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Down regulation of IFIH1 Gene Expression in Early COVID-19 Infection

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Abstract: COVID-19 have not been completely elucidated, non-adaptive inflammatory responses appear to play a substantial part in the disease's progression. The aim of the study Down regulation of IFIH1 Gene Expression in Early COVID-19 Infection. One hundred coronaviruses infectious disease (COVID-19) patients and 58 healthy individuals were enrolled to assess the gene expression of Interferon Induced With Helicase C Domain 1 (IFIH1) by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), with some emphasis on clinical and biological markers of disease like chemokine (CCL-2), and tumor protein (P^{53}). The finding showed that CCl2 and P53 levels have a significant decrease in patients (185 ng/L and 24.5 ng/ml) from control (229 ng/L and 30 ng/ml) (p = 0.001). The ratio of relative *IFIH1* gene expression in COVID-19 patients *versus* healthy control was 0.95. Although the $2^{-\Delta\Delta Ct}$ of IFIH1 gene expression showed no significant variation between the age group (≤45 and > 45 years): sex, and severity in patients and controls, also non-statistical increasing $2^{-\Delta\Delta Ct}$ means of IFIH1 with disease progression like critical (17.5) than mild-moderate (15.8) in patients. The findings showed the CCL2 and P53 levels were non-significantly correlated with the IFIH1 expression (p > 0.05): although there is a negative correlation (r_s= - 0.104) between CCL-2 and IFIH1 values making that prognostic indicator for reducing the development of infection. When IFIH1 expression increased, the P53 level non-significant increased (p = 0.099). It was concluded that IFIH1 expression are an impact factor correlated with P53 levels reducing the COVID-19 risk.

Keywords: IFIH1, P^{53}, CCL-2, gene expression, c-reactive protein.

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Introduction

Patients who have been diagnosed with severe COVID-19 typically require the support of mechanical ventilation in addition to other forms of acute treatment in order to continue to breathe. The method in which the immune systems of these patients react to the infection will influence the outcome for many of these individuals. It is possible that the patient will experience extra damage as a consequence of this if the inflammatory response that is triggered by the immune system is too severe. Although the precise mechanisms that lead to the development of severe kinds of COVID-19 have not been completely elucidated, non-adaptive inflammatory responses appear to play a substantial part in the disease's progression. Only steroid treatment and medication that inhibits the IL-6 pathway have been demonstrated to be effective in lowering

the death rate in this population (1). Both of these treatments focus on taming overactive immune responses in an effort to forestall organ loss and save the patient. One example of a gene that plays a vital role in the inflammatory response is the IFIH1 gene. This gene is responsible for encoding a protein that works to aid the body in detecting viruses. There are many distinct variations of this gene, and each one of them generates a protein that is only very slightly different from the others. It is quite likely that this mutation has an effect on the manner in which the immune system responds to the virus that is responsible for COVID-19. The MDA5 helicase is a cytoplasmatic viral receptor, and it is encoded by the human gene IFIH1, which is located on the opposite strand of chromosome 2. After associating itself with a strand of viral RNA, MDA5 initiates a dialogue with a mitochondrial adaptor known as MAVS, which is an abbreviation for mitochondrial antiviral signalling protein. This dialogue takes place after MDA5 has associated itself with the viral RNA. This discussion kicks off the transcription of genes that code for type-1 interferon, which eventually results in the activation of the systemic inflammatory response. The rs1990760 polymorphism is present in humans, and it is responsible for encoding a variant of the IFIH1 gene (NM_022168: c.2836G > A (p.Ala946Thr)). This variant of the IFIH1 gene has been associated to varied levels of vulnerability to autoimmune disorders and viral infections (2). IFIH1 participates in a feedback loop that eventually governs both the viral clearance and the inflammatory responses of the host. The control of IFN-dependent pathways by IFIH1 is the mechanism that makes this possible. As a consequence of this, the goal of the study was concentrated on analysing whether or not the levels of IFIH1 gene expression are associated with the severity and progression of COVID-19, as well as its linkage with a number of other clinical and biological markers of infection.

