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DC-SIGN Receptor Level in Rheumatoid Arthritis Patients in Baghdad; Serological study

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Abstract:

Rheumatoid arthritis (RA), is an autoimmune, and inflammatory disease that is closely related to the destruction of cartilage and bone. DC-SIGN are important types of C-type lectin receptors (CLRs), expressed on dendritic cells and macrophages, and have a central role in regulating innate and adaptive immunity, function as pattern recognition receptors, and as cell adhesion molecules. Recent evidence has demonstrated that DC-SIGN is involved in the pathophysiological of chronic inflammation, so DC-SIGN has been linked to several autoimmune and may play an essential indicator in the pathogenesis and progression of RA. Therefore, the purpose of this study is to determine the serum level of DC-SIGN in RA patients, as well as the level of DC-SIGN based on demographic characteristics. Fifty Iraqi RA patients were enrolled in the study, and a control sample of 38 healthy individuals (ascertain by laboratory and clinical tests) were included and matched by gender, age, and ethnicity with the patients. The DC-SIGN concentration was calculated in the patients' serum and compared to control using the ELISA assay and the results revealed significantly increased serum level of DC-SIGN (12.047 ± 1.114 vs. 6.863 ± 0.806 ng/ml) was recorded in RA patients compared to controls. When correlating results, it was shown that the concentration of DC-SIGN in the serum did not record a significant difference between gender and age, as well as the blood groups. To determine the impact of the therapeutic status in RA patients on the DC-SIGN level, it was found that the concentration of DC-SIGN level was higher in untreated patients compared to treated patients. Regarding viral infection, when an investigation was conducted in RA patients infected with SARS-CoV-2, the serum level of DC-SIGN in RA patients with COVID-19 showed no change in concentrations compared to uninfected RA patients.

Keywords: Autoimmunity, CD209, COVID-19, DC-SIGN, Rheumatoid arthritis

Introduction:

Arthritis is a prevalent disease that comes in numerous forms, the most common of which is rheumatoid arthritis (RA), it is an autoimmune disease and chronic inflammation, in which inflammatory and immune cells infiltrate into the synovial membrane, cartilage, and bone tissue resulting in pain, stiffness, and deformity of the joints [1, 2]. C-Type Lectin Receptors (CLRs) are a large and functionally diverse group of transmembrane and soluble proteins that involve many members with diverse functions [3]. CLRs are able to bind damage-associated molecular patterns (DAMPs) as well as pathogen-associated molecular patterns (PAMPs), and function in modulating cell activation [4]. Several members of CLRs are associated with severe autoimmune diseases, and antigens are taken up by different CLRs such as DC-SIGN, mannose receptor, or dectin-1 and loaded for presentation to CD4 + T cells [5]. Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN or CD209) is a type II CLRs that acts as an adhesion molecule situated on dendritic cells (DCs), which recognizes antigens and can promote TLR to induced

activation of NF-κB [6]. DC-SIGN is participating in DCs migration and activation of T lymphocytes, in addition, it can be a goal of some microorganisms or tumor cells, which may result in avoidance from immune surveillance or immune suppression [7]. Serum levels of DC-SIGN in RA are not yet determined in the Iraqi patients, therefore the purpose of our study is assess the serum levels of DC-SIGN in a sample of Iraqi RA patients, which might have a broad influence on host immune responses.

Materials and Methods:

Subjects

The study included fifty Iraqi patients with RA. They were referred to the Rheumatology Consultation Clinic, Baghdad Teaching Hospital in Baghdad for diagnosis and treatment during the period December 2020 to April 2021, informed consent was obtained from the Ministry of Health (the approval number is 30005 on 23/11/2020). The diagnosis was done by the consultant medical staff at the institute, which was based on clinical and laboratory examination and an information sheet was filled for each participating subject. A control sample was also included (38 healthy individuals), which was matched with patients for gender, age, and ethnicity, who were seronegative for RA tests.

Sample Collection

From each participating subject, 6 ml of venous blood was collected. The blood was distributed into two aliquots tubes (the first was a plain tube, while the second was an anticoagulation tube). Serum was collected using a serum separator plain tube, then centrifuged at 2000 rpm for 10 minutes to separate serum, which was distributed into aliquots in tightly closed Eppendorf tubes, and kept frozen at -20ºC until analyzed.

