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Independent Tendency of *ACE2* and *GRP78* Expression in SARS-CoV2 Infection

Abstract: Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV2) is a virus that attacks respiratory tract and causing COVID-19 pandemic. This virus utilizes host receptor as cellular entry. Angiotensin-converting enzyme 2 (*ACE2*) has been assumed to be the essential host receptor for SARS-CoV2 infection. Furthermore, another costimulatory molecule has also been reported, such as glucose-related protein 78 (*GRP78*). However, there are several inconsistent clinical data that could be observed regarding these molecules involvement during SARS-CoV2 infection. This study aimed to observe the possible involvement of both *ACE2* and *GRP78* during infection phase through gene expression profile analysis. Clinical specimens used in this study were taken following the standard swab sampling procedure from both oropharyngeal and nasopharyngeal swab in positive and negative clinical samples. Subsequently, nucleic acid samples were proceeded by conventional Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) in order to analyze the expression of *ACE2* and *GRP78*. Agarose gel electrophoresis was then performed prior to the densitometric analysis. Statistical analysis using Mann-Whitney Test and Independent Sample t-Test were applied to justify the gene profile difference of *ACE2* and *GRP78*. Our study showed the enhancement tendency in both *ACE2* and *GRP78* expression from the positive SARS-CoV2 samples in non-significant different.

Keywords: Gene expression; *ACE2*; *GRP78*; SARS-CoV2

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has been known as a global issue these days. The disease with pneumonia-like characteristics was firstly reported in Wuhan, China on December 2019 which was declared as pandemic on March 2020.[1] Originally it attacks respiratory tract and causes acute respiratory disease with the clinical manifestation such as fever, cough, shortness of breath, and myalgia.[2] Some other symptoms such as sputum production, headache, haemoptisis, and diarrhea are also reported.[3] Furthermore, the existence of collective symptoms could be assumed to a person who is infected by SARS-CoV2.[4]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) as a cause of COVID-19 is a human Coronavirus (hCoV) which belongs to betacoronaviridae family.[4] SARS-CoV2 is an enveloped virus and composed of several structural proteins including membrane (M), envelope (E), nucleocapsid (N), and also spike (S).[5] Considering viral genetic background, including its identity, SARS-CoV2 has been reported to have around 79,5% similarity in its nucleotide sequence against to the previous SARS-CoV which also has 96% similarity to the bat coronavirus.[6] Despite of both nucleotide sequence similarities, SARS-CoV2 tends to be transmitted easily with less severity.[3]

Infection of SARS-CoV2 is initiated through the attachment of viral spike (S) protein to the responsible receptor in the host cell.[7] SARS-CoV2 receptor that is believed to be responsible for the infection is angiotensin-converting enzyme 2 (*ACE2*) where then similar to the previous SARS-CoV receptor.[5,8] Further reports also concern for the involvement of other molecules during SARS-CoV2 infection since there are some complexities in determining only a single causative related cell surface receptors.[9]

Structural analysis which has been performed to SARS-CoV2 spike protein, shows that this protein could also bind to the glucose-related protein (*GRP78*) on the cell surface.[9] Glucose-related protein (*GRP78*) is a chaperone molecule that is abundantly found in eucaryote endoplasmic reticulum.[10] Normally, *GRP78* is located in endoplasmic reticulum lumen area. However, when the cell is undergoing stress, this molecule escapes and translocates to the cell membrane. Thus, being easily reached by the virus and initiate the viral infection.[11]

SARS-CoV2 can be detected in various sites in upper and lower respiratory tracts, including throat, nasal nasopharyngeal, sputum, and bronchial fluid. Practically, nasopharyngeal swab is the common procedure to obtain SARS-CoV2 containing specimens in combination with oropharyngeal swab.[12] Recently, there is a screening study reporting about gene expression pattern in both upper and lower respiratory tract which shows that *ACE2* is the most vary expression while *GRP78* is the highest level of expression in human respiratory epithelial cells.[8] Therefore, there are some other possibilities for the involvement of other molecules in SARS-CoV2 infection besides *ACE2* receptor.