Material and Methods

Study participants

The ethics committee at the Iraqi Ministry of Health and Environment approved the study. and all participants provided written consent. This study included 100 nasal swabs and blood specimens and 58 healthy control. Patients were referred to the patient at Al-Shifa Medical Center at Baghdad Teaching Hospital/ Medical City. during the period from February 28 to April 30, 2022. For the patients suffering from upper and/or lower respiratory tract symptoms during ten days of symptoms onset the nasal swabs were using viral transport media (VTM) for maintaining viral viability during transportation to a laboratory for diagnosis. all of The specimens (swab and serum after separation) were stored at -70 ºC until the time of the ELISA test, Patients and control were stratified by age group, gender (male and female): and Severity (Mild, moderate, and Severe). In addition, The Healthy Control sample included age- and gender-matched volunteers who were blood donors and health service personnel. They had no neurological or autoimmune disorders.

Blood sample collection

Five milliliters of blood were collected from each participant in an EDTA and gel tube. The tubes were left to stand for 30 minutes at room temperature were spin and then centrifuged (the gel tube) for 15 min at 4°C to separate the serum. The serum

was divided into aliquots and kept frozen at -20°C using in the Immunoassay of CCL2 and P53. While the blood in the EDTA tube was added Triazole was used for RNA extraction.

qRT PCR for detection of IFIH1 Gene Expression

The RNA sample were extracted from the blood samples using kits then The synthesis of complementary DNA (cDNA) was carried out by following the instructions contained inside Easy Script (Transgene, China). The RT-PCR tubes that contained RNA (3-5): 1 l Anchored Oligo (dT) Primer (0.5 g/l): and Random Primer (0.1 g/l) were then placed in a thermocycler and heated to 65 degrees Celsius for five minutes before being cooled to 4 degrees Celsius for ten minutes. Following the incubation step, it was added to 10 of EX reaction mix, followed by of gDNA remover, of Easy Script®RT/RI enzyme mix, and 3 of RNase-free water to bring the total volume up to 20 . Incubation in a thermocycler at 25 degrees Celsius for ten minutes, then at 42 degrees Celsius for thirty minutes, and finally at 85 degrees Celsius for five seconds. The cDNA was amplified using particular primers and a kit called TransStart® Green qPCRSuper mix kit, all of which were manufactured by Transgene in China. The genes IFIH1 (Forward-5-ATGGAAAAAAAAGCTGCAAAAG A-3) and Beta-actin (Housekeeping genes) (Forward-5-CTCCATCCTGGCCTCGCTGT-3 and Reverse-5-GCTGTCACCTTCACCGTTCC-3) (3). The reaction that included 10 microliters of master mix SybrGreen, 1 microliter of each primer, 3 microliters of cDNA, and 5 microliters of nuclease-free water. The initial denaturation of the amplification process consisted of the following steps: a temperature of 94 degrees Celsius for 30 minutes, followed by 40 cycles of 94 degrees Celsius for 5 seconds, 58 degrees Celsius for 15 seconds, and 72 degrees Celsius for 20 seconds. Finally, the dissociation contained the following: temperature between 55 and 95 degrees Celsius, and a period of one minute for each cycle.

Statistical analysis

The continuous variable was presented with a mean, median, and standard deviation or interquartile range, and the Mann-Whitney U-test was utilised to analyse the data and determine whether or not there were statistically significant differences between the groups. The Fisher exact test and the Pearson Chi-square test were used to analyse the data and determine whether or not there was a significant difference between the groups based on the categorical variables that were presented as numbers and percentages. In this particular investigation, patients were separated into low-production and high-production groups according to a median of biomarkers tested (defined as below and above the mean, respectively): and the control group served as the reference category. Logistic regression analysis was used to construct odds ratio (OR) and 95% confidence intervals (CI). In order to investigate the degree of similarity between the biomarkers found in COVID-19 and those in the healthy control group, a Spearman rank-order correlation was carried out. It was determined that the data was statistically significant if the probability value (p) was less than 0.05. For the purpose of conducting these analyses, the statistical programme IBM SPSS Statistics 25.0 (Armonk, New York:

IBM Corp.) and GraphPad Prism version 8.0.0 (San Diego, California, USA) were utilised.

