Original Article

Clinical Significance and Different Expression of Dipeptidyl Peptidase IV and Procalcitonin in Mild and Severe COVID-19

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Abstract

Background: Coronavirus has become a global concern in 2019-20. The virus belongs to the coronavirus family, which has been able to infect many patients and victims around the world. The virus originated in the Chinese city of Wuhan, which eventually spread around the world and became a pandemic.

Materials and Methods: A total of 60 Patients with severe (n=30) and mild (n=30) symptoms of COIVD-19 were included in this study. Peripheral blood samples were collected from the patients. Real-time PCR was used to compare the relative expression levels of Procalcitonin and dipeptidyl peptidase IV (DPPIV) in a patient with severe and mild Covid-19 infection.

Results: Procalcitonin and dipeptidyl peptidase IV markers in the peripheral blood of patients with severe symptoms, were positive in 29 (96.60%) and 26 (86.60%), respectively (n=30); however, positive rates in the mild symptoms patients group were 27 (90%) and 25 (83.30%), respectively. There was a statistically significant difference between these two groups in terms of DDPIV and Procalcitonin ($p<0.001$).

Conclusion: Procalcitonin and DPPIV increase in patients with COVID-19 infection, significantly higher in the patients with more severe clinical symptoms than those with milder ones. More studies will be needed to verify the reliability of the current findings.

Keywords: Procalcitonin, DPPIV, Severe symptoms, Mild symptoms, COVID-19

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Introduction

Coronavirus disease 2019 (COVID-19) caused by coronavirus has become the biggest global concern in 2019-20. The Evidence indicates that the virus originated in wild animals and birds (1). Two coronavirus epidemics have occurred in the world with typical features of a respiratory syndrome called severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) (2, 3).

The coronavirus-induced epidemic of acute respiratory syndrome (SARS-CoV-2) was first identified in December 2019 in Wuhan, China (4, 5). The COVID-19 patients may show severe or mild forms of the disease. People with serious infections often have other systemic diseases, such as diabetes, immunodeficiency, or cardiovascular diseases, which in turn accelerates the progression of the infection in them and sometimes leads to death (6-10).

ARDS is the main cause of mortality in patients with Covid-19. ARDS is a common immunological event for SARS-COV, 2-SARS-COV, and MERS-COV infections (6, 11). Like most other coronaviruses, viral outer membrane spike glycoproteins are the most important proteins that can react with host target proteins such as ACE2, CD26, Cyclophilins, and others, which are important for cell adhesion and virulence (12, 13).

Dipeptidyl peptidase IV (DPPIV) also called CD26 is a 110 kDa cell surface glycoprotein with dipeptidase activity in the extracellular domain (14). DPPIV is a multifunctional cell surface protein in most cell types, including T lymphocytes, bronchial mucosa, and brush border of proximal tubules therefore, according to studies, it may play a role in the systemic spread of viral infections in humans (15-17).

Procalcitonin is a diagnostic indicator of systemic infections and sepsis. There is a link between increased levels of Procalcitonin and leukocyte-derived cytokines during infection and sepsis (18).

Therefore, DPPIV and Procalcitonin markers may be useful as diagnostic markers to differentiate the patients with mild form COVID-19 from severe. Therefore, in the present study, the expression evaluation of DPPIV and Procalcitonin genes in patients with severe Covid-19 and mild type was compared.

Methods

This study was approved by the Research Ethics Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.NRITLD.REC.1399.043), and all patients filled in informed consent. A total of 60 patients with mild (n=30) and severe forms (n=30) were included in this study. Blood samples were taken from the patients with both severe COVID-19 who were admitted to the ICU as well as mild patients who were admitted to the infectious-internal medicine wards.

Table 1: Sequence and the primers' characteristics.

