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SHORT COMMUNICATION

Retinoic acid-inducible gene 1 (RIG-1) and IFN-β promoter stimulator-1 (IPS-1) significantly down-regulated in the severe coronavirus disease 2019 (COVID-19)

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Abstract

Introduction Retinoic acid-inducible gene 1 (RIG-1) and melanoma differentiation-associated protein 5 (MDA5) are the well-known cytoplasmic sensors that recognize microbial DNA or RNA and active down-stream molecules, including IFN-β promoter stimulator-1 (IPS-1) and receptor interacting protein 1 (RIP1). The roles played by the networked molecules on the infection with SARS-CoV-2 needs more investigations.

Material and method In this project MDA5, RIG-1, IPS-1 and RIP1 mRNA levels were evaluated in 45 hospitalized patients suffering from coronavirus disease of 2019 (COVID-19) and 45 healthy subjects using Real Time-qPCR technique.

Result The results showed significant decreased RIG-1 and IPS-1 in the SARS-CoV-2 infected patients when compared to healthy cases. MDA5 and RIP1 did not change when compared two groups. Male patients had similar expression of MDA5, RIG-1, IPS-1 and RIP1 when compared to female patients.

Conclusion Based on the results, it seems that RIG-1 and its signaling molecule, IPS-1, play key roles in the peripheral blood immune cells against SARS-CoV-2 and, their down-regulation may be induced by the virus to escape from immune responses.

Keywords Innate immunity · SARS-CoV-2 · MDA5 · RIG-1 · RIP1 · IPS-1

Introduction

Innate immune responses play significant roles against SARS-CoV-2 [1]. The virus can be recognized by innate immune cells, and then inflammation can occur, which is the primary cause of hospitalization [2]. The pattern recognition receptors (PRRs) via recognition of damage-associated molecular patterns (DAMPs) that are produced by own human cells and pathogen-associated molecular patterns (PAMPs) can induce the related inflammation [3–5]. Retinoic acid-inducible gene 1 (RIG-1) and melanoma differentiation-associated protein 5 (MDA5) are the most well-known PRRs, which recognize cytoplasmic viral dsRNA [6–8], like the SARS-CoV-2 genome. However, the roles played by the internal sensors against SARS-CoV-2 and the roles of the molecules in the induction of inflammation in hospitalized SARS-CoV-2 infected patients are yet to be clarified completely. It has been reported that following interactions of MDA5 and RIG-1 with their ligands, their card caspase domains can be activated and lead to phosphorylation of IFN-β promoter stimulator-1 (IPS-1) and receptor-interacting protein 1 (RIP1), as their adapter molecules [9, 10]. The signaling pathways are responsible for the expression of some cytokines, such as interferons, via activation of interferon regulatory factor 3 (IRF3) and IRF7 [11]. Additionally, increased inflammation in hospitalized SARS-CoV-2 infected patients is the main pathological condition [12–14]. The roles played by MDA5 and RIG-1 and their signaling molecules, IPS-1 and RIP1, in the induction of inflammation in the SARS-CoV-2 infected patients needs more investigation. Therefore, this project aimed to

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Table 1 Relative expressions of MDA5, RIG-1, IPS-1, and RIP1 in the male and female SARS-CoV-2 infected patients

Gender	MDA5	RIG-1	IPS-1	RIP1
Male (Mean Rank)	11.42	9.60	11.92	8.50
Female (Mean Rank)	14.46	14.57	16.73	12.50
P value	0.301	0.089	0.123	0.131

The analysis using Mann-Whitney test showed that the relative expressions of MDA5, RIG-1, IPS-1, and RIP1 did not significantly different in the male when compared to female patients.

evaluate the expression levels of MDA5, RIG-1, IPS-1, and RIP1 in Iranian hospitalized SARS-CoV-2 infected patients.

Materials and methods

Subjects

In this cross-sectional study, 45 healthy controls and 45 hospitalized SARS-CoV-2 infected patients at the age of 30–60 years old were enrolled and referred to Afzalipour Hospital, Kerman, Iran. An Informed consent form was filled out by the participants before sampling. Expression levels of MDA5, RIG-1, IPS-1, and RIP1 were explored in the healthy controls and SARS-CoV-2 infected patients and also between male and female SARS-CoV-2 infected patients. Healthy controls of similar age and sex had no symptoms of the coronavirus disease of 2019 (COVID-19), and a negative PCR test was selected. The patients were selected according to their symptoms of COVID-19 and positive PCR test for SARS-CoV-2. The patients' existence of SARS-CoV-2 RNA was explored using a Real-Time PCR test. To analyze the mRNA levels of MDA5, RIG-1, IPS-1, and RIP1. Blood samples were collected from the SARS-CoV-2 infected patients and healthy controls in 5.5 ml anti-coagulant pre-treated tubes to extract genomic RNA.

Real-time PCR for detection of SARS-CoV-2

According to the manufacturer's guidance, a viral-RNA extraction kit and a Real-Time PCR kit (Karmania Pars Gene, Kerman, Iran) were used to purify SARS-CoV-2 RNA and SARS-CoV-2 N gene amplification, respectively. RNase P is the internal control of the kit.

