THE RELATION OF ACE2 AND TMPRSS2 NASOPHARYNGEAL EXPRESSION WITH DISEASE SEVERITY IN VIETNAMESE COVID-19 PATIENTS

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Summary

Introduction: ACE2 and TMPRSS2 are two crucial proteins of host cell membrane helping SARS-CoV-2 viral infection to cause COVID-19 disease. ACE2 is a receptor for binding of the viral spike (S) protein and TMPRSS2 following cleaves the S protein at the S1/S2 and S'2 sites for membrane fusion.

Aim: This study aimed to evaluate the expression of nasopharyngeal ACE2 and TMPRSS2 upon admission in relationship with the subsequent clinical course during hospitalization of COVID-19 patients.

Material and methods: Representative 29 and 23 nasopharyngeal fluid samples collected upon admission from typical severe and mild COVID-19 patients hospitalized in the National Hospital for Tropical Diseases (Vietnam), respectively, were relatively compared RNA expression of ACE2 and TMPRSS2 by using Realtime-PCR with specific primers. SARS-CoV-2 cases were diagnosed clinically, blood and biochemical parameter, X-ray/CT and confirmed by RT-PCR.

Results: The RNA expression of TMPRSS2 was relative higher (1.36 fold) in severe group in comparison with mild patients (p = 0.0004), but the level of ACE2 expression was no difference between two groups.

Conclusion: TMPRSS2 but not ACE2 gene expression in the nasopharyngeal mucosa may promote the severity of COVID-19 patients but requires further study in larger samples.

Keywords: *ACE2. TMPRSS2, nasopharyngeal fluid sample.*

INTRODUCTION

The COVID-19 disease is severe acute respiratory syndrome caused by the SARS-CoV-2 virus that has been rapidly spreading and become to a global pandemic. In Vietnam, until June 2022, the number of cases has exceeded 10.7 million cases and there are more than 43 thousand deaths[1]. COVID-19 patients present mild to severe symptoms. Most infected individuals showed common respiratory viral infections such as dry cough, headache, and fever… but someone may progressive respiratory distress and even death due to even more critical condition of respiratory failure, septic shock and multi-organdisfunction[2] Epidemiological risk factors such as age, sex (male), hypertension, obesity, diabetes and smoking habit associated to hospitalization or death caused by COVID-19[3,4].

The angiotensin-converting enzyme 2 (ACE2) has been known as the entry receptor for SARS-CoV-2 and the trans membrane serine protease 2 (TMPRSS2) is an important priming enzyme required for the viral invasive process[5], both of them characterize potential initial target sites for SARS-CoV-2 replication in humans[6]. ACE2 and TMPRSS2 genes have been

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detected on many organs such as nasal mucosa, lungs, small intestine, liver, kidney[6,7]… However, ACE2 and TMPRSS2 expression level on nasopharyngeal mucosa of SARS-CoV-2 infected individuals and their potential impact on COVID-19 susceptibility is still questionable. Previous studies have still contradictory about the role of ACE2 expression. Some of evidences supposed that ACE2 may exacerbate the disease: lower ACE2 expression in upper respiratory tract could reduce susceptibility to SARS-CoV-2 in childen[8], higher ACE2 expression in individuals with hypertension and upon angiotensin receptor blocker therapy or smoker may be a factor involving in exacerbation of the disease[9-11]. On the other hand, many studies supported that ACE2 plays the anti-inflammatory role that may improve progression: ACE2 and the angiotensin II type 2 receptor (AT2) ameliorate acute lung injury/inflammation in mice[12]; ACE2 deficiency exacerbated inflammation in adipose tissue upon high calorie diet induced obesity in mice, reinforcing its potential anti-inflammatory effects[13].Transmembrane protease serine 2 (TMPRSS2) plays the role as a contributing factor to the more severe outcomes noted for COVID-19[14]. In which, the intron variant rs383510 in the gene TMPRSS2 is associated with an increased risk to SARS-CoV-2 infection[15]; TMPRSS2 gene is upregulated by androgenic hormones also in the lungs[16]. TMPRSS2 expression is higher in bronchial epithelial cells of males than females[17] that explained why man were more severe than women, and using inhibiting TMPRSS2 activity is a promising strategy to block viral infection[18]. In addition, TMPRSS2 effect on modelling disease severity may be dependent on ACE2 levels, higher TMPRSS2/ACE2 ratios showed a prominent risk effect for respiratory distress requiring oxygen therapy during COVID-19 suggesting that the impact of this serine protease on COVID-19 might be better explored in combination to its partner ACE2[19].