	Procalcitonin	**Dipeptidyl peptidase IV**	**18srRNA**
Forward primer	GGAGAGCAGCCCA GCAGACCC	GCACGGCAACACATTG AA	GTAACCCGTTGAAC CCCATT
Length	21	18	20
Reverse primer	GTTGGCATTCTGG GGCATGCTAA	TGAGGTTCTGAAGGCCT AAATC	CCATCCAATCGGTA GTAGCG
Length	23	22	20
Product length	328	65	152
Annealing	60	56	56

RNA extraction and cDNA synthesis: RNA extraction was performed using the RNA Blood Mini Kit (Qiagen Cat no.52304, Germany) according to the manufacturer's protocol. RNAs purity and concentration were determined using a Nanodrop spectrophotometer (Bio- Tek-USANanodrop). Viva 2-steps RT-PCR Kit (Vivantis, Malaysia) was used to synthesize cDNA according to cDNA Kit's protocol. All cDNAs were stored at -20℃. The expression levels of DPPIV and Procalcitonin genes were determined using Cinna Green qPCR Mix, 2X (Cina Colon, Iran) by real-time PCR (Qiagen Rotor-Gene, Germany). 18srRNA was used as the reference gene to normalize the RT-qPCR data. The sequences of the used primer are presented in Table 1.

Quantitative Real-time PCR: before real-time PCR, the primers were prepared for DPPIV and Procalcitonin markers. The properties of which are shown in Table 1. To determine the efficiency of the primers, the real-time PCR reaction was performed with different primer dilutions (1 - 0.1 - 0.01 - 0.001), and the slope of the standard curve, which indicated the efficiency of the primers, was obtained. Moreover, the efficiencies of primers were confirmed by positive and negative control. The negative control experiment was performed without reverse transcriptase in each reaction buffer to confirm the specificity of the RT-PCR assay. Target cDNAs obtained with reverse transcriptase were amplified by PCR for 40 cycles (one cycle: 95°C for 5 min) followed by 40 cycles consisting of denaturation, primers connection, amplification, and final amplification at 95°C for 15, 56°C for 1 minute, 72℃ for 25 seconds, and 72℃ For 5 min, respectively. 18srRNA gene was used as an internal control gene. Three synthesized cDNA samples from each patient were tested to express the reference and the studied genes. To determine the gene expression levels in the blood samples of both severe and mild patients the $2^{-\Delta\Delta Ct}$ formula was used.

Statistical analysis: The results were analyzed using SPSS Version 20. Data were expressed as the mean and standard deviations. The student t-test was used to analyze the difference or correlation between gene expression levels and Clinicopathologic Features. The difference was considered significant at $P \leq 0.05$.

Results

The severe patients consisted of 21 (70%) males and 9 (30%) females, and the group of patients with mild symptoms consisted of 20 (66.6%) males and 10 (33.4%) females. There was no significant difference between the two groups in terms of average age.

The Analysis of the studied biomarkers' expression: Dipeptidyl peptidase IV and Procalcitonin markers in the peripheral blood of patients with severe symptoms, were positive on 29/30 (96.60%) and 26/30 (86.60%), respectively. The positive rate of these markers in the group of patients with mild symptoms was 27/30 (90%) and 25/30 (83.30%), respectively. There was a statistically significant difference between these two groups in terms of DDPIV and Procalcitonin ($p<0.001$) (Figure 1 & 2).

The relative expression of both markers in patients with severe symptoms was compared to the patients with mild symptoms. In the patients with severe symptoms, the relative expression of Dipeptidyl peptidase IV and Procalcitonin was up-regulated to 1.18 and 1.42 compared to patients with mild symptoms, respectively (Figure 3).

Discussion

This study has evaluated the relative expression of DPPIV and Procalcitonin in peripheral blood of patients with severe and mild-moderate symptoms of COVID-19. We have found that in the peripheral blood of patients with severe symptoms the expression levels of DPPIV and Procalcitonin are significantly up-regulated compared to patients with mild-moderate symptoms. Procalcitonin is an amino acid polypeptide 116, and its blood concentration is less than detectable in healthy individuals. However, it is increased in systemic inflammation, especially microbial infections (19). Increased Procalcitonin production mechanism

