Evaluation and gene expression of CD4 in COVID_19 patients

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Abstract

Background: Respiratory failure, heart failure, and sepsis/multiorgan failure are some of the factors that contribute to COVID-19 death. In particular, CD4+ T cells perform a crucial antiviral role by balancing the fight against infections with the risk of developing autoimmunity or extreme inflammation. CD4+ T cells have a variety of helper and effector capabilities. The ability of CD4+ T cells to differentiate into a variety of helper and effector cell types allows them to direct B cells, assist CD8+ T cells, attract innate cells, directly engage in virus defense, and aid in tissue repair. Virus-specific CD4+ T cells frequently develop into T follicular helper cells and Th1 cells (Tfh). Objective: Study relationship between CD4 gene expression and disease severity in SARS-CoV-2 patients . Materials and methods: At the Al Hussain Teaching Hospital in Samawah, Southern Iraq, 130 blood samples were taken from patients. the COVID-19 infections of the hospitalized patients. In addition to the interim WHO recommendations, all COVID-19 patients included in this study had their diagnoses made in accordance with the Iraqi National Guidelines for the diagnosis and treatment of COVID-19. qRT-PCR experiments were carried out using Trans Script!® Green one-Step qRT-PCR Super Mix, which targeted gene expression CD4by forward and reverse primers in accordance with the manufacturer's instructions. Total RNA was isolated from whole blood using EasyPure® Blood RNA Kit Cat.No.ER401. Results: Our results showed that expression levels of CD4 was (0.09) comparing to genes Fold of control samples, the mean age of gene expression for CD4 to the age group (20-29 years) are 29.086 ,followed by the age group (30-49 years) 30.24, (50-60 years) are 33.45 and the age group > 70 the mean are 34.31,and proportion of gene expression for CD4 was higher in males than the females (31.55 vs. 32.21). Conclusion: Gene expression of CD4 is down-regulated in each of the severe ,moderate and recovery phases with a significant difference from the control group and the amount of gene expression obtained through this study depends on the proteins(gene fold), which in turn depends on mRNA which the more the amount of protein increases and the latter decreases in COVID patients because they have chronic diseases.

Keywords: SARS COV-2, Gene expression, CD4+ T cells, COVID-19 patients.

Introduction

The SARS-CoV-2 coronavirus, which causes Coronavirus Disease 2019 (COVID-19), was originally discovered in Wuhan, Hubei Province, China, and swiftly spread to other nearby cities and nations (Zhu et al., 2020). Coronaviruses (CoVs) are single-stranded , positive-strand RNA viruses that infect the respiratory tracts of domestic and companion animals, as well as humans, causing mild to serious respiratory diseases (Su et al., 2016). Fever , cough, myalgia, and fatigue have been identified as common confirm symptoms in cases. These symptoms, however, are not specific to COVID19; they are similar to those of other virus-infected diseases, such as influenza (Huang et al., 2020). Real-time polymerase chain reaction (RT-PCR) on viral nucleic acids, computed tomography (CT) imaging, and certain hematological parameters are now the gold standards for making a definitive diagnosis of the infection (Jin et al., 2020). By striking a delicate balance between the fight against infections and the danger of developing autoimmunity or excessive inflammation, T cells, CD4+ T cells, and CD8+ T cells in particular play a crucial antiviral function (Cecere TE et al., 2012). Protective immunity may be mediated by T cells that target any viral protein. T cell responses against SARS coronavirus type 2 spike protein (Spike) are of special relevance, however, since practically all prospective COVID-19 vaccines solely include Spike (Krammer, 2020). Spike-specific CD4+ Tcells are required for induction of anti-Spike antibodies, and CD4+ Tcells specific for other virion structural proteins may also contribute (Crotty, 2015; Elsayed et al., 2018). Differentiated CD4+ T cells may train B cells, aid CD8+ T cells, attract innate cells, have direct antiviral activity, and aid in tissue healing, among other functions. CD4+ T cells that have encountered a virus often develop into either Th1 cells or T follicular helper cells (Tfh). IFNg and similar cytokines are produced by Th1 cells, which contribute to their antiviral activity. Tfh cells are specialist suppliers of B cell assistance that are required for the maturation of memory B cells, long-term humoral immunity, and the majority of neutralizing antibody responses (Crotty, 2019). CD4+ T cells aid CD8+ T cell responses as well as antibody responses. IL-21 may be crucial for CD4+ T cell aid to CD8+ T cells, even if the particular CD4+ T cells delivering the CD8+ help are yet unknown (Buchholz and Busch, 2019; Zander et al., 2019),

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2. Material and Method

A cross sectional study was conducted on the following study groups during the period from December 2021 to May 2022. One hundred and thirty sample of Blood were collected from patients at Al_ Hussain Teaching hospital, Samawah-Southern Iraq .The sample studied included (N=130) cases including 20 individuals from a control sample. The mean age was (28.22) years ranging between 20 to 85 years.