Laboratory Tests

After a clinical examination of the patient by the medical staff at the Rheumatology Consultation Clinic, the serum was screened by RA standard tests (ESR, CRP, RF, and Anti-CCP) and also ABO test and COVID-19 rapid test. Tests were carried out on all the subjects (patients and control) and the tests were carried out at the laboratory Department of Biology, College of Science for Women, University of Baghdad.

Assessment of DC-SIGN Serum Level

Sera of RA patients and controls were assessed for level of DC-SIGN using commercially available kits (Bioassay Technology Laboratory; China), by sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed for quantitative measurement of human DC-SIGN receptors in sera, and the standard curves are as illustrated in Fig 1.

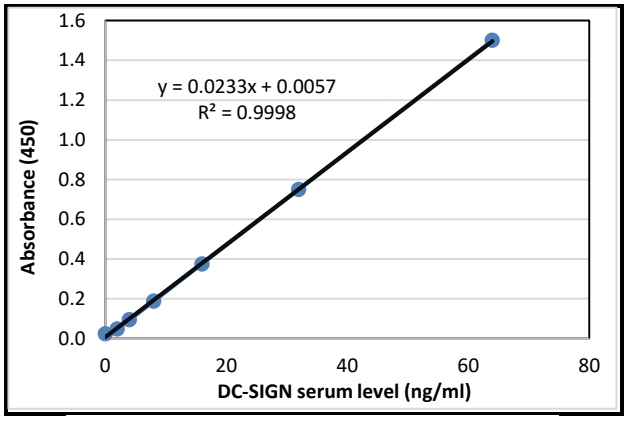

Figure 1. Standard Curve of DC-SIGN.

Statistical Analysis

For statistical analysis after recording the data in excel for the survey and then importing it into the file, it was statistically analyzed. Demographic variables were characterized using frequencies and percentages. The serum level of DC-SIGN was statistically analyzed using the computer program SPSS version 14. The data were given as mean ± standard error (S.E.), and then differences between means were evaluated by ANOVA, and P-value less than 0.05 was considered statistically significant.

Results and Discussion:

The results of the study were presented in two main sections, which included; serum levels of DC-SIGN and the relationship between demographic traits and DC-SIGN serum level. During the period from December 2020 to April 2021, blood samples and information were collected from 50 patients who were confirmed to have RA and attending the Rheumatology Consultation Clinic / Baghdad Teaching Hospital in Baghdad, and the diagnosis was confirmed by laboratory tests that included CRP, ESR, RF, and anti-CCP, in addition to the clinical signs and symptoms, which were evaluated by the consultant medical staff. All laboratory tests for RA, as well as COVID-19 and blood type examination, were performed for all subjects (patients and healthy controls). The presented resulted revealed that serum level means of DC-SIGN (12.047 ± 1.114 vs. 6.863 ± 0.806 ng/ml) was increased in RA patients compared to healthy controls, with a significant difference ($P \leq 0.05$) was observed, Fig 2.

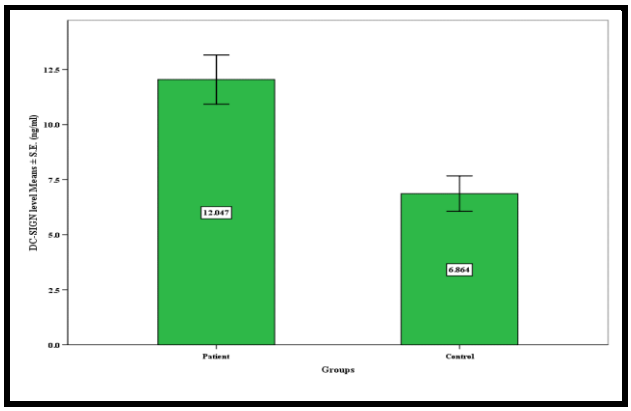

Figure 2. Serum level of DC-SIGN in RA patients compared to healthy controls.

DC-SIGN is a member of the CLRs and is primarily expressed in DCs and macrophages and has an important role in innate immunity. Several studies have shown that DC-SIGN has a significant association with many chronic inflammatory diseases. In contrast to osteoarthritis, the synovial macrophages of RA patients highly expressed CD-SIGN. CD-SIGN functions in the RA synovium regulate and activate T-cells as well as modulate adaptive immune responses to induce osteoclastogenesis, and bone structural damage in RA. Therefore, the increased levels of CD-SIGN indicate its involvement in the pathogenesis of RA [8]. When Chan *et al.,* [9] studied the single nucleotide polymorphisms of CD-SIGN in the Taiwanese population they observe that CD-SIGN has a significant factor for the development of RA because CD-SIGN has higher expression in immune cells and correlates with the severe cartilage damage in patients with RA.