SAMPLES AND METHOD

Study design

This study was performed with 52 specimens at first positive SARS-CoV-2 collected from 29 and 23 subsequent severe and mild COVID-19 patients, respectively, admitted to the National Hospital for Tropical Diseases (NHTD) between May 2021 and January 2022 during COVID-19 outbreak in Vietnam. All patients had confirmed SARS-CoV-2 routine diagnosis by using the real-time RT-PCR for detecting E gene and Rdrp gene of SARS-CoV-2 (Super Cript lll one step RT PCR, Invitrogen, Thermo Fisher, USA). The assay was performed on the Biorad CFX96 realtime PCR system (USA).

Specimen collection, RNA extraction and cDNA synthesis

Nasopharyngeal swabs (NT)collected in Universal Transport Medium after using for SARS-CoV-2 diagnosis were kept at -80°C. Human RNA was extracted from 1000 µL of the samples according to the manufacturer's instructions (New England Biolabs, USA). Twenty-microliter eluate was used to cDNA synthesis immediately with ProtoScript®II First Strand cDNA Synthesis Kit (New England BioLabs, USA) according to the manufacturer's protocol on the PCR machine (Eppendorf®Mastercycler® Pro S, Germany). Briefly, each 20-µL reaction contained 1 µg of RNA, Oligo d(T)23VN 2 µl, ProtoScript II Reaction Mix (2X) 10 µl, ProtoScript II Enzym Mix (2X) 2 µl and Nuclease-free H2O. Cycling conditions were as followed: 60°C for 30 seconds, 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds, 60°C for 1 minute; and 60°C for 30 seconds. cDNA products werekept at -20°C for further analysis.

Quantitation of ACE2 and TMPRSS2 mRNA

Monoplex qRT-PCR using specific primer pairs (TMPRSS2 F-AATCGGTGTG TTCGCCTCTAC and R-CGTAGTTCTTCGTTCCAGTCGT; ACE2 F-GGGATCAGAGATCGGAAGAAGAA and R - AGGAGGTCTGAACATCATCAGTG); and normalized with GAPDH F-TGAAGGTCGGAGTCAACGGATTTGGT and R-CATGTGGGCCATGAGGTCCACCAC) was performed according to the manufacturer's protocol (Luna Universal qPCR Master Mix kit, New England Biolabs, USA) on the Realtime PCR intrument (Lightcycle 96 ROCHE, Tokyo, Japan). Briefly, each reaction contained Master Mix (1X) 10 µl, primers

(0.25 µM) 0.8 µl x2, cDNA (≤ 100 ng) and Nuclease-free Water for just 20 µl reaction mixture. Cycling conditions were as followed: 95°C for 1 minutes, then 40 cycles of 95°C for 10 seconds, 60°C for 30 seconds, 72°C for 10 seconds, and 72°C for 5 seconds.

Statistical analysis

Relative comparison in level of ACE2 and TMPRSS2 expression between severe and mild patient groups were performed by using the T-test statistical test. Only a p-value less than 0.05 was considered statistically significant.

Ethics statement

The experimental procedures used in this study were approved by the Ethics Committee, National Hospital for Tropical diseases, Hanoi, Vietnam.

RESULTS

Clinical characteristics of COVID-19 patients

The 52 COVID-19 patients at the equivalent age involved in this study presented mild symptom at admission and after that 29/52 patients developed severity with typical clinical symptom and blood test. Severe group were enrolled the patients who required oxygen support over the course of SARS-CoV-2 infection while mild group presented only mild symptoms of the disease. Of the study population, there were 29 (55.8%) patients in the mild group and 23 (44.2%) in the severe and critical group. In the mild group, there were 11 (47.8%) male and 12 (52.2%) female patients with mean age 50.1 ± 11.5. Similarly, the severe and critical group consisted of 17 (58.6%) males and 12 (41.4%) females with a mean age of 47.5 ± 9.9 years. The mean age between the 2 groups had no statistically difference (p = 0.13). The mean of biochemical and hematological indicators during hospitalizing (CRP, Ferritin, LDH, URE, AST, ALT, Calcium, GGT, Na+, K+, Cl-, Fibrinogen, D-Dimer, LYM, INR, RBC, WBC, HCT, PLT, PT%) were all different statistical significantly between the 2 groups (p < 0.01).