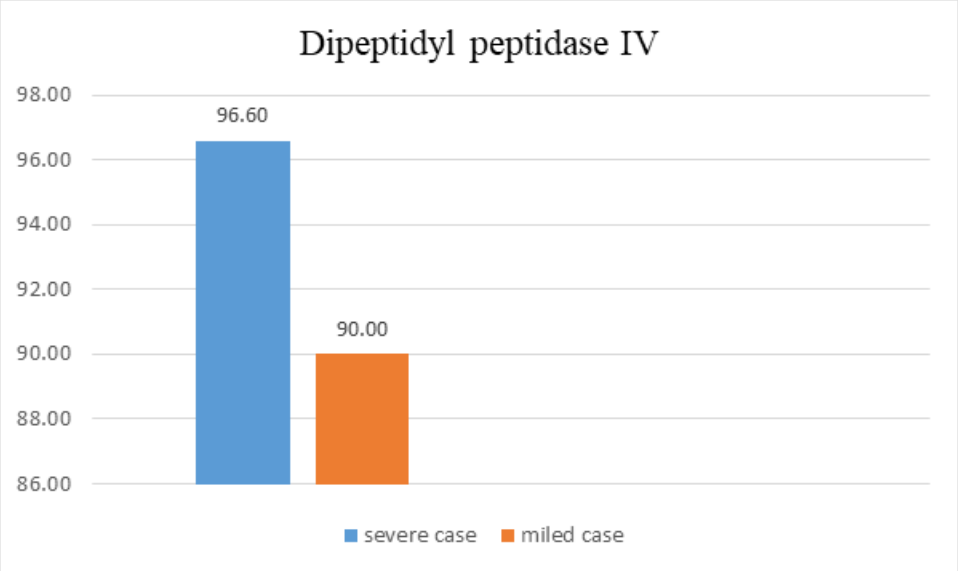

Figure 1. The percentage of positive Procalcitonin marker in patients with severe and mild symptoms.

is not yet fully understood (20). DPPIV is an encoded protein by the DPP4 gene. This protein is an enzyme that is expressed at most levels of cells and is associated with immune regulation, signal transmission, and apoptosis (21) and as mentioned, it may play a role in the systemic spread of viral infections in humans. Levy et al, have examined the reduction of Procalcitonin in patients admitted to the intensive care unit (ICU) and compared the clinical outcome of patients with that. They reported that an increase in the survival of these patients was closely related to a decrease in serum Procalcitonin between two or three days of hospitalization (22). In the performed studies the increase of Procalcitonin expression in patients admitted to the ICU may indicate a lack of response to treatment in this group of patients. Yukioka et al., in their study on Prolactinonin, have reported that procalcitonin serum levels are a sensitive criterion for a clear distinction between microbial

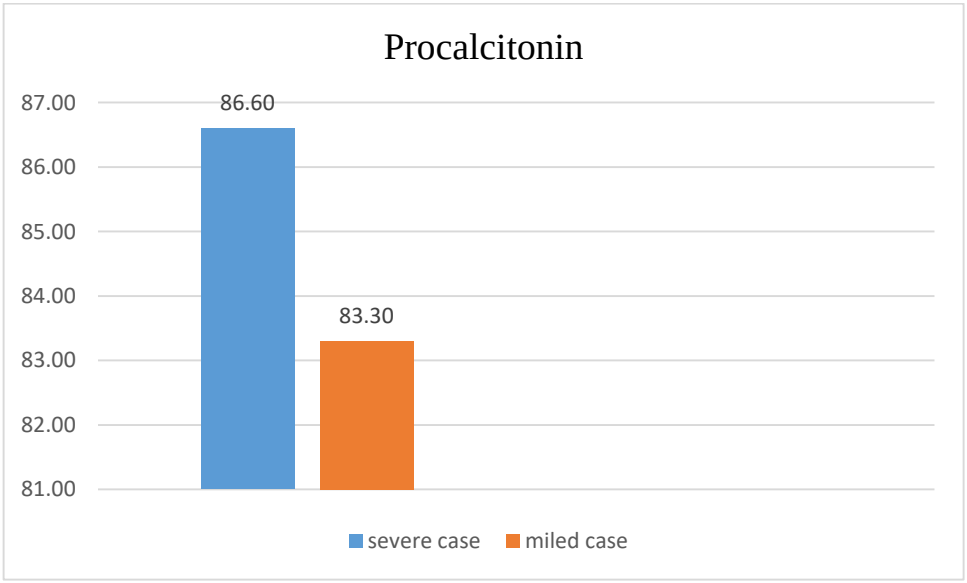

Figure 2. The percentage of positive Procalcitonin marker in patients with severe and mild symptoms.