This study was performed using SARS-CoV2 positive and negative both nasopharyngeal and oropharyngeal swab specimens which were collected from RS Asri Medical Center Yogyakarta. It was purposed for the gene expression analysis in both positive and negative SARS-CoV2 conditions in order to observe other functional viral receptors. Therefore, the existence in both gene expression discrepancies and alterations may suggest the direct involvement of *GRP78* along with *ACE2* during the SARS-CoV2 infection.

MATERIALS AND METHODS

This research is an observational analytic laboratory study. This study used 20 positive samples (n=20) and 10 negative samples (n=10). The specimens were collected by healthcare professionals in Asri Medical Center Hospital, Yogyakarta. All samples were taken according to the consecutive blinded sampling. This study was conducted under the ethical approval from the Ethics Committee of Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta (No. 029/EC-EXEM-KEPK FKIK UMY/IV/2021).

The obtained specimens were further extracted by the laboratory technical supports from the Molecular Medicine and Therapy (MMT) Laboratory. The RT reaction was performed by adding 6 µl RNA into the PCR tube as a total initial mixture. Then, RNA denaturation was performed using thermocycler (Scilogex, AS) for 5 minutes at 65ºC. Subsequently, 2 µl Mix A (gDNA Remove Solution) was added, then vortex and spindown for 2-3 seconds prior to gDNA removal for 5 minutes at 37ºC. 2 µl 5X Master Mix II (Bioline, England) was sequencially added to the mixture, then vortex and spindown for 2-3 seconds. The RT reaction was conducted under the conditions of 37ºC for 15 minutes, 50ºC for 5 minutes, and 98ºC for 5 minutes.

The PCR amplification was proceeded by mixing 25 µl 2X My Taq HS Mix (Bioline, England), 20 µl nuclease free water (Bioline, England), and 3 µl template cDNA previously prepared. 1 µl forward and reverse primer of each gene target were used as previously described.[13,14] PCR amplification steps were performed following the conditions of 95ºC for 4 minutes, 94ºC for 30 seconds, 42ºC for 30 seconds, and 65ºC for 30 seconds in 35 cycles. Followed by 65ºC for 4 minutes and hold at 4ºC for *ACE2*. Similar conditions were applied for *GRP78* with modification in cycling and extension as follow, 95ºC for 30 seconds, 56ºC for 20 seconds, and 72ºC for 30 seconds in 36 cycles, followed by 72ºC for 4 minutes and hold at 4ºC.

The PCR products were undergone electrophoresis with 1% agarose gel (Vivantis, Malaysia), 1X TAE running buffer (Vivantis, Malaysia), and 1 µl Gel Red (Biotium, USA). 2 µl novel juice (GeneDireX, USA) were added into 10 µl PCR product for loading preparation. 5 µl of ladder marker (Vivantis, Malaysia) was subsequently loaded as an indicator marker. Electrophoresis analysis was performed for 20-30 minutes using MiniRun GE, China. Running result was analyzed by using UV transluminator (GeneDireX, USA) and 48 megapixels mobile camera (Samsung Galaxy A51) for image detection. The expressions signals were then quantified under densitometric analysis using ImageJ version 1.52 (Wayne Rasband, National Institute of Health, USA). The data were then analyzed with Saphiro-Wilk for data normality

test and followed by Independent Sample t-Test for the parametric data and Mann-Whitney Test for the nonparametric data using SPSS version 20.0 (IBM, USA).

RESULT

The expression profile of *ACE2* and *GRP78* in each sample were shown in Figure 1 to Figure 2. *β-actin* was considered as an internal control. Normality statistical data indicated that *ACE2* data were not normally distributed (p 0.009, p<0.05), while *GRP78* data were normally distributed (p 0.609 for positive & p 0.604 for negative, p>0.05).

