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SHORT COMMUNICATION

The dipeptidyl peptidase-4 expression in some MERS-CoV naturally infected dromedary camels in Saudi Arabia 2018–2019

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Abstract MERS-CoV usually causes respiratory and renal failure in some patients, which may be the underlying cause of death. Dromedary camels are the only known reservoir of the virus until now. They shed the virus in their body secretions thus potentiate a risk for human infection. MERS-CoV tropism and replication is mainly affected by the presence of certain receptor ligands on the target tissues. The dipeptidyl peptidase-4 (DPP-4) is believed to act as receptors for MERS-CoV. The main objective of this study was to determine the expression levels of the DPP-4 in various organs of some naturally infected camels. We conducted a surveillance study to identify some positive MERS-CoV infected camels. Three positive animals identified by the Real time PCR. Our results are clearly showing the high level of expression of the DPP-4 in various organs of these animals' particularly nasal turbinate, trachea, and lungs. The expression level may explain at least in part the pathogenesis of MERS-CoV in these organs. These findings confirm the pivotal roles of the DPP4 in the context of the MER-CoV infection in dromedary camels. Further studies are needed for a better understanding of the molecular pathogenesis of MER-CoV infection.

Keywords MERS-CoV · Natural · Infection · DPP4 expression · PCR · Immunohistochemistry

MERS-CoV is one of the respiratory coronaviruses of human. As of Feb 2020, there are 2494 laboratory-confirmed human cases reported from 27 countries across the globe. About 858 were passed away. The majority of the infection and fatalities were reported in the Arabian Peninsula [1]. MERS-CoV is belonging to the lineage C of betacoronaviruses. It is a positive sense single stranded RNA virus. The virus replicates in the cytoplasm of the infected cells. The virus replication cycle occurs in several steps starting with the adsorption of the virus to the cell surface. This step requires the presence of some specific viral receptors on the target cells. The DPP-4 was reported to be one of the main viral receptors utilized by the virus to facilitate the virus entry to the target cells in addition to the sialic acid in transgenic mice model [2]. Some studies proved the upregulation of the DPP4 in heavy smokers in the context of MERS-CoV infection which contributed to severe lung complications and finally death of the affected patients [3]. Interestingly, glycosylation of the DPP-4 protein resulted in marked inhibition of MERS-CoV replication in mouse model [4]. Some recent studies reported the correlation between the expression of DPP-4

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and the success of MERS-CoV infection both in vitro in Illama and pigs [5]. In similar manner, experimental infection of dromedary camels resulted in extensive ciliary loss of the respiratory tract as well as severe loss of the DPP-4 in the respiratory tract of the infected camels [6]. However, few studies were conducted to explore the roles of DPP-4 in the context of natural MERS-CoV infection in dromedary camels. We conducted this study to show the expression levels of the DPP-4 during the active natural MERS-CoV infection in dromedary camels in Saudi Arabia.

We conducted this study according to the guidelines of the Animal Ethics protocols and the National Committee of Bio-Ethics, King Abdul-Aziz City of Science and Technology, Royal Decree No. M/59 (http://www.kfsh.med.sa/KFSH_WebSite/usersuploadedfiles%5CNCBE%20Regulations%20ENGLISH.pdf). Meanwhile, sampling protocol approved by the Ethics Committee of the Ministry of Environment.

This study was conducted as a part of a molecular surveillance of MERS-CoV among dromedary from March to April 2018 at the Ministry of Environment, Water and Agriculture (MEOWA), Riyadh, in collaboration with the King Faisal University, Saudi Arabia. A total of 75 dromedary camels were examined in the south Riyadh slaughterhouse. The target animal population was less than 2 years old. Two nasal swabs were collected per each animal, the first was collected on the buffer of Rapid MERS-CoV Ag detection kit while the second was collected on the viral transport media (COPAN Italia, Italy), and tested by the (real time PCR technique (rtRT-PCR). All collected swabs were transferred to the Riyadh veterinary laboratory within 2-h after collection to confirm the presence of the MERS-CoV-RNA by RT-PCR. Three positive as well as other two negative MERS-CoV animals were selected for further testing.