Results and discussion

Levels of CCL-2 and P53

The finding showed that CCl2 and P53 levels have highly significant differences in patients (185 ng/L and 24.5 ng/ml) from than control (229 ng/L and 30 ng/ml) (p = 0.001): meaning that the infection was down-regulating to induce inflammation with the highest viral load lower than their levels in healthy individuals. More cases of mild-moderate were observed with low levels of CCL2 and p53, while these were reported in severe and critical with high levels. Significant variation was shown in severe and critical with CCL-2 (OR=3.45 and 3.23; p = 0.008 and 0.019, respectively): but no significant differences between stratum with p53. Thus, CCL-2 is an effecter factor for disease progression. Moreover, the results obtained in the current study revealed that there was a non-significant difference in the amount of CCL2 between patients and the control groups, the median CCL2 levels for patients aged ≤45 and >45 are 181 ng/mL (interquartile range IQR: 157.1 – 261.6) and 185ng/mL (IQR: 157 – 236.7): respectively. For healthy controls, the median levels are 278.5 ng/mL (IQR: 210.8 – 350.5) for those ≤45 and 224.8 ng/mL (IQR: 196.9 – 309.4) for those >45. In the case of gender, results revealed a correlation between the male and female levels of CCL2 183ng/mL (IQR: 155.6-238) and 228.8 ng/mL (IQR: 185 – 312.4): respectively. This indicates that the levels of CCL2 in female patients were higher than in males, but there are no significant differences in CCL2 levels between males and females. Furthermore, severity, there are three categories mild-moderate, severe, and critical. The median CCL2 levels for patients with mild-moderate cases is 173 ng/mL (IQR: 157.4 – 236.9): while for severe and critical patients, the median levels are 177 ng/mL (IQR: 157.1 – 197) and 196.9 ng/mL (IQR: 160.1 – 273.5): respectively, and there are no significant differences in CCL2 levels between the severity groups. Regarding, the p53 levels in patients aged 45 and younger were 22.8 ng/mL (interquartile range (IQR) 12.2 – 27.1) while in patients aged over 45, they were slightly higher at 24.6 ng/mL (IQR 9.8 – 27.4); however, the p-value was not significant (p =0.8). Among males, the median level of p53 rise to 25.7 ng/mL (IQR 17 – 28.9) compared to females who had a median level of 22.3 ng/mL (IQR 16.4-26.7); but, this increase was not significant (p =0.23). In terms of disease severity, the median levels of p53 were lower in mild-moderate and severe, meanwhile revealing not significant (p=0.248) rise in critical cases 22.3 (IQR7.2 – 27.1): 22.8 (IQR 17.6 – 26.8): and 26.7 (IQR 19.3 – 28.9) ng/mL, respectively.

Detection of IFIH1 gene expression

The folding expression ($2^{-\Delta\Delta Ct}$) of *IFIH1* gene was decreased in total COVID-19 patients compared to control by16.2 (14.4-18.3) and 17.1(15.1-18.9): respectively, but these decreasing in folds values no significantly (p = 0. 112): this could be induced the caused down regulation for IFIH1 gene expression to give more time for viral multiplication and inflammation at the infection site (Figure 1). The ratio of relative IFIH1 gene expression was 0.95 in COVID-19 patients versus healthy control.

