Hantak MP, Li K, McCray Jr. PB, Perlman S, Gallagher T. The tetraspanin CD9 facilitates MERS-coronavirus entry by scaffolding host cell receptors and proteases. PLoS Pathog 2017;13(7):e1006546https://doi.org/10.1371/journal.ppat.1006546
[39] Corman VM, Albarrak AM, Omrani AS, Albarrak MM, Farah ME, Almasri M, et al. Viral Shedding and Antibody Response in 37 Patients With Middle East Respiratory Syndrome Coronavirus Infection. Clin Infect Dis 2016;62(4):477–83. https://doi.org/10.1093/cid/civ951. PubMed PMID: 26565003; PubMed Central PMCID: PMCPMC7108065.
[40] McFee RB. Middle East Respiratory Syndrome (MERS) Coronavirus. Dis Mon 2020;66(9):101053https://doi.org/10.1016/j.disamonth.2020.101053. PubMed PMID: 32773137; PubMed Central PMCID: PMCPMC7386480.
[41] Tynell J, Westenius V, Ronkko E, Munster VJ, Melen K, Osterlund P, et al. Middle East respiratory syndrome coronavirus shows poor replication but significant induction of antiviral responses in human monocyte-derived macrophages and dendritic cells. J Gen Virol 2016;97(2):344–55. https://doi.org/10.1099/jgv.0.000351. PubMed PMID: 26602089; PubMed Central PMCID: PMCPMC4804640.
[42] Chu H, Zhou J, Wong BH, Li C, Chan JF, Cheng ZS, et al. Middle East Respiratory Syndrome Coronavirus Efficiently Infects Human Primary T Lymphocytes and Activates the Extrinsic and Intrinsic Apoptosis Pathways. J Infect Dis 2016;213(6):904–14. https://doi.org/10.1093/infdis/jiv380. PubMed PMID: 26203058; PubMed Central PMCID: PMCPMC7107330.
[43] Xu YY, Liu HH, Zhong YW, Liu C, Wang Y, Jia LL, et al. Peripheral blood mononuclear cell traffic plays a crucial role in mother-to-infant transmission of hepatitis B virus. Int J Biol Sci 2015;11(3):266–73. https://doi.org/10.7150/ijbs.10813
[44] Rouzioux C, Hubert JB, Burgard M, Deveau C, Goujard C, Bary M, et al. Early levels of HIV-1 DNA in peripheral blood mononuclear cells are predictive of disease progression independently of HIV-1 RNA levels and CD4+ T cell counts. J Infect Dis 2005;192(1):46–55. https://doi.org/10.1086/430610
[45] Alhetheel A, Albarrag A, Hakami A, Shakoor Z, Alswat K, Abdo A, et al. In the peripheral blood mononuclear cells (PBMCs) of HCV infected patients the expression of STAT1 and IRF-1 is downregulated while that of caspase-3 upregulated. Acta Virol 2020;64(3):352–8. https://doi.org/10.4149/av_2020_313