Tissues from upper and lower respiratory tract, and kidney were fixed in 10% neutral-buffered formalin for 5 days. Tissues were processed and paraffin sections were stained with Haematoxylin and Eosin (H&E) for histopathological examination as previously described [6].

Tissue sections from trachea, turbinate bones, lungs and kidneys were screened for the presence of cell surface receptor dipeptidyl peptidase 4 (DPP4 by immunohistochemistry. We used the polyclonal rabbit anti DPP4 (ABIN213568, antibodies-online.com, Germany), to detect the DPP4, proteins. The technique was carried out as per the manufactures instructions as well as previously described [6].

Several dromedary camels were kept in isolation yard in the slaughterhouse for testing (Fig. 1a). We identified three animals to be slaughtered for further processing. Before slaughtering, these animals were physically inspected to identify any obvious clinical signs (Fig. 1b). We collected some nasal swabs from these animals to be tested by the quick MERS-CoV latex agglutination test to identify some positive animals. Positive animals showed double bands on the strips while the negative animals showed only one band (Fig. 1c). Three out of the tested positive animals were subjected to necropsy examination after slaughtering.

Immunohistochemical results of the examined tissues elucidated detectable signals of the cell surface receptor Di-Peptidyl Peptidase 4 (DPP4) in different organs (Table 1). The DPP4 receptor signals was detected in the all examined tissues (nasal turbinate, trachea, lung, and kidneys) with moderate to strong signals (Table 1). The evaluation of the signals depended on both the distribution as well as the strength of the reaction in the examined tissues. Most of the reactions were obviously detected in

Table 1 Scoring system for the DDP-4 expression in some naturally infected dromedary camel MERS-CoV

Organ	DPP4
Nasal turbinate	+++
Trachea	++
Lungs and bronchi	++
Kidneys	+++

Fig. 1 Clinical examination and identification of some MERS-CoV naturally infected of dromedary camels. **A** Dromedary camels kept in isolation units before slaughtering. **B** Technique for the collection of nasal swabs from some dromedary camels. **C** MERS-CoV latex agglutination test showing positive animals (double bands) and negative animals (one band)