ACE2 and TMPRSS2 transcription levels

Fifty-two specimens of nasopharyngeal mucosa obtained from 29 subsequent severe and 23 mild COVID-19 patients at the time of admission to NHTD for SARS-CoV-2 diagnosis were analyzed ACE2 and TMPRSS2 mRNA expression, therefore the expression of ACE2 and TMPRSS2 in all samples were not associated with any interferences of medical treatment. Relative mRNA expression of ACE2 and TMPRSS2 were determined by qRT-PCR with using GAPDH gene as reference performing simultaneously on all samples with the selected standard sample. The results showed that the expression level of TMPRSS2 was higher significantly (1.306 + 0.333) in severe group compared with that of mild group (p = 0.0004) (Figure 1) while the expressions of ACE2 was not different between two groups.

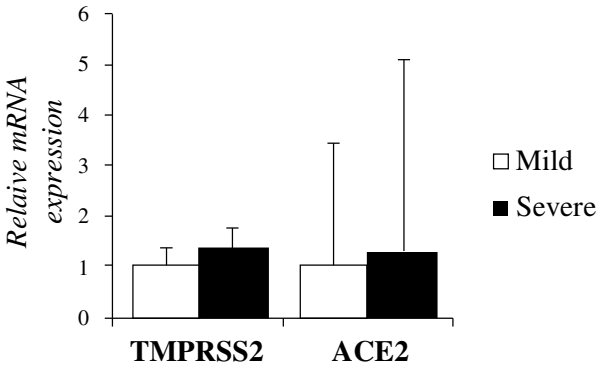

Figure 1. Expression of TMPRSS2 and ACE2 in nasopharyngeal mucosa of mild and severe COVID-19 patients

DISCUSSION

The finding of prognostic markers at the onset of symptoms is so important for clinical treatment. Epidemiological factors such as age and chronic diseases (systemic arterial hypertension, diabetes and, obesity) combined with the routine blood and biochemical tests such as CRP, ferritin, white blood cell count…, can help predict disease severity[2,4]. However, all available factors are not accurate for all cases, it is important to find new factors to help predict clinical progression more accurately.

Our study in total 29 severe and 23 mild cases were analyzed ACE2 and TMPRSS2 expression in nasopharyngeal swabs collected for SARS-CoV-2 diagnosis at the first day of hospitalization. All

samples in both groups were obtained at symptom onset or the first SARS-CoV-2 positive result, thus unaffected by pathological manifestations or medical treatment. In addition, two cohorts were not difference in age, gender, comorbidities, and other epidemiological factors.

ACE2 is well known as the entry receptor for SARS-CoV-2 infection and TMPRSS2 as a major priming enzyme contribute to its fusion on the cell membrane[5,20]. Therefore, ACE2 and TMPRSS2 were considered as potentially major host factors influencing the pathogenesis of COVID-19. The expression levels of these host factors at nasopharyngeal mucosa, the primary site of exposure, could impact to respiratory viral infection. ACE2 and TMPRSS2 expressions are lower in children than in young adults' upper airways[8,21], positively correlated to age while no association with sex[19], supporting that they may influence age-related susceptibility to COVID-19. Thus, the expression levels of these genes in nasopharyngeal mucosa at the onset of symptom may be potential predictor for disease severity.

In the present study, we characterized the transcription levels of both genes in nasopharyngeal samples of COVID-19 patients and investigated their association with aggressive progression. We found that, TMPRSS2 but not ACE2 expression levels at the nasopharyngeal epithelium associated to severity of SARS-CoV-2 infected individuals. Nasopharyngeal expression of ACE2 was not significantly different between the two cohorts which may explain that the patients in the two groups were of similar age and sex, consistent with previous results although its functional activity on viral fusion depends on ACE2-mediated viral adsorption[19]. Presumably, the virus only needs ACE2 as the entry port as long as it persists on the cell surface and not belonging to the number of ports. In contrast, TMPRSS2 were considered as key point enrolled to COVID-19 pathogenesis. TMPRSS2 expression in nasopharyngeal were higher 1.3 folds significantly in severe group compared with mild patients. The results reflect that TMPRSS2 may contribute to critical step of viral invasion and may have important role in pathological mechanism of COVID-19. TMPRSS2 levels can be used as predictors of disease severity for the management of individuals infected with SARS-CoV-2.

An important limitation of this study is small sample size because it is difficult to obtain patients at the equivalent age with a sufficient range of symptoms from mild to severe throughout the hospitalized course, most of the hospitalized patients presented severe symptom on admission. In future investigation, we need to co-operate with other hospitals for more data.

In conclusion, the present study suggests that nasopharyngeal expression of TMPRSS2 genes but not ACE2 may be a potential predictor of COVID-19 severity.

CONCLUSION

TMPRSS2 but not ACE2 gene expression in the nasopharyngeal mucosa may promote the severity of COVID-19 patients but requires further study in larger samples.

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