With respect to age in RA patients, the mean age of RA patients was 49.580 ± 1.863 years; they were divided into six age groups to determine the incidence of the disease. The age group 41-50 is the most affected, reaching 32% of the total patients, followed by 30% in the age group 51-60 years. This was followed by the age group above 60 years with 26%, then the age group from 31 to 40 years with 6%, followed by the age group less than 20 years with 4%. The lowest incidence of the disease was observed at 2% in age groups 21-30 years. Therefore, the disease may affect all ages without exception with its peak in the third and fourth decades of life, and this was confirmed by several studies on the spread of the disease by age, as it was found that the age group 40-50 years is the most affected [10 ,11]. The results of DC-SIGN serum levels in RA patients within each age group are not statistically significant, but the DC-SIGN serum level showed an increasing trend in the mean values with aging and peaking in the 41-50 years age group with a mean value of 14.36 ± 2.43 ng/ml, while the level decreased within the age group 31-40 years to 7.58 ± 2.65 ng/ml. There is no study yet on the relationship of these receptors with age, but we can explain the evaluated level of DC-SIGN in age groups above 40 because it contributes to the inflammatory process and disease progression.

The prevalence of RA among gender, a higher prevalence of female was observed in the RA patients compared to the male (84% vs. 16%, respectively). Global studies confirmed that the ratio of females to males with RA is about 75% and 25%, respectively, which is approximately close to the current ratio of our study. This could be explained by the important role of female sex hormones in the induction of self-reactive T and B cells. The reaction of the immune response is also stronger in females than in males, and the overflow of the immune response is explained by the effect of female sex hormones that amplify the immune response against infectious agents and autoimmune diseases in susceptible people [12, 13]. Sex hormones bind to their intracellular receptors, the binding of the steroid to the suitable receptor provokes the translocation of the complex steroid receptor to the nucleus, where it acts as a transcriptional complex. Estrogens receptors are found in cells of the breast, ovary, uterus, and bone, as well as present in cells of the immune system such as T lymphocytes (helper, suppressor, and cytotoxic), and B lymphocytes. These receptors are also found in mature lymphocytes that explain the immune changes that happen in the presence of estrogens [14, 15]. When determining the DC-SIGN level according to gender, there was no significant difference in serum level of DC-SIGN between females and males in RA patients or in controls. While the mean serum DC-SIGN levels were observed slightly higher in the male patient group than in females, Fig 3. Sex hormones regulate Th1/Th2 balance, steroid hormones for example androgens (such as testosterone and androstenedione) stimulate T-helper cells to produce type 1 cytokines, which reduces T-helper 2 activity and stimulates T CD8 cells as well as stimulated monocytes to increase IL-1 and IL-12 production. Whereas estrogens enhance autoimmune diseases with type 2 cytokine [16].

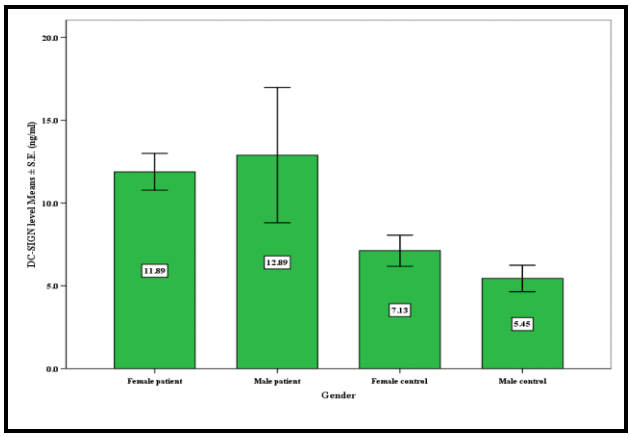

Figure 3. Serum level of DC-SIGN in RA patients and healthy controls distributed by gender.