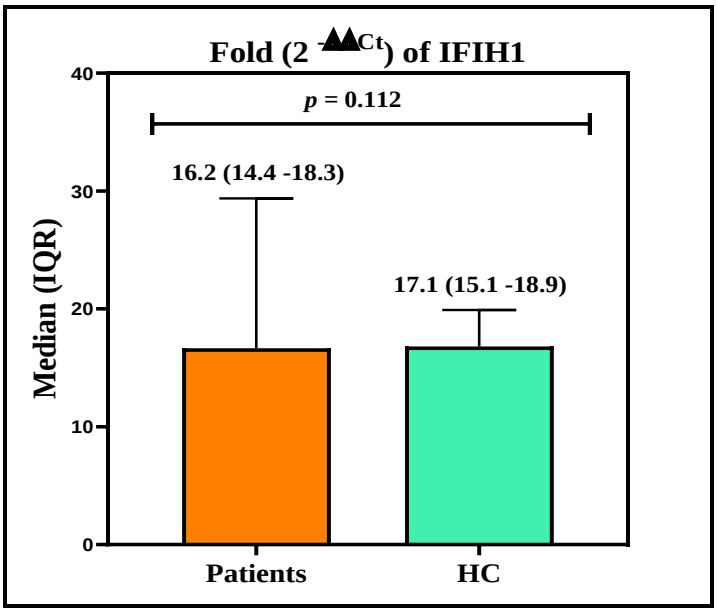

Figure (1): Fold rate of IFIH1 in COVID-19 patient and healthy control.

The $2^{-\Delta\Delta Ct}$ means of gene expression showed no significant variation between the age group variation (≤45 and > 45 years): sex, and severity in patients and controls, although the results showed increasing $2^{-\Delta\Delta Ct}$ means of IFIH1 in male (16.5) than female (15.8) patients, also the IFIH1 expression increased with disease progression like critical (17.5) than mild-moderate (15.8) in patients (Table 1).

Table (1): Median levels of IFIH1fold stratified according to COVID-19 patients and healthy control

Character		IFIH1median (IQR)of Fold ($2^{-\Delta\Delta Ct}$)	
		Patient(no.100)	Control(no.58)
Age group	≤ 45	15.8 (13.9 – 18.1)	17 (15.4 – 19)
	> 45	16.4 (14.9 – 18.7)	17.1 (14.4 – 19.1)
	p-value	$p = 0.374$	$p = 0.906$
Sex	Male	16.5 (15 – 18.7)	17 (14.6 – 20.2)
	Female	15.8 (14.1 – 17.7)	17.4 (15.3 – 18.5)
	p-value	$p = 0.251$	$p = 0.835$
Severity	Mild-moderate	15.8 (13.3 – 17.6)	NA
	Severe	16.5 (15.2 – 17.8)	
	critical	17.5 (14 – 18.7)	
	p-value	$p = 0.462$	

IQR: Interquartile range; NA: Not applicable; *p*: Kruskal-Wallis test and Mann-Whitney *U* test probability.

Multinomial logistic regression analysis

Multinomial logistic regression model where the dependent variable contains three or more potential values, according to Table 2 shows that the cases were stratified by unadjusted, age group and gender. No significant variation was observed in each stratum with the exception of the unadjusted. The IFIH1 is a susceptible factor against the development of COVID-19 (OR =1.02; $p = 0.003$).

Table (2): Logistic regression analysis of IFIH1 fold in COVID-19 patient *versus* HC.

Logistic regression analysis model	OR	95% CI	*p*-value
I (unadjusted)	1.02	0.89 - 1.4	**0.003**
II (age-adjusted)	0.91	0.82 - 1.01	0.083
III (age and gender adjusted)	1.2	0.27 – 4.8	0.851

†: The reference category is > Median; OR: Odds ratio; CI: Confidence interval; *p*: Probability (significant p-value is indicated in bold).