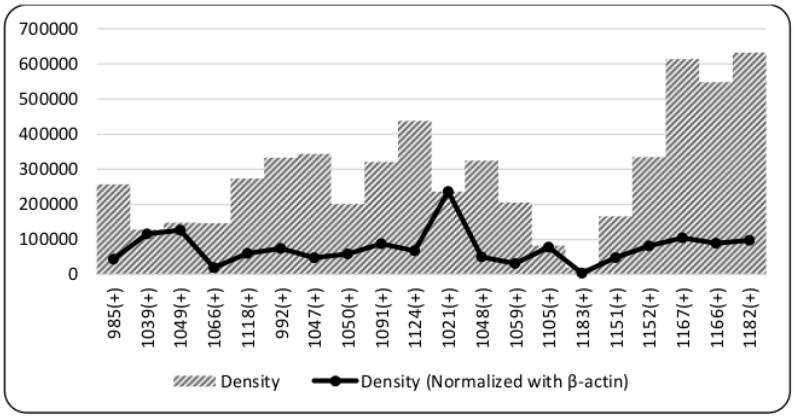

Figure 1. Density of *ACE2* in Positive Samples

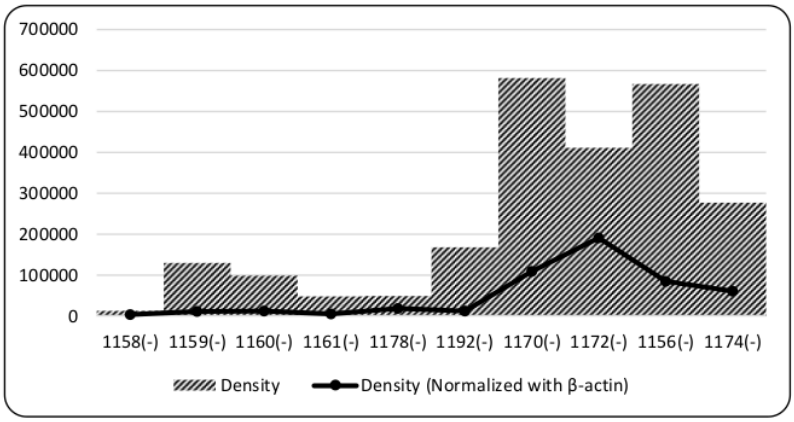

Figure 2. Density of *ACE2* in Negative Samples

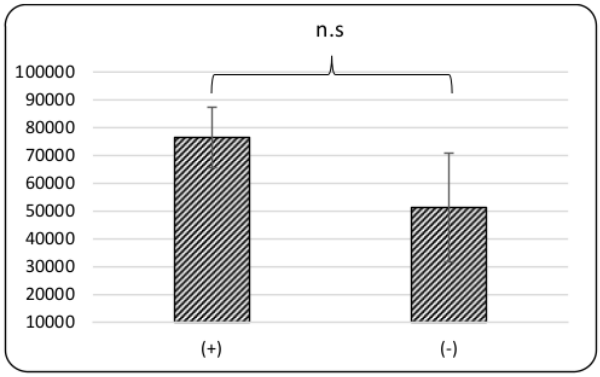

Figure 3. The Mean Density of *ACE2*

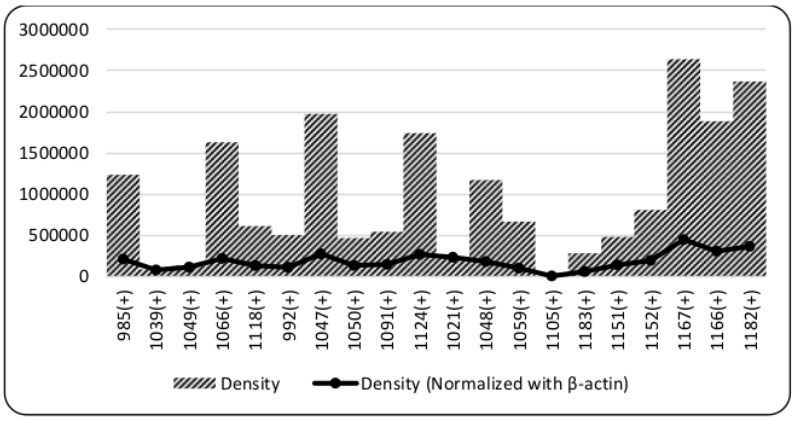

Figure 4. Density of *GRP78* in Positive Samples

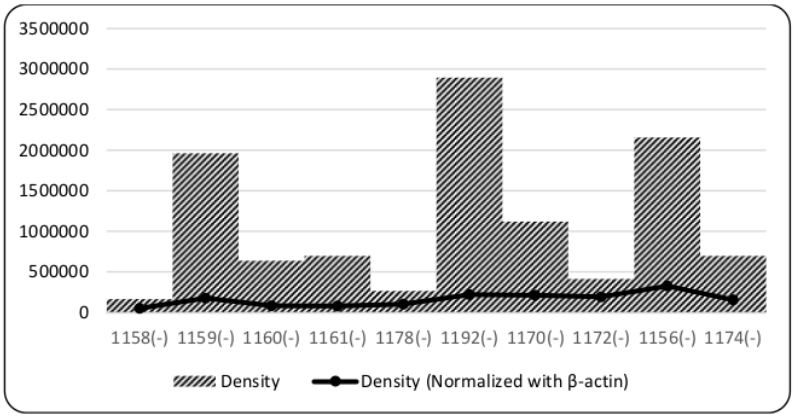

Figure 5. Density of *GRP78* in Negative Samples

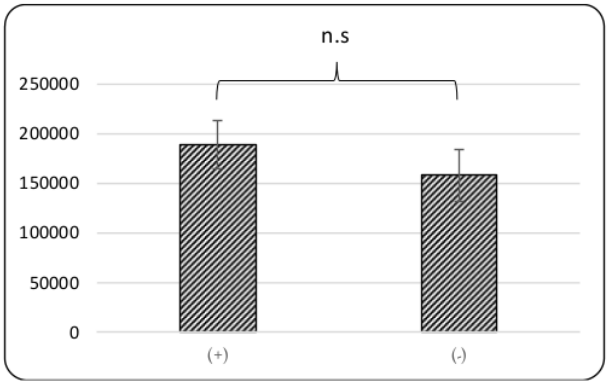

Figure 6. The Mean Density of *GRP78*

The signal density showed the gene expression of *ACE2* and *GRP78* which could be observed under the length at 238bp and 278bp respectively. The higher the density, the higher the expression accordingly. Statistical analysis of *ACE2* gene expression had a *p* value 0.104 ($p>0.05$), which indicated that there was no significant difference between both positive and negative samples. Similarly, there was also no significant difference could be observed in *GRP78*, which had a *p* value 0.436 ($p>0.05$).

In the positive group, the mean density of *ACE2* was 76594.82 with the standard of error was 10966.9067, while in negative group showed 51217,48 with standard of error 19484.783. As for *GRP78*, in the positive group, showed 189070.53 with the standard error 24068.762, while in negative group was 158287.10 with the standard error 26412.411.

DISCUSSION

In this study showed relative gene expression related to both *ACE2* and *GRP78* which were previously reported as a part of responsible genes in COVID-19 infection. Since our data showed quite similar expression level in both samples condition, it suggested that the SARS-CoV2 infection had no effects on the expression level of *ACE2* and *GRP78*. However, the result also suggested that there was an enhancement tendency in the SARS-CoV2 positive samples, as shown in Figure 3 and Figure 6.

