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Association of interleukin 23 with systemic lupus patients: with C3, C4 and anti-ds DNA levels

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Abstract:

An important part of the pathogenetic pathways underlying systemic lupus erythematous (SLE) is the dysregulation of both humoral and cellular immunity. Interleukin 23 (IL-23), one of the proinflammatory cytokines, is involved in the promotion of this imbalance. Using multiarray ELISA, the IL-23 serum levels of 100 Iraqi SLE patients and 96 healthy controls were evaluated in this study.

Serum levels of IL-23 were considerably higher in SLE patients than in healthy individuals. It has a high correlation with complement consumption (C3, C4), rheumatoid arthritis, and seizures.

Measurements of C3, C4, and anti-dsDNA were made. Non-parametric testing and multivariate analysis utilizing partial least squares (pLs) models were used to analyze the data.

Serum IL-23 levels are greater in SLE patients. In addition, SLE patients have elevated levels of C3, C4, and anti-dsDNA. Research aims to identify a biomarker for a potential therapeutic target in SLE and discover a function in the pathophysiology of the disease. In addition, laboratory results (ESR (mm/h), hemolytic, leucopenia, leucocytic count, lymphopenia, thrombocytopenia, platelets count, hypocomplementemia 3, hypocomplementemia 4, diabetes mellitus, and hypertension) are recorded. Age and disease duration are also evaluated. The demographic criteria of both patients and controls are presented in Table 1. Where P-value is (0.329, 0.097) in age and sex respectively is non-significant. Study group included 100 (82%) females and 18 (18%) males with mean age 31.75±7.04 years. The duration of the disease ranged from 1–17 years. The age at disease onset ranged from (17–61) years with the mean 26.96±6.92. The clinical and laboratory data within the patients group are presented in Table 2. Anti-ds-DNA antibodies were present in 49% of patients. The comorbidities were hypertension in 37% patients and diabetes mellitus in 8% of patients (10%). While IL-23 in patients is 21.78±29.19 (0.1–117.4) compared with control 3.67±4.08 (0.1–16.3), where P value= 0.001*

العلاقة بين مستويات الابين ابيضاض 23 و المضاد للدنا مع الذئبة الحمراء

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مستخلص

جزء مهم من المسالك الممرضة لداء الذئبة الحمراء بعدم التنظيم لكلا المناعة الهورمونية والخلوية. انترلوكين 23 هو واحد من السايتوكاين الالتهابية هي متظمنة في تحفيز هذه الموازنة. استخدام الاليزا مستويات الانترلوكين 23 في مصل 100 مريض عراقي بالذئبة الحمراء و 96 سليمين كانوا قيموا في هذه الدراسة. مستويات الانترلوكين في مصل مرضى هو كانت أعلى بكثير في مرضى الذئبة الحمراء مقارنة بمستويات المصل في الافراد الاصحاء. انه يمتلك علاقة قوية مع استهلاك المتمات سي3، سي4، روماتزم، الصرع. قياسات سي3، سي4 و مضاد الدنا كذلك عملت. كذلك اختبارات متعددة التكافئ تستخدم موديلات المربعة كانت تستخدم لتحليل البيانات. مستويات الانترلوكين 23 في المصل كانت اعلى في مرضى الذئبة الحمراء. بالاضافة الى، مرضى الذئبة الحمراء تمتلك مستويات عالية ل سي3، سي4 و مضاد الدنا. الهدف من البحث ان يؤشر المؤشرات البايولوجية لعلاج مرضى الذئبة الحمراء و اكتشاف الوظيفة في الفسلجة الامراضية للمرض. بالاضافة الى اختبارات المختبر (نسبة ترسيب كريات الدم، انحلال الدم، قلة الكريات البيض، عدد الكريات البيض، قلة اللمفاويات، نقص الصفيحات، عدد الصفائح الدموية، نقص تكملة الدم 3 ، نقص تكملة الدم 4 ، داء السكري، وارتفاع ضغط الدم) كان يسجل. العمر و فترة المرض كذلك قيمت النتائج المظهرة في الجدول 1 لكلا المرضى و الاصحاء، حيث الاحتمالية هي(0.329، 0.097) في العمر و الجنس بالتعاقب هو غير مهم. الدراسة تتضمن 100 امراة (82٪) و 18 رجل (18٪) مع معدل عمر 7.04±31.75 سنة، فترة المرض تمتد من 1 الى 17 سنة، العمر عند حالة المرض ممتد من 17 الى 61 سنة مع معدل6.92±26.96. النتائج المختبرية و السريرية في مجاميع المرضى تعرض في جدول 2. الاجسام المضادة للدنا كانت موجودة في 49٪ من المرضى، كذلك ارتفاع ضغط الدم في 37٪ من المرضى و السكر في 8٪ من المرضى بينما الابين ابيضاض 23 هي29.19±21.78 (0.1–117.4) مقارنة مع السيطرة 4.08±3.67 (0.1–16.3) حيث قيمة الاحتمالية هي 0.001

Introduction

The most prevalent kind of lupus, known as systemic lupus erythematous (SLE), is an autoimmune disease in which the immune system targets its own tissues, resulting in extensive tissue destruction and inflammation in the organs impacted (1).

Systemic lupus erythematous is clearly associated with T-lymphocytes through signal transduction, abnormal cytokine release, activation and accumulation of B lymphocytes and dendritic cells (2).The inflammatory cytokine IL-23 has been demonstrated to be essential for the upkeep and proliferation of T helper type 17 cells (Th17 cells). Th17 cells can release their effector cytokines, which mediate protection against external fungus and bacteria and assist in barrier immunity, when IL-23 stabilises RORγt. These cytokines include IL-17, IL-21, IL-22, and GM-CSF (3). Type 3 innate lymphoid cells, which actively express Th17 cytokines in response to IL-23 stimulation, have been shown to exhibit effects akin to those that IL-23 has on Th17 cells [4]. The IL-23 receptor is also expressed by natural killer cells. In response, they secrete more interferon-γ and increase the cytotoxicity of cells that are dependent on antibodies. Additionally, CD4 memory T cells proliferate when exposed to IL-23 (but not naïve T cells) [5]. In addition to inducing inflammation, IL-23 stimulates angiogenesis [6]. However, we discovered that IL-23 might have a more significant impact on the phenotype of SLE T cells; it stimulates the production of B cell autoantibodies by extrafollicular T helper cells (eTfh), while inhibiting the production of IL-2 and the development of regulatory T cells (Tregs) (7). Evidenced by the ability of murine lupus lymphocytes treated in vitro with IL-23 to cause moderate nephritis in mice that are otherwise healthy but lymphopenic (8). The objective of the current study was to examine the relationship between IL-23 levels, C3, C4, and ds, and SLE symptoms by comparing the serum levels of IL-23 in Iraqi SLE patients with those in healthy controls (9).

Patients and Methods

Study Design and Participants Recruitment

The present case-control research was carried out on 100 adult Iraqi patients with SLE diagnosed according in Hospital Medenat AL-tib in 2022 clas-

sification criteria for Systemic Lupus Erythematosus (9). Patients were regular attendees following up at the outpatient clinic and consulting in Medenat AL- tib. Patients were excluded if they had any malignancy, other autoimmune disease, mixed connective tissue disease, overlap syndrome, or were pregnant at the time of study. One hundred adults (age- and sex-matched) were included as controls. All individuals signed their informed consent before enrollment.

Data Collection

For all patients, age, sex, detailed history taking and clinical and rheumatological examination were performed. Five ml of venous blood was taken from each participant; 3 ml put in a plain tube which was subjected to centrifugation where the serum was obtained and preserved at -20°C until use. Patients and controls sera were screened for the serum level of il-23 by ELISA technique (Sandwich Elisa) (China); 2 ml of which was kept in EDTA tube (used in immunological tests and kept at -20° C).

Demographic data, disease duration, medication history, presence of concomitant chronic diseases, such as hypertension (HTN) and diabetes mellitus (DM). Laboratory investigations included erythrocyte sedimentation rate (ESR), platelets count, hypocomplementemia 3, hypocomplementemia 4, complete blood count (CBC), as well as immunological profiles (including immunofluorescence antinuclear antibodies (ANA) and anti-double stranded antibodies (ds-DNA), complement 3 (C3), and complement 4 (C4).

Table 1: Demographic Characteristics of Patients and Controls

Characteristics	Patients (n=100)	Control (n=96)	P-value
Age (years), mean±SD	31.75±7.04	33.45±7.66	0.329
Male, No. (%)	18 (18%)	8 (8.3%)	0.097
Female, No. (%)	82 (82%)	88 (91.7%)	

Notes: Data expressed as mean±standard deviation or frequency (%), as appropriate. Significance of age was made by unpaired Students “t” test and comparison of gender was made by Pearson Chi-Square test.

Table 2 : Clinical and Laboratory Characteristics within the Patients' Group (N=100) Domain Values

Age at onset, mean±SD (range, years)	26.96±6.92 (17–61)
Disease duration, mean±SD (range, years)	4.9±4.96 (1–19)
Laboratory data	
ESR (mm/h), mean±SD (range)	40.28±24.08 (9–87)
Hemolytic No. (%)	3 (3%)
Leucopenia < 3×113/mm3, No. (%)	14 (14%)
Leucocytic count, mean±SD (range)	8.67±6.44 (3–37) (×103/mm3)
Lymphopenia < 1×105/mm3, No. (%)	15 (16.3%)
Thrombocytopenia <100×103/mm3, No. (%)	3 (3%)
Platelets count, mean±SD (range)	274.70±102.91 (55–507) (×103/mm3)
Hypocomplementemia 3 (lowC3), No. (%)	57 (57%)
Hypocomplementemia 4 (lowC4), No. (%)	39 (39%)
DNA, No. (%)	49 (49%)
Comorbidity	
DM, No. (%)	8 (8%)
Hypertension, No. (%)	37 (37%)

Note: Data expressed as mean±standard deviation (minimum–maximum) or frequency (%), as appropriate.

Abbreviations: ESR, erythrocyte sedimentation rate; RBCs, red blood cells; C3, complement 3; C4, complement 4.

Table 3 : Concentration level of IL-23 in patients Compared to control

Parameter	Patients (n=100) Mean±SD (Range)	Control (n=96) Mean±SD (Range)	P-value
IL-23 (pg/mL)	21.78±29.19 (0.1–117.4)	3.67±4.08 (0.1–16.3)	0.001*

Notes: Data expressed as mean±standard deviation (minimum–maximum) or frequency (%), as appropriate. * P-value is significant, i.e. <0.05.

IL-23 serum levels were significantly elevated among patients versus controls (P<0.001) (Table 3). IL-23 showed a significant positive correlation with disease duration (r=0.289,

P=0.007).

Determination of Human Interleukin 23 (IL-23) in Serum of SLE Patients

IL-23 level was estimated in sera of patients and controls using ELISA kit provided by SunLong Biotech Co., LTD (Catalogue Number: SL0989Hu), Gangzhou, China. This ELISA kit utilizes Sandwich-ELISA as the method.

This kit includes a Microelisa stripplate that has been pre-coated with