Correlation between *IFIH1* gene expression along with CCL2, and P53 levels

Figure (2) explains Spearman's rank correlation coefficient (rs) between *IFIH1* expression in patients. The findings showed the CCL2 and P^{53} levels were non-significantly correlated with the *IFIH1* expression ($p > 0.05$): although there is a negative correlation (rs= - 0.104) between CCL-2 and IFIH1 values making that prognostic indicator for reducing the development of infection. When *IFIH1* expression increased, the P^{53} level non-significant increased ($p = 0.099$). The results observed that *IFIH1* expression is an impact factor correlated with P^{53} levels reducing the risk of infection.

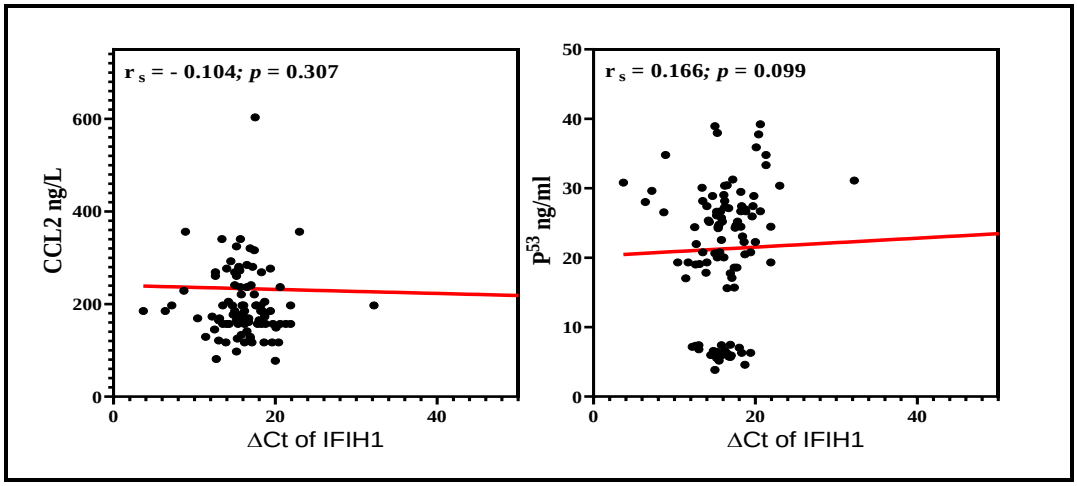

Figure (2): Scatter plot Spearman rank-order correlation coefficient (rs) for analysis between CCL2 levels, and P53 levels with IFIH1 gene expression in COVID-19 patients.

During the course of our study, we detected a decrease in the total quantity of IFIH1 gene expression that was present in COVID-19 patients. It is probable that this took place because MDA5 is the principal sensor of SARS-CoV-2 in lung epithelial cells. If this is the case, then this would explain why this took place. This would control the early reaction of interferon, which in turn would limit the replication of the virus. The most important thing that we found out as a result of our inquiry was this. It is possible that polymorphisms that promote lower expression of IFIH1 and/or MDA5 function could decrease the IFN antiviral response, which would subsequently elevate the risk for viral replication as well as the severity of the disease (4,5). It is possible that polymorphisms that promote lower expression of IFIH1 and/or MDA5 function could reduce the IFN antiviral response. After inhibiting ATPase, a more favourable response to IFN was observed, and it was discovered that the existence of a mutation was the cause of the change in ATPase activity. The