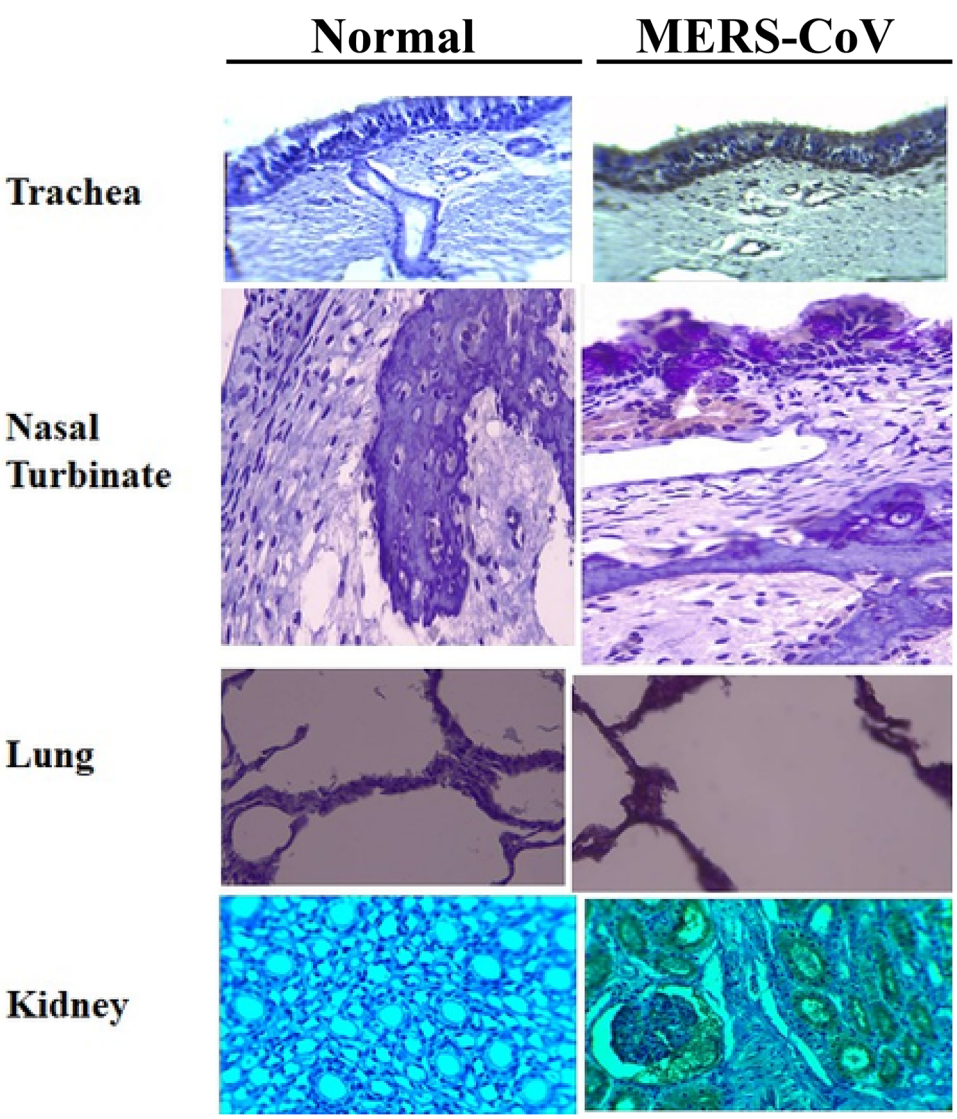

Fig. 2 Results of the DPP4 expression in various organs of MERS-CoV naturally infected dromedary camels. Results of the immunohistochemistry testing of some organs from MERS-CoV naturally infected dromedary camels. Positive signals were detected at various levels in various organs including nasal turbinate, trachea, lung and kidney. This in comparison to the similar organs from non-infected camels

both the apical epithelial layers and submucosal glands of the turbinate bones, trachea and bronchi. In the lungs, the reaction of DPP4 receptors and was detected in the alveolar walls (Fig. 2).

Viral replication and tropism is mainly governed by many factors including the availability of specific viral receptors and transcription factors. It is believed that MERS-CoV utilizes the DPP-4 as main receptors and sialic acid as accessory receptors [7]. MERS-CoV infection usually starts with the attachment of the virus by its spike glycoproteins to the target cell DPP4 ligands [7]. One study have found that DPP4 was detected on cells in the cortical apical proximal tubular epithelium and arteriolar smooth muscle of kidney of camel [8]. Our study considered natural infected animals and others studies done under experimental conditions using different animals (rhesus macaques, common marmosets, and dromedary camels) are confirming the importance of the 14 amino acids within the DPP4 which binds to the RBD of the MERS-CoV in the successful viral infections [9, 10]. Extensive ciliary loss in the respiratory organs of the dromedary camels was recently shown under experimental infection using the recombinant MERS-CoV/Vaccinia virus- Ankara (MVA-S) [6]. Our results showed high level of DPP4 expression levels in nasal turbinate and kidney. This may explains the multi-organs failure in some MERS-CoV patients particularly lung and kidneys [11]. Our results is very much consistent with the pathogenesis of MERS-CoV infection in human as well as the experimental infection of MERS-CoV in dromedary camels and other members of family *Camelade* [12]. Silencing of the DPP4 could be a novel approach to reduce the risk of

MERS-CoV infection in the future. This direction worth investigation in the future.

The DPP4 is highly expressed in the respiratory system as well as in the kidney of the naturally infected MERS-CoV dromedary camels. This is confirming their potential roles as receptors for the MERS-CoV as well as highlighting their roles in the molecular pathogenesis of the virus.

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Data availability statement Data will be available upon request.

Compliance with ethical standards

Conflict of interest All authors declares no conflict of interest.

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