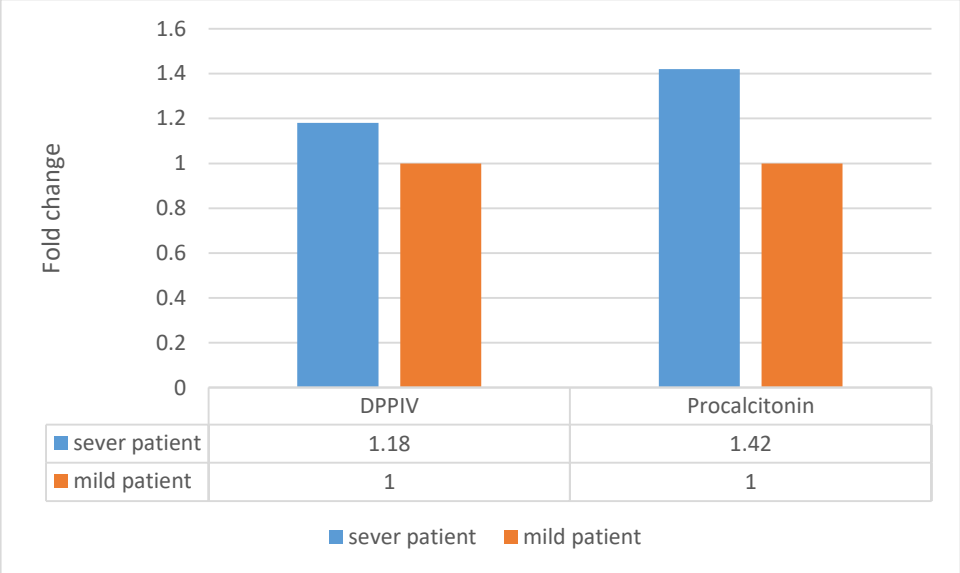

Figure 3. The difference between marker expressions in patients with severe symptoms compared to patients with mild symptoms.

infections and predictive reaction to treatment (23). In our study, there was an increase in the level of Procalcitonin expression in a group of patients with more severe symptoms than that of the group with mild symptoms, and this may be due to the appropriate reaction of a group of patients to the treatment protocol, who were hospitalized in the infectious-internal medicine wards and improves the disease and shows milder symptoms, and the level of Procalcitonin decreases in this group.

DPPIV is a multifunctional cellular surface protein in most cells. It has been shown that the S protein (spike) of the MERS-CoV virus using DPPIV as a cell surface receptor can cause infection (24, 25). The receptor remains intact among different species such as bats and humans, which in turn could cause extensive host suffering for viruses such as MERS-CoV (26). Therefore, depending on the expression level of this protein at the level of cells such as T lymphocytes, bronchial mucosa, brush border of proximal tubules and such Coronavirus can infect more or fewer cells and cause disease, and this can be a treatment for MERS-CoV infection, in such a way that not only by blocking this receptor the virus can be prevented from the cells of the respiratory system, but also it can be removed from bloodstream (27, 28).

Vankadari et al. have demonstrated the structure of the glycoprotein S (spike) model for the COVID-19, which is involved in adhesion to the host cell. They have also shown glycosylation of spike glycoprotein sites in the COVID-19, which distinguishes it from SARS, and COVID-19 coating structure in hiding from the immune system. There is also a key finding in this study: the second S1 of COVID-19 protein S potentially interacts with human CD26 (DPPIV) and the virus enters the cells through this(29-30). Therefore, according to this study, if DPPIV protein is increased, more viruses will enter the cells and the disease will be more severe. Our study also showed an increase in DPPIV expression in patients with more severe symptoms than in patients with milder symptoms. The marker can also be used as an effective treatment, and antibodies can be designed to block the CD26 marker and prevent COVID-19 from entering cells to prevent disease.

Conclusion

In the present study, using Ct values obtained from Real-time PCR reactions and calculations, it was shown that Procalcitonin and DPPIV proteins are markers that increase in patients with COVID-19 infection. This increase is significantly higher in patients with more severe clinical symptoms than those

with milder symptoms. Obviously, due to the novelty of this disease, other studies are needed to complete the information to obtain ideal results for the diagnosis and treatment of this disease.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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