Genomic RNA extraction, cDNA synthesis, and real-time PCR

Total mRNA was extracted from blood using a commercial kit from Karmania Pars Gene (Kerman, Iran). Spectrophotometer UV at 260-280 nm was used to analyze total RNA quantity, and agarose gel electrophoresis was used to evaluate the RNA quality. A cDNA synthesis kit (Karmania Pars Gene, Kerman, Iran) was used to convert mRNA to cDNA. The primers for MDA5, RIG-1, IPS-1, RIP1, and beta-actin were designed using Primer 3 online software for real-time PCR (Table 1). The real-time PCR and data analysis protocols were described in our previous investigations [15–17].

Data analysis and statistical methods

Due to the using Kolmogorov-Smirnov test, it was demonstrated that the raw data of MDA5, RIG-1, IPS-1, and RIP1 has not normal distribution. Thus, a non-parametric test, Mann-Whitney U test, under SPSS software version 22, was used to analyze the differences between SARS-CoV-2 infected patients and healthy controls. The P value level less than 0.05 was considered to be significant. In this project, the mRNA quantifications of MDA5, RIG-1, IPS-1, and RIP1 were performed using $2^{-\Delta\Delta ct}$ formula [18].

Results and discussion

MDA5, RIG-1, IPS-1 and RIP1 mRNA levels

The Mann Whitney's test showed that the Mean Rank of both RIG-1 ($P=0.049$) and IPS-1 ($P=0.010$) mRNA levels were significantly decreased in the patients when compared to healthy controls. Accordingly, the Mean Rank of RIG-1 mRNA levels were 15.33 (0.0043 (0.0011–0.0314)) in the patients and 22.77 (0.0282 (0.0066–0.9801)) in the healthy controls (Fig. 1). The Mean Rank of IPS-1 mRNA levels were 16.71 (0.0281 (0.0137–0.3114)) in the patients and 27.30 (0.4322 (0.1607–1.6770)) in the healthy controls (Fig. 1).

The Mean Rank of MDA5 in the patients and controls were 17.96 (0.4322 (0.1607–1.6770)) and 18.10 (0.4322 (0.1607–1.6770)), respectively ($P=0.971$). The Results also demonstrated that there were no significant differences between patients (15.20 (0.4322 (0.1607–1.6770))) and controls (16.10 (0.0593 (0.0313–0.4540))) regarding mRNA levels of RIP-1 ($P=0.792$). Figure 1 illustrates the expressions of MDA5, RIG-1, IPS-1, and RIP1 in the patients and controls.

Data are presented as Mean Rank (Median (25th -75th)).

Expression of MDA5, RIG-1, IPS-1, and RIP1 in male and female patients

Analysis of the male and female in the SARS-CoV-2 infected patients revealed that male was not different regarding mRNA levels of MDA5 ($P=0.301$), RIG-1 ($P=0.089$), IPS-1 ($P=0.123$), and RIP1 ($P=0.131$) when compared to healthy controls (Table 1).