Our results tend to be similar with the study of Gutiérrez-Chamorro et al., which reported that the *ACE2* expression was not significantly different in both positive and negative SARS-CoV2.[15] However, study by Zhuang et al. stated that *ACE2* had an enhancement in SARS-CoV2 patient.[16] This disagreement in *ACE2* expression profile could be related to the possible multi signaling pathway in the upstream mechanism of *ACE2* promoter activity such as complex of Interferon-Stimulated Gene (ISG). In general, interferon is a host antiviral responsive molecule that could be induced by viral infection. The enhancement of interferon expression, particularly type I, may increase *ACE2* expression through Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) pathway. Janus Kinase 1 (JAK1) and TYK2 will phosphorylates STAT1 and STAT2, which subsequently forms IFN-Stimulated Gene Factor 3 (ISGF3) complex. This complex may access to the Interferon Stimulating Regulatory Elements (ISRE) on the *ACE2* promoter region, thus control *ACE2* expression. Regarding the cell environment signaling, this mechanism may lead to the enhancement of *ACE2* regulation, as could be seen in Figure 7.[17]

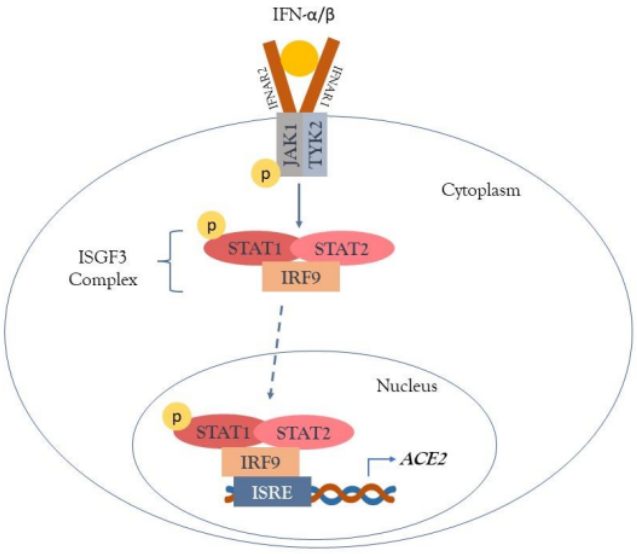

Figure 7. JAK/STAT Signaling Pathway (Modified from Lodhi, et al. (2021))

Similar to *ACE2*, *GRP78* also showed a similar tendency of enhancement in the positive samples. In the positive samples, SARS-CoV2 replicates in the host cell using host translational machinery, subsequently interfering endoplasmic reticulum and inhibiting protein synthesis to produce viral proteins. This condition could lead to the endoplasmic reticulum stress (ER stress).[18] According to Carlos et al., ER stress activates the unfolded protein response (UPR) in endoplasmic reticulum and leads to the enhancement of *GRP78* expression level.[19] The enhancement tendency in both genes expression in positive samples could be observed through the biological traits of the data, following the data distributions and central tendency. Since, the value of standard error would be reduced by increasing the number of data.[20]

In accordance to the non-significant expression difference from our data was also consistent to the previous result by Aguiar et al., who demonstrated the most expressed genes in the upper respiratory tract which are *GRP78* and *ACE2* in the SARS-CoV2 positive condition. This condition also happened in the lower respiratory tract. Furthermore, Chaudry et al., also explained that *ACE2* expression in the upper respiratory tract is lower than in the lower respiratory tract. *ACE2* expression commonly abundant to be found in type II alveolar cells. Meanwhile, *GRP78* is most abundantly expressed by bronchus, bronchial, and alveolus cells.[11] Since oropharynx and nasopharynx are not an ideal sites to evaluate precisely the expression of *ACE2* and *GRP78* in SARS-CoV2 infection, however, the specimen collection for SARS-CoV2 detection was performed by oropharyngeal and nasopharyngeal swab method.

The limitation of this study is the less number of samples could be analyzed and the possible variation during preanalytical procedures. However, the analyzed samples had also been normalized with human *β-actin* as a housekeeping gene.

CONCLUSION

According to our finding, it could be summarized that the expression of *ACE2* as the putative recognized functional receptor and *GRP78* as a potential co-receptor in SARS-CoV2 infection slightly tended to increase while also concerning the cellular and tissue environment signaling condition.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

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