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Impact of COVID-19 on Gene Expression of CTLA-4 and Its Potential Role in the Development of Rheumatoid Arthritis: A Post-Infection Analysis.

Maryam Hassan Sanguor[1], Hatham W Atwan[2], Iman Hussein[3], Zeenah W Atwan[4].

1-Department of Nursing Techniques, Technical Institute, University of Technology, Basrah, Iraq.

2- Department of Inspection, Basrah Health Directorate, Basrah, Iraq.

3- Department of Biology, College of Science, University of Basrah, Basrah, Iraq.

4-Central Laboratory, department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq.

Corresponding Author Email Address: Maryam.sanguor@stu.edu.iq

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Abstract

Since the emergence of SARS-CoV-2, a high number of mortalities around the world has been reported. Post-infection, the immune system is activated abnormally which leads to an excessive release of cytokines and interleukins implicated in eliminating the viruses including CTLA-4. Such responses sometimes cause autoimmune diseases including Rheumatoid Arthritis (RA). The study aimed to analyze gene expression of with CTLA4 in patients diagnosed with RA post Covid-19 infection. Blood samples were collected from 61 RA patients diagnosed post Covid-19 patients RAI, and 40 control (C) experienced at least one Covid-19 infection. RNA was extracted and used to prepare cDNA from each sample to analyze the cellular expression of CTLA-4 using relative gene expression. The study involved 61 patients, 17 of them were vaccinated without prior Covid-19 infection (RAV) as well as 44 were diagnosed with RA following their recovery from Covid-19 (RAI). The results showed a significant increase in CTLA-4 gene expression only in RAI samples. However, a decrease in the RAV sample compared to control was noticed. In summary, COVID-19 seems to have an important role in the development of the RA disease, as during its occurrence immuno-changes disorders can happen, one of obvious influences of which is the significant changes in the gene expression of CTLA-4 gene, which in turn could be involved in the development of the RA.

Keywords: Covid-19, Vaccination, Rheumatoid Arthritis, CTLA-4.

Introduction

COVID-19 which is caused by SARS-CoV-2 is a very common cause of an excessive pro-inflammatory response. The Similarity between cytokine hyperactivation in COVID-19 and in rheumatoid arthritis (RA), a chronic autoimmune disease was reported. RA is characterized by T cell hyperactivation and synovial inflammation (1). Many viruses, including respiratory viruses, are associated with arthritis-like symptoms and joint pain. Studies showed that COVID-19 results in muscle and joint pain in 44% of infected individuals (2).

Furthermore, individuals with inflammatory arthritis may experience a relapse of symptoms due to infections (3). Consequently, the development of RA or its attacks may be triggered/enabled by SARS-CoV-2 infection. Limited data is currently available and it is not yet proven that SARS-CoV-2 infection and the development of RA are directly related (4). When SARS-CoV-2 infection occurs, it could lead to increased release of inflammatory mediators such as C-X-C motif chemokine ligand 10 (CXCL10), interleukin (IL)-17, and tumor necrosis factor-alpha (TNF-α). These mediators are implicated in initiating osteoclast genesis and reducing osteoblast differentiation and proliferation, which ultimately decreases bone density (5). In RA patients, insufficient expression of CTLA-4 in T reg cells due to the methylation of the CTLA-4 promotor's DNA at the NF-AT binding site occurs. Such an event makes Treg cells unable to activate the kynurenine pathway, which triggers the development of RA (6).

While SARS-CoV-2 vaccines are generally safe for healthy individuals, a small percentage of people may experience rare side effects, including autoimmune disorders (7). Vaccines primarily aim to prevent infection, with clinical trials proving their safety and efficacy. However, real-life data have reported adverse effects, possibly due to short study durations and the urgent need to start immunizations. Reports showed that immune-mediated disease attacks or new inflammatory diseases post-vaccination (8). Some reports listed the development of autoimmune disorders after SARS-CoV2 vaccination, including autoimmune glomerulonephritis (9), autoimmune rheumatic diseases (7), and autoimmune hepatitis (10). So, the aim of the study was to study the potential role of SARS-COV-2 infection in the progression of RA by evaluating CTLA-4 gene expression in rheumatoid arthritis patients.

Materials and Methods

Sample Collection: Blood samples (2 ml) were obtained from 61 diagnosed rheumatoid arthritis patients and 40 control subjects (pre-infection without developing RA) at the Biological Pharmaceutical Unit of Al-Basrah Teaching Hospital between September 2023 and December 2023. The patients were diagnosed by the physician (arthrologist) based on clinical features mainly warm tender joints. Laboratory diagnosis was further supported by the clinical part including: Rheumatoid factor (RF), Anti-citrullinated Peptide Antibody (ACCP), and Erythrocyte Sedimentation Rate (ESR).

RNA extraction and cDNA synthesis: Using the Solarbio (Cat No. R1200, China) following the manufacturer's instructions, total RNA was isolated from whole blood. According to the manufacturer's instructions, cDNA was then synthesized from total RNA using the Universal RT-PCR Kit (M-MLV, free Taq polymerase) purchased from Salorbio, Cat No: RP1105, China.

Real Time PCR for Genes: The specific primers of CTLA-4 For.5′-CTACCTGGGCATAGGCAACG-3′ Rev.5′-CCCCGAACTAACTGCTGCAA-3′ (11) and GAPDH For.5′-CTTTTGCAGACCACAGTCCATG-3′ Rev. 5′-TTTTCTAGACGGCAGGTCAGG-3′(12) were used. The kit from SolGent Co., Ltd. (cat. No. 34014, Korea) was employed to prepare the Real Time PCR experiment. SYBER green master mix (10 µl), Forward and reveres primers (100 pmol), nuclease-free water (5 µl), and cDNA template (7 ng/µl) were mixed to prepare the PCR reaction mixture. It was first denatured for 10 minutes at 95 °C, followed by 35 cycles of denaturing for 10 seconds at 95 °C, annealing for 30 seconds at 60 °C, extension for 30 seconds at 72 °C, and a final extension step of 5 minutes at 72 °C.

Statistical Analysis : The t-test (two sample unequal variance) was used to statistically analyze the data at $P \leq 0.05$.

Results

CTLA4 Gene expression analysis

Due to the importance of CTLA4 gene in the immune response and the development of immune diseases, CTLA4 gene expression in both RA positive and negative samples was quantified using qPCR relative assay with SYBR green master mix After subtracting the signal of GAPDH as the housekeeping gene, CTLA4 expression was higher in RAI samples than in the control sample while lower in the RAV samples compared with control (A). After normalizing the control sample to 1 it was shown that the CTLA4 expression was reduced to about 25% in RAV group while the RAI expressed the gene to about 0.7 compared to C group. Figure (B).

Discussion

One of the most important factors that can cause a failure in the T-cell function is the expression of the CTLA-4 by Tregs which transduces inhibitory signals and maintains an immune response homeostasis. CTLA-4 haplotype absence could cause autoimmune diseases, multiorgan invasion by lymphocytes as well as peripheral B cells, and immunoglobulin deficiency (13,14). It was also found that Single-nucleotide polymorphisms in CTLA-4 have been correlated with some autoimmune diseases including, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, and type 2 diabetes (15,16,17). The results found upregulated gene expression in RAI samples compared to the control. The results are well supported by the conclusion of Jury et al., that high levels of CTLA-4 in FOXP3- T cells from patients with systemic lupus erythematosus (SLE) compared to healthy people. The suggested mechanism is that FOXP3- T cells in (SLE) patients are unable to control the activation and proliferation of effector T cells (18).