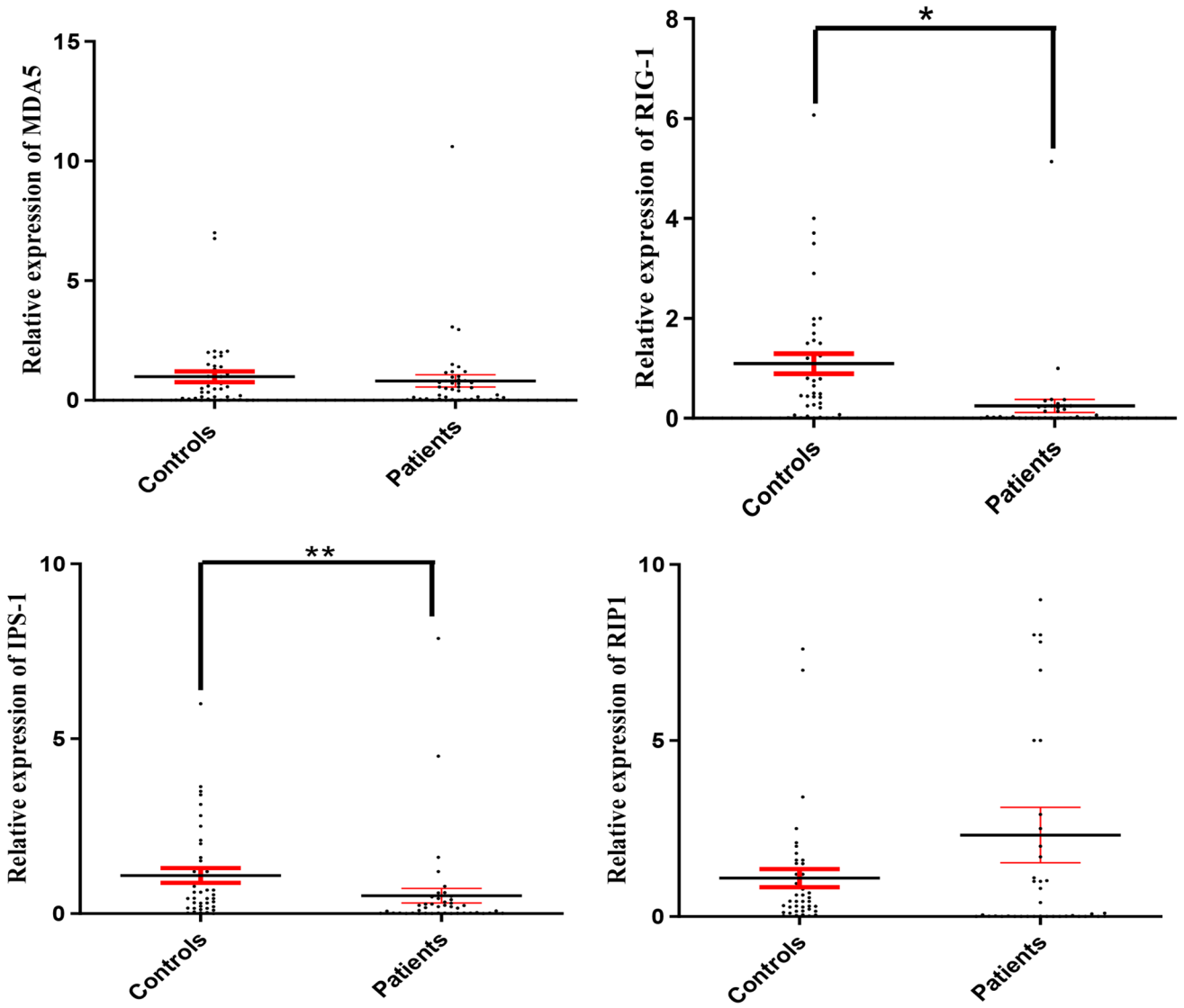

Fig. 1 Relative expression ofMDA5, RIG-1, IPS-1, and RIP1 in the SARS-CoV-2 infected patients in comparison to healthy controls. The statistical analysis showed that relative expression of RIG-1 (*) and IPS-1 (**) significantly decreased in the patients when compared to healthy controls

The immune system is important for defense against viral infections. Unfortunately, SARS-CoV-2 infection leads to suppression of type I and III responses and affects the pro-inflammatory response, thereby accelerating viral replication and damaging host tissues and organs [19]. The results showed that IPS-1 and RIG-1mRNA levels significantly decreased in the SARS-CoV-2 infected patients. Previous investigations revealed that IPS-1 is a crucial molecule that plays a key role against RNA viruses [19, 20]. The molecule activates type I interferons or pro-inflammatory cytokines through activation of IRFs and nuclear factor kappaB (NF-κB), respectively [19]. Hospitalized SARS-CoV-2 infected patients suffer from an unprotected immune response against SARS-CoV-2; IPS-1 may be a key mechanism for viral replication that can make a hypothesis. Previous investigations proved the significant roles played by IPS-1 against SARS-CoV-2 [21]. However, the downregulation of RIG-1 in the patients indicates that SARS-CoV-2-induced inflammation is independent of RIG-1 and IPS-1 and that molecules are suppressed in patients and cause SARS-CoV-2-replication and escape from immune responses. Yang et al. reported that RIG-1 is an important molecule against SARS-CoV-2, and decreased expression of the molecule results in replication of the virus [22].

Several investigations have also reported the significant roles played by the receptor against SARS-CoV-2 [23]. Since interaction between RIG-1 and SARS-CoV-2-RNA is an important stage in inducing expression of anti-viral interferons, hence it appears that down-regulation of RIG-1 and its signaling molecule, IPS-1, may be associated with

defected innate immune responses against SARS-CoV-2. Therefore, based on the fact that the patients were hospitalized and suffered from acute inflammation, we can hypothesize that RIG-1 and IPS-1 may not be involved in the induction of acute inflammation. For example, the roles played by other PRRs, including toll-like receptors in the induction of inflammation, have been demonstrated by several investigations [24].

The results showed that mRNA levels of MDA5 and RIP1 did not change between patients and controls. Thus, it seems that the molecules neither defected during immune responses against SARS-CoV-2 nor participated in the induction of acute inflammation. However, previous studies revealed that in parallel with RIG-1, MDA5 also plays a key role against SARS-CoV-2. However, the sensors apparently recognize SARS-CoV-2 in cell type-specific manners [25]. Based on the fact that our samples were the peripheral blood immune cells, it may be concluded that the sensors may be important in the infected cells but not for immune cells. The results demonstrated that gender could not change expression levels of MDA5, RIG-1, IPS-1, and RIP1 and cannot be considered an important factor. Several investigations proved the results and revealed that gender could not affect immune-related molecules in the peripheral blood immune cells against viral infections [24, 25]. In conclusion, in the Iranian hospitalized SARS-CoV-2 infected patients, RIG-1 and IPS-1 are down-regulated. Hence, it may be associated with decreased immune responses to SARS-CoV-2 and eradication of the virus.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The ethical Committee of the Islamic Azad University, has certified the study protocol (Code: IR.IAU.Kerman.REC.1400.028).

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