Five ml of blood from patients was taken using a disposable syringe, and the blood was then added to an EDTA tube for Real-time PCR gene expression to (CD4). Using the EasyPure® Blood RNA Kit, total RNA was isolated from 50 l to 1.5 ml of fresh or anticoagulated blood using a quick and easy column-based process. DNA is digested with DNase I after blood has been lysed. Silica membrane is attached with RNA. High grade RNA is eluted after washing. For qRt-PCR, Northern blot, and RT-PCR, purified RNA is appropriate. qRT-PCR reactions were performed using Trans Script !® Green one-Step qRT-PCR Super Mix,which targeted gene expression CD4by forward and reverse primers according to the manufacturer's protocol.For one-step qRT-PCR with high sensitivity, high synthesis efficiency, and high amplification efficiency, Trans Script!® Green one-Step qRT-PCR Super Mix is created. In order to complete the process of going from reverse transcription to aPCR in a single tube, this kit first synthesizes first-strand cDNA using RNA as templates and reverse gene-specific primers. It then performs qPCR using the synthesized cDNA as templates and both forward and reverse gene-specific primers. Utilizing a Nanodrop spectrophotometer from THERMO-USA, DNA from blood samples was examined. This device evaluated DNA content (ng/l) and DNA purity by measuring absorbance at (260/280nm). PCR thermo cycler conditions for rRNA gene were done by using conventinal PCR thermocycler system as in the following table (2-1) .

Table (2-1). qPCR Conditions

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	3 min	1
Denaturation	95 °C	10 sec	40
Annealing\ Extension Detection(scan)	55 °C	30 sec	
Dissociation stage Melting	60-95°C	0.5 min	1

To determine the copy number of PCR fragments, sequentially attenuated gene-specific cDNA generated from CD4 amplifications were used, explain as in the table (2-2) All prefixes were designed with Primer3 software. These primers were designed by this study by using NCBI Gen Bank data base and Primer 3 online. These primers where provided by (Alpha DNA , Kanada) as following table:

Table(2-2): Oligonucleotide Primers Sequence Used for RT. qPCR Amplification of Specific gene

Gene name	Primer's sequences	PCR products	GenBank Code
CD4	F 5' TGGAGCATGGGACTGTTCTT'3 R 5' GATGTGAGTCTGTGGTCCCA'3	185bp	NC_000012.12 REGION: 6789528..6820799

3. Results

The Result for estimating level were obtained from 107 patients. SARS COV2 patient were classified into three main phases , severe , moderate and recovery phases and control group . Genes of interest were quantified using qRT-PCR SYBR Green. Our results showed that expression levels of CD4 was (0.09) comparing to genes Fold of control samples and the *t*-value for CD4+ T cells is -5.29 and The *p*-value is < 0.0001 is significant at $p < 0.5$ compared with control samples.

In this study, CD4+ gene expression was examined in the adaptive immunity of COVID-19 patients. Real-time transcription-polymerase chain reaction (RT.PCR) analysis has been used to determine gene expression in adaptive immune cells and their role in improving or deteriorating a person's health. The results showed a high level of gene expression in SARS-CoV2 patients in general from the CD4+ T cells, compared to the gene expression of the healthy patients control.

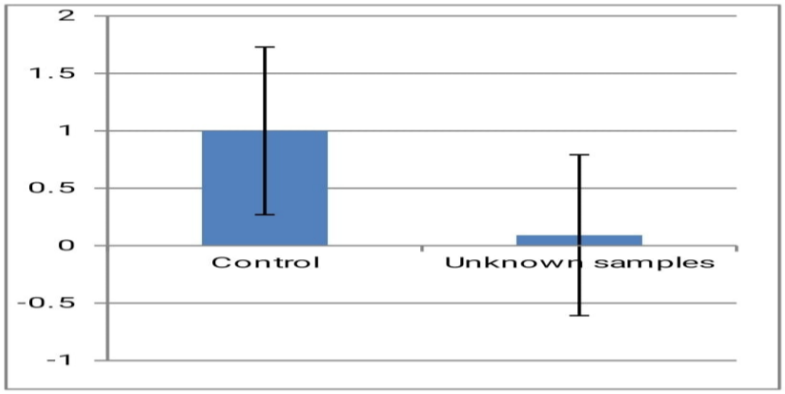

Figure (1) That Expression levels of CD4(Fold change) was (0.09) comparing to genes Fold of control samples