IFIH1 polymorphism has been linked to changes in gene expression as well as baseline protein levels (6): in addition to the structural modifications that it is responsible for producing. Even though it does not impact the quantities of protein, this is still the case. In addition, these findings lend credibility to the notion that pangolins do not possess the gene for IFIH1, and as a result, their reactions to Z-RNA and long double-stranded RNA are far less robust than the reactions of other species. Because of this, it is highly likely that the pangolin's resistance to RNA viruses has reduced over the course of their evolutionary history. The multiplication of RNA viruses requires cytoplasmic Z-RNA and lengthy double-stranded RNA. This is due to the fact that RNA viruses can only multiply if they have cytoplasmic Z-RNA and lengthy double-stranded RNA. We put up the concept that a potent antiviral defence was detrimental, and that the absence of IFIH1 offered an evolutionary benefit by raising the host's tolerance to illnesses caused by particular RNA viruses, such as coronaviruses. This idea was based on the observation that a powerful antiviral defence is associated with an increased risk of infection. In order to accomplish this goal, it was hypothesised that the deletion of IFIH1 increased the host's tolerance to infections induced by certain RNA viruses. In specifically, this benefit was accomplished as a direct result of the removal of IFIH1.

In addition, it was shown that a child who possessed a different homozygous missense IFIH1 mutation (7) had a connection between IFIH1 deficiency and life-threatening infections with HRV and other respiratory viruses. These illnesses were caused by respiratory viruses. A study that wasn't connected to either party showed that there is a connection between the two. This observation provides additional backing for the theory that a deficiency in IFIH1 plays a causative role in an extraordinarily high susceptibility to respiratory viruses in general. Another study indicated that the expression of IFIH1 was higher in both patient groups; however, the effects were not very significant, and the differences between the groups are not likely to account for the variations in cytokine responses. This finding was made by a different researcher using a different methodology. Using Luminex, we analysed an additional 42 cytokines that were present in the whole blood stimulations in order to determine whether or not these immunological alterations were primarily owing to IFN-I responses. In addition, the researchers found that factors such as age or severity did not play a significant effect in the conclusions of the study. In spite of this, because previous research had revealed that age and gender were significant risk factors, we recruited severely ill and critically ill patients who were the same age (8). In addition to this, the connection of IFIH1 with P53 is a significant factor that contributes to the reduction in the likelihood of becoming infected. This is because P53 is capable of having both favourable and unfavourable effects on a wide variety of viral infections (9, 10). Because double-stranded RNA are produced during RNA virus infections, and because these double-stranded RNA trigger antiviral responses mediated by type I interferon (IFN-I) signalling, which is a process in which p53 appears to reduce the replication of some viruses, certain viruses have developed mechanisms to counteract the effects of p53 in infected cells (11, 12). Maintain tight control on the expression of the IFN that is responsible for viral

replication. Coronaviruses may be able to evade the immune system in their hosts by stimulating low-level IFN responses. It is probable that the insufficient IFN response is the result of the decreased amount of p53 that is hydrolyzed by the papain-like proteases (PLPs) that are produced by the coronavirus. PLPs are a specific form of cysteine protease that lowers innate immune function. They are able to accomplish this by enhancing the connection that exists between murine double minute 2 (MDM2) proteins and p53, which ultimately results in the ubiquitination of p53 (13). It is believed that MDM2 is the principal negative regulator of p53. Additionally, MDM2 is engaged in a variety of distinct intracellular signalling pathways. These pathways include the inhibition of the growth of tumours, the reaction of the nucleus of the cell to stress, and the control of interferon type I. (14, 15) Studies have found that cells that have been infected with influenza are connected with accumulation of MDM2 as a result of the fact that influenza infection triggers cells to create viral non-structural protein 1, which is linked to nucleolar stress (16,17). Concerning COVID-19, it was hypothesised that SARS-CoV-2 would also interfere with the control of MDM2/p53. This was due to the fact that other coronaviruses, such as SARS-CoV and MERS-CoV, induce low quantities of Type I interferon to be produced in the body (18,19). In spite of the fact that MDM2 was found to be down-regulated in COVID-19 lung tissue, which indicates that there is a dysregulation of the balance between MDM2 and p53, our data contradict these assumptions because a variety of Type I interferons were found to be up-regulated in COVID-19. This shows that there is a dysregulation of the balance between MDM2 and p53.

Conclusion

The results observed that *IFIH1* expression is an impact factor correlated with P^{53} levels reducing the risk of infection.

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