Upregulated CXCL10 Gene Expression in SARS-CoV-2 Infected People

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ABSTRACT

Interferon and interferon-induced genes play a crucial role in early-stage post-infection virus defense. C-X-C10, also known as interferon gamma-induced protein 10 or small-inducible cytokine B10, is encoded by the CXCL10 gene and is essential for T-helper cell recruitment. The purpose of this study was to assess the gene expression of CXCL10 in SARS-CoV-2-positive and -negative individuals using qPCR. The results demonstrated a 35-fold increase in CXCL10 expression in SARS-CoV-2-positive individuals vs to negative samples. In conclusion, the elevated gene expression of CXCL10 in SARS-CoV-2 patients is a signal for the immune system to respond to the invading virus and may be taken into account in the design of future vaccines.

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1. Introduction

Late in December 2019, Severe Acute Respiratory Syndrome (SARS-CoV-2) was discovered as a new virus, emerged in Wuhan, China. SARS-CoV-2 infects both humans and vertebrates, such as cattle, dogs, chicken, pigs, birds and cats. The respiratory, intestinal, and central nervous systems in humans and other mammals are amenable to SARS-CoV-2.The genome sequence analysis showed that COVID-19 belongs to β-coronavirus family similarity in its genome with SARS-like bat coronaviruses suggests that SARS-CoV-2 might originate from bats [1-4]. CXCL10 or what is so called IP10 is an interferon inducible gene; it is an attractive protein to recruit T-helper cells. It belongs to a group of chemokines that are classified according to their structure. They play crucial function in recruiting of white blood cells to sites of inflammation [5]. CXCL10 is selective ligand for CXCR3, which is mainly expressed on T-lymphocytes, macrophages, natural killer cells, dendritic cells and B cells. The CXCL10-CXCR3 axis has essential functions for the immune system. It has been revealed that normally it controls immune cell development, activation, and migration [6].CXCL-10 belongs to a group of genes that code for small peptides that play a role in the immune response's proinflammatory and chemotactic activities [7-8]. On the other hand, CXCL10 with CXCR3 play critical roles in selective cells, recruitment to inflamed regions and in increase of inflammation and also tissue damage. One of the main functions of CXCL10 is to promote the recruitment of CD8+ and Th1-type CD4+ effector T cells to infected or inflamed tissues. Furthermore, CXCL10 stimulates the accumulation of CD4+ and CD8+ T cells in infected tissues. Thus, over activity of the CXCL10–CXCR3 axis is associated with the development of some diseases. The abnormal activity of the axis has been seen in autoimmune disorders including rheumatoid arthritis or systemic lupus erythematosus as well as cancers e.g., skin or brain tumors [9-12]. So, the aim of the study was to assess the gene expression of CXCL10 in SARS-CoV-2 infected or uninfected people by quantitative real time PCR, RT-qPCR.

2. Experimental

2.1 Materials & Method

Total RNA samples were collected from 20 patients suspected of infection with COVID-19 at the Public Health Laboratory in Basrah, Iraq. The age range of the patients was 21-55 years old, whereas that of the non-COVID-19 infected individuals was 20-66 years old. The infected patients

included 3 females and 7 males whereas the non-infected individuals consisted of were 5 females and 5 males.

2.2 Gene Expression by Real-Time PCR

2.2.1 Isolation of Total RNA from Cell Lines

RNA was extracted using G-spin columns according to the manufacturer's instructions AddBio, Korea.

2.2.2 One- step protocol

RNA was converted to cDNA qPCR using Kits: Go Taq® qPCR and RT-qPCR from (Promega: 72050) was used relying on reverse transcriptase enzymes.To prepare Real Time PCR reaction, the kit components were added to the reaction mixture and completed to a final volume of 20 µl according to qPCR Kits: Go Taq® qPCR and RT-qPCR from (Promega) instruction table and the reaction was achieved at cycles of temperatures table. Kit number: 72050.

qPCR Primers

CXCL-10 gene specific primers were: forward: 5′-CGCTGTACCTGCATCAGCAT-3′, reverse: 5′-GCAATGATCTCAACACGTGGAC-3′ [13]. Data were analysed using fold change analysis.

Table 1: Components of Real Time PCR reaction

No.	Materials	Size
1	Go Taq® qPCR Master Mix	170µL
2	Go Script™ RT Mix for 1-Step RT-qPCR	6.8µL
3	Forward Primer for (STAT-1, CXCL-10, IFNG, IFIT-2, IFIT-3, GAPDH)	17µL
4	Reverse Primer for (STAT-1, CXCL-10, IFNG, IFIT-2, IFIT-3, GAPDH)	17µL
5	Template RNA	5µL
6	Nuclease-Free Water	6µL

Table 2: Real Time -PCR program.

No.	Steps	Temperature °C	Time	No. of Cycles
1	Reverse transcription	37	15 min.	1
2	Denaturation 1	94	10 min.	1
3	Denaturation	94	10 sec.	40
4	Annealing (all the primers)	60	30sec	
5	Extension 1	72	30 sec.	
6	Extension 2	72	1 min.	1

3. Results and discussion

CXCL10 relative gene expression in clinical samples

Due to its critical role in the maturation and differentiation of B cells and antibody production, CXCL10 gene expression was also analyzed in SARS-CoV-2 infected or uninfected people

patients (10 each group). Using qPCR, SYBR green and CXCL-10 specific primers CXCL10 gene expression upregulated to 35 fold in SARS-C0V-2 infected compared to the uninfected people (Fig.1). Data were analyzed by ΔΔ CTs and normalized to (GAPDH) house-keeping gene.

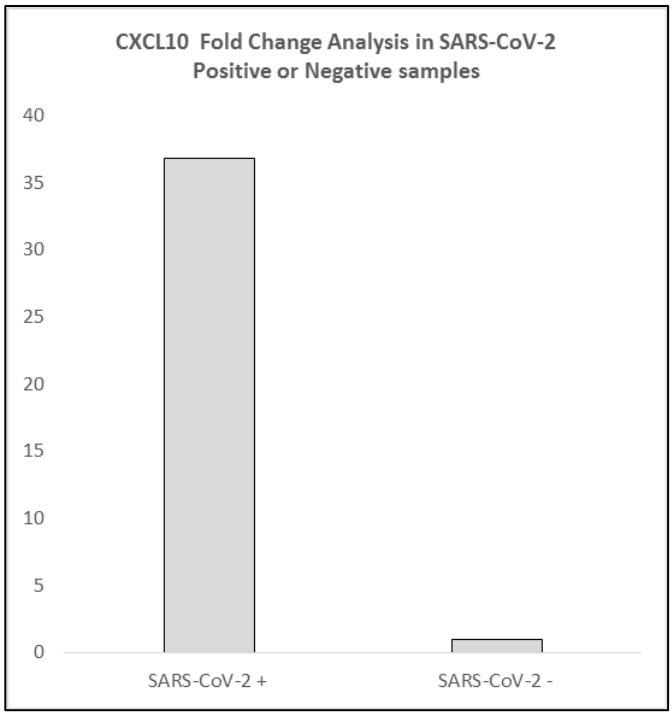

Figure 1: Fold change analysis of CXCL10 in SARS-C0V-2 infected or uninfected people.

Total RNA was reverse transcribed and the synthesized DNA was used as a template for qPCR relative expression assay using SYBR green master mix. Data were analyzed by ΔΔ CTs and normalized to (GAPDH). CXCL - 10 gene expressions was upregulated to 35 times in SARS-CoV-2 infected or uninfected people. CXCL10/IP10 functions as biomarker and very well correlated with the severity of COVID-19. CXCL10 functions in thrombosis and inhibiting the endothelial repair. Severity of COVID-19 is correlated to the level of CXCL10 in the serum [14]. Chemokines play a key role in innate defense following viral infection, according to new researches. In addition, CXCL10 induces natural killer (NK) cell migration post viral entry, and works as chemotactic factor on T cells [15-18].

In 2007, a study published by Law *et al.* showed that SARS coronavirus nsp-1 can stimulate the expression of CXCL10 in NF-κB dependent manner in human cells (A549) [19]. CXCL10 and CXC chemokine ligand 9 (CXCL9) expressions has been reported to contribute to antiviral immune responses, In the absence of T and B cells [20]. Indeed, recent studies have shown that CXCL10 plays a vital role in directing effector T-cell migration toward tumor development, allowing established tumors to be regressed [21]. CXCL10 attracts activated NK cells as an innate immune response to vaccinia virus infection [20].

4. Conclusions

In accordance with earlier results, the increase in the CXCL-10 gene led to an increase in immune cell activity and viral destruction. In conclusion, our data shows that the CXCL10 increase might be utilized or at least evaluated in the construction of SARS-CoV-2 vaccine candidates.

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التعبير الجيني المنتظم ل CXCL10 في الأشخاص المصابين بـ SARS-CoV-2

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المستخلص

تلعب الجينات التي يسببها الإنترفيرون دورًا مهمًا في التقليل من حدة الفيروسات في المرحلة المبكرة بعد الإصابة .يتم تحفيز البروتين المستحث بالانترفيرون جاما أو السيتوكين المحفز الصغير بواسطة جين CXCL10 وهو ذو اهميه حاسمه في تجنيد الخلايا التائية المساعدة.هدفت الدراسة إلى تقييم التعبير الجيني لـ CXCL10 بواسطة qPCR في الأشخاص المصابين أو السليمين لـ SARS-CoV-2 .أظهرت النتائج زيادة كبيرة في تعبير CXCL10 في مرضى SARS-CoV-2 المصابين وصل إلى 35 ضعفًا مقارنة بالعينات السلبية. نستنتج من هذا ان التعبير الجيني المنتظم لـ CXCL10 في مرضى SARS-CoV-2 هو تنبيه لجهاز المناعة في الجسم للاستجابة للفيروس ويمكن اعتماده عند تصميم اللقاحات المستقبلية.