Table (3-1) dividing COVID_19 patients with chronic disease into three phases severe, moderate and recovery according CT value for CD4				
No. Samples	Chronic disease	Severe phase	Moderate phase	Recovery phase
1	HTN	27.08	28.98	26.9
2	_____	27.93	28.96	28.4
3	DM,HTN	28.18	27.30	28.8
4	DM,HTN	27.87	28.08	26.97
6	DM,HTN	28.47	28.00	27.29
6	HTN	29.78	29.22	26.82
7	HTN	27.42	28.04	28.24
8	HTN	28.68	26.82	26.56
9	DM,HTN	28.24	28.18	31.05
10	DM,HTN	28.14	27.40	28.43
11	DM	31.54	28.96	33.55
12	_____	31.05	35.24	31.44
13	DM,HTN	30.96	33.87	33.12
14	DM,HTN	32.20	33.55	30.96
15	DM,HTN	32.46	33.58	33.78
16	HTN	36.37	31.39	34.97
17	HTN	31.43	30.30	31.04
18	DM,HTN	33.11	34.13	34.97
19	DM,HTN	34.11	35.27	35.19
20	HTN	34.97	32.92	32.32
21	DM, HTN	34.44	32.69	32.12
22	DM, HTN	33.52	34.80	35.19
23	HTN	33.69	38.83	35.88
24	_____	35.19	38.57	33.21
25	DM	33.52	34.80	31.05
26	DM, HTN	35.75	31.05	31.44
27	DM, HTN	35.24	31.44	33.87
28	DM, HTN	35.96	33.87	34.8
29	DM, HTN	32.20	33.55	34.8
Mean	----------	31.7069	31.7169	31.48828

Discussion

Immunity study

In the current study*(table3.1)* We discovered that individuals with COVID-19, particularly those with severe COVID-19, had a normal laboratory finding of a reduction in T-lymphocytes in peripheral blood. Infected T-lymphocytes, particularly CD4+ T cells and CD8+ T cells, may be the major target of SARS-CoV-2. (Guan W-J et al.,2020, Chen G et al.,2019).

T-lymphocytes are essential to the development of antiviral immunity. Interferon (IFN) was a key effector cytokine released by the CD4+ T cell subsets that was necessary for viral eradication (Sarawar SR et al.,1994, Topham DJ et al.,1996). A prior research also revealed that a sharp drop in total lymphocytes was a sign that the coronavirus had destroyed cellular immune function by consuming immune cells. However, there is inadequate proof that CD4+ T cells may predict the outcome in COVID-19 patients (Chen N et al.,2019).

These data suggest in table (3-1) that chronic diseases, like HTN and DM, are important comorbidities that may complicate and influence the susceptibility of people to COVID-19. the proportion of cases who had chronic disease exceeded 50%; therefore, the risk potential of HTN and DM in COVID-19 progression can be indicted. In addition, HTN and DM were linked to more severe disease and a worse COVID-19 prognosis(Chatterjee and Cheng, 2020; Lim et al., 2021). Since VID-19 first appeared, DM has been acknowledged as an exceptional risk factor for the severity of VID-19. Additionally, it has been connected to the admission of patients with VD-19 to intensive care units (ICUs), which has been associated with greater rates of morbidity and mortality (Wu and McGoogan, 2020). The reason behind that is noticed, but several factors might be involved. It has been shown that diabetics with poorly managed hyperglycemia have a weakened immune response to viral infections, especially when T-cell performance against viruses is compromised. As a result, the likelihood of virus clearance may be decreased (Nyambuya et al., 2020). In addition , DM patients show elevated levels of plasminogen, which is a protein that can cleave the SARS-CoV-2 S (spike) protein. By such mechanism, virus entry to the target cell is facilitated, and consequently, the virulence and infectivity of SARS- CoV-2 is increased (Ji et al., 2020). Furthermore, DM patients with COVID-19 have shown up-regulated levels of some inflammatory mediators (for instance, IL-6, D-dimer and C-reactive protein (CRP) in comparison with COVID-19 patients who had no DM (Guo et al., 2020).

Conclusion

Gene expression of CD4 is down-regulated in each of the severe ,moderate and recovery phases with a significant difference from the control group and the amount of gene expression obtained through this study depends on the proteins(gene fold), which in turn depends on mRNA which the more the amount of protein increases and the latter decreases in COVID patients because they have chronic diseases.

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