Regarding the distribution of blood groups in RA patients, in this study, the ABO blood group in RA patients was O^+ (50%), A^+ (16.7%), B^+, O^- (11.1%) and A^-, AB^+ (5.6%), there did not exist B^- and AB^- blood groups among RA patients. The results revealed that the highest percentage was for the blood group O^+, this is due to the fact that blood group O with Rh-positive is the most commonly prevalent among the Iraq population, this agrees with results of [17 ,18]. The study revealed that the serum levels of DC-SIGN did not differ significantly in their effect on patients according to blood groups, but slightly increased levels of DC-SIGN were observed in A^- and O^- blood groups (17.24 ± 2.42 & 16.80 ± 3.61 ng/ml respectively). Previous studies have indicated that Rh-negative and Rh-positive individuals differ in their tolerance to certain health conditions and biological factors, such as infection, and physiological disorders. It has been reported that an individual with Rh-negative has recurrent immune problems such as hypersensitivity, hematological and neurological diseases. In addition, individuals who are Rh-negative have an increased risk of developing certain autoimmunity diseases, for example, RA. The general pattern indicates that Rh-negative individuals may have autoimmune problems, and maybe more resistant to viral infections, and less resistant to bacterial infections [19].

In this study, we also examined the effect of treatment on the serum level of DC-SIGN. DC-SIGN serum levels were significantly altered in patients first diagnosed with RA and still untreated (14.65 ± 3.67 ng/ml), compared to immunotherapy-treated patients (9.63 ± 0.91 ng/ml). Early treatment, especially at the beginning of the symptoms of RA, is possible to preserve the articular cartilage from cracking and thus reduce disease symptoms. RA patients are traditionally treated with immunosuppressive drugs such as corticosteroids, DMARDs such as methotrexate, and nonsteroidal anti-inflammatory drugs (NSAIDs) [20]. According to the results of this study, DC-SIGN levels were higher in untreated patients and decreased after treatment. This suggests that the role of RA therapy in reducing disease progression by controlling inflammation and reducing joint damage.

The results also highlighted DC-SIGN levels as important markers with a potential relevance when dealing with the prognostic of COVID-19 infection. A few RA patients with COVID-19 were observed, and these cases did not suffer an exacerbation of symptoms but were resembling the common cold symptoms. Therefore, when correlating positive COVID-19 cases and DC-SIGN serum levels in RA patients, the results of RA patients with COVID-19 showed no change or significant difference in the concentrations of DC-SIGN serum level (11.34 ± 2.09 ng/ml). Similar results were obtained for other types of CLRs, Mohammed et al., reported that there are no associated differences in mannose receptor serum level between RA patients infected with COVID-19 and uninfected RA patients [21].

Some microbial infections via molecular mimicry or bystander activation affect specific and nonspecific immunity through the production of cytokines, thus promoting autoimmune diseases. Coronaviruses are pathogens that can cause the common cold, a new type of coronavirus has been discovered known as Severe Acute Respiratory Syndrome Virus 2 (SARS-CoV-2) and the disease is named coronavirus disease 2019 (COVID-19) [21, 22]. COVID-19 uses the surface protein Spike to attach and enter the host cell Human angiotensin-converting enzyme 2 (ACE2) is known to interact with the viral spike protein and functions as an entry receptor for SARS-CoV-2 [23].

The affinity surface glycoproteins of viruses such as HIV, influenza, hepatitis, SARS-CoV-2 and others, are recognized by various CLRs like DC-SIGN with the prognostic application when detected at diagnosis. DC-SIGN mediated attachment and entry, therefore DC-SIGN acts as an ACE2-independent entry receptor for SARS-CoV-2 and is involved in the attachment of SARS-CoV-2 to innate immune cells. Several studies have confirmed that DC-SIGN expression levels are increased in severe COVID-19 patients beside elevated amounts of inflammatory cytokines and chemokines. Previously, this strategy was exploited for the treatment of viral infections. In view of SARS-CoV-2, DC-SIGN-targeted antivirals could represent a promising option for host-directed therapy for COVID-19 [24 -26]. No significant

associations in DC-SIGN with COVID-19 were observed in this study, and this could be explained by the fact that patients with rheumatoid arthritis are taking treatments such as disease-modifying anti-rheumatic drugs (DMARDs) that reduce the activation of inflammatory processes. Recent studies on the use of DMARDs are not only for treat RA, but also for combating COVID-19 by blocking virus entry, preventing excessive immune activation, and reducing cytokine storm. Thus, DMARDs that target a wide range of individual pro-inflammatory pathways may find broader implications not only for the management of RA but also in the control of COVID-19 [27].

Conclusions:

Based on the results of the current study, we found that the serum levels of DC-SIGN were increased in the serum of RA patients, and it is possible to reach CD-SIGN which is an important indicator for the development of RA, and the DC-SIGN concentration level is influenced according to levels and type of treatment in RA patients, while there is no change in DC-SIGN level in RA patients during SARS-CoV-2 infection. In addition, DC-SIGN serum level is impacted by changes in age, gender, and blood type in RA patients.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Authors sign on ethical consideration's approval
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.
- The research got approval from the Medical Research Ethics Committee of the Ministry of Health, Baghdad Teaching Hospital/ Baghdad/ Iraq, the approval number is 30005 on 23/11/2020.

Authors' contributions statement:

H.A. Carried out the experiment and verified the analytical methods, and also wrote the manuscript with support from Z.A. and A.A. Z.A. Conceived the original idea, encouraged H.A. to investigate, and supervised the findings of this work and the whole project. A.A. Contributed to the preparation and diagnosis of the sample as well as contributed to the interpretation of the results. All authors discussed the results and contributed to the final manuscript.

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مستوى مستقبلات DC-SIGN في مرضى التهاب المفاصل الروماتويدي في بغداد، دراسة مصلية

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[2] وزارة الصحة، مديرية صحة بغداد، مستشفى ابو غريب، بغداد، العراق

الخلاصة:

التهاب المفاصل الروماتويدي هو أحد أمراض المناعة الذاتية الالتهابية الجهازية التي ترتبط ارتباطًا وثيقًا بتدمير الغضاريف والعظام. يعد DC-SIGN من مستقبلات C-type lectin receptors (CLRs) المهمة و يعبر على سطح الخلايا المتغصنة و البلعمية و له دور اساسي في تنظيم المناعة الفطرية والمكتسبة ، كما يعمل كمستقبل للتعرف على الأنماط و ايضا كجزيئة التصاق. أثبتت الدراسات تورط الـ DC-SIGN في الفسيولوجية المرضية للالتهابات المزمنة، لذلك تم ربط علاقة الـ DC-SIGN بالعديد من امراض المناعة الذاتية، لذا قد تلعب عاملاً ومؤشرًا أساسيًا في التسبب وتطورمرض التهاب المفاصل الروماتويدي. لذا الغرض من هذه الدراسة هو تحديد المستوى المصلي للـ DC-SIGN في مرضى التهاب المفاصل الروماتويدي، وكذلك دراسة المستوى المصلي للـ DC-SIGN اعتماداً على الخصائص الديموغرافية للمرضى. شملت الدراسة خمسون مريضا عراقيا يعاني من التهاب المفاصل الروماتويدي، وتضمنت الدراسة ايضا عينة سيطرة لاشخاص اصحاء (تم التأكد عن طريق الفحوصات السريرية والمختبرية)، وتمت مطابقتهم حسب الجنس والعمر والعرق مع المرضى. تم حساب تركيز DC-SIGN في مصل المرضى ومقارنته بالسيطرة باستخدام اختبار ELISA وأظهرت النتائج زيادة معنوية في مستوى مصل DC-SIGN (12.047 ± 1.114 مقابل 6.863 ± 0.806 نانوغرام / مل) في مرضى التهاب المفاصل الروماتويدي مقارنة بالسيطرة. وعند ربط النتائج، تبين أن تركيز DC-SIGN في المصل لم يسجل فرقًا معنوياً بين الجنس والعمر وكذلك الحال لمجاميع الدم. و لتحديد تأثير الحالة العلاجية لمرضى التهاب المفاصل الروماتويدي على مستوى DC-SIGN ، وجد أن تركيز مستوى DC-SIGN كان أعلى في المرضى الغير المعالجين مقارنة بالمرضى المعالجين. و عندما أجريت دراسة على مرضى التهاب المفاصل الروماتويدي المصابين بـ SARS-CoV-2 ، لم يظهر مستوى مصل DC-SIGN في مرضى التهاب المفاصل الروماتويدي و المصابين بـ COVID-19 أي تغيير في تركيز DC-SIGN مقارنة بمرضى التهاب المفاصل الروماتويدي الغير مصابين.

الكلمات المفتاحية: المناعة الذاتية، CD209، كوفيد-19، DC-SIGN ، التهاب المفاصل الروماتويدي