Furthermore, the literature supports that CTLA-4 gene is significantly highly expressed in SLE patients compared to controls (19). A study was conducted in Egypt on Type 1 diabetes (T1D) patients and compared with controls they found a significant increase in CTLA-4 (+49 A/G), especially in younger people and females (20). A possible mechanism that could be involved in developing RA could be the decrease in Mitogen-activated protein kinase (MAPK) phosphorylation and an increase in Small mothers against decapentaplegic 2/3 (Smad2/3) nuclear localization. Overexpression of the cytoplasmic domain of CTLA-4 (cdCTLA 4) transforms naive T-cell preferred to Foxp3+ T cells under Th17 differentiation circumstances (21).

However, the results disagree with Liu et al who found that CTLA-4 is low in severe aplastic anemia (SAA) patients (22). Lower levels of sCTLA-4 are present in RA patients in the quiescent stage of the disease compared to those in the activation stage. However, this study differs in age categories, severity of the disease, and time of diagnosis (23).

On the other hand, CTLA4 gene expression was low in RAV samples compared with control which open the door for evaluating the exact mechanisms that new fashion vaccination is working through. However, an increasing body of evidences reported adverse effects of the vaccination such as myocarditis, and pericarditis (24).

Conclusion

COVID-19 seems to have an important role in the development of the RA disease, as during its occurrence immuno-change disorders can happen. This could be not very surprising since evidences correlated different viral infections with joint pain and inflammation. The study provided evidence about the mechanism underlying the development of RA by assessing the relative and fold change expression, one of the obvious influences of which is the significant changes in the gene expression of CTLA-4 gene, which in turn could be involved in the development of the RA. The increased expression of CTLA-4 was reduced by vaccination which could explain the importance of vaccination to lessen the adverse effects of SARS-CoV2 infection.

Acknowledgment

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Consent for publication

Each patient signed a form has the following statement: I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without cost. I understand that I will be given a copy of this consent form.

Limitations

It is not easy to generalize the results of this study due to the small sample size. The mechanisms underlying the observed gene expression changes require further investigation. Finally, the study did not explore the potential confounding factors

such as comorbidities or genetic variability among patients.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

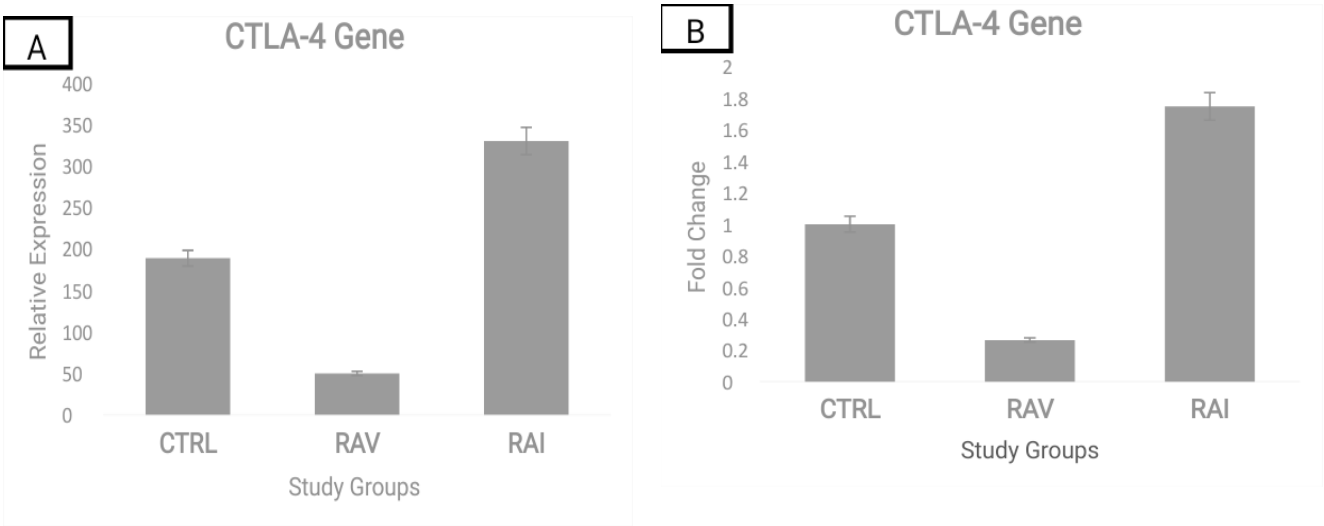

Figure (1): Gene expression of CTLA-4 in RAV and RAI compared to the control group. Gene expression was measured using RT-PCR. The t-test was used to determine the statistical significance, showing * P = 0.045 that the difference is statistically (P > 0.01).

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تأثير فايروس كوفيد-19 على التعبير الجيني للجين CTLA-4 ودوره المحتمل في تطوير التهاب المفاصل الروماتيزم: تحليل مابعد العدوى.

مريم حسن صنكور[1], حذام وحيد عطوان[2], ايمان حسين ربيعة[3], زينة وحيد عطوان[4].

1- قسم تقنيات التمريض, المعهد التقني الطبي, جامعة التقنية الجنوبية, العراق.
2- دائرة صحة البصرة - قسم التفتيش والمتابعة - القطاع الخاص.
3- قسم علوم الحياة، كلية العلوم، جامعة البصرة، العراق.
4- المختبر المركزي , كلية الطب، جامعة البصرة، العراق.

الخلاصة

منذ ظهور فايروس SARS-CoV-2، تم تسجيل عدد كبير من الوفيات حول العالم. بعد الإصابة، ينشط الجهاز المناعي بطريقة غير طبيعية، مما يؤدي إلى إطلاق مفرط للسيتوكينات والإنترلوكينات التي تلعب دورًا في القضاء على الفايروسات، بما في ذلك البروتين 4 المرتبط بالخلايا الليمفاوية التائية السامة. CTLA-4 في بعض الأحيان، تتسبب هذه الاستجابات في حدوث أمراض مناعية ذاتية مثل التهاب المفاصل الروماتيزم. هدفت الدراسة إلى تحليل التعبير الجيني لـ CTLA-4 في المرضى الذين تم تشخيصهم بالروماتيزم بعد الإصابة بفايروس كورونا. تم جمع عينات دم من 61 مريضًا مصابًا بالتهاب المفاصل الروماتيزم بعد الإصابة بفايروس كورونا (RAI)، من ضمنهم 40 عينة (مجموعة السيطرة C) الذين تعرضوا لإصابة واحدة على الأقل بفايروس كورونا. تم استخراج الحامض النووي الريبي (RNA) من العينات واستخدامه لتحضير cDNA من كل عينة لتحليل التعبير الجيني لـ CTLA-4 باستخدام تقنية التعبير الجيني الكمي. شملت الدراسة 61 مريضًا، كان من بينهم 17 مريضًا تلقوا اللقاح دون إصابة سابقة بكوفيد-19 (RAV)، و44 مريضًا تم تشخيصهم بالتهاب المفاصل الروماتويدي بعد تعافيهم من كوفيد-19 (RAI). أظهرت النتائج زيادة معنوية في التعبير الجيني لـ CTLA-4 فقط في عينات RAI، بينما لوحظ انخفاض في مجموعة RAV مقارنةً بالمجموعة السيطرة. في الختام, يلعب كوفيد-19 يلعب دورًا مهمًا في تطور مرض التهاب المفاصل الروماتيزم، حيث يمكن أن تحدث اضطرابات مناعية أثناء الإصابة. ومن أبرز هذه التأثيرات التغيرات الملحوظة في التعبير الجيني لـ CTLA-4، مما قد يكون له دور في تطور المرض .

الكلمات المفتاحية: كوفيد-19, اللقاح, التهاب المفاصل الروماتيزم, البروتين 4 المرتبط بالخلايا الليمفاوية التائية السامة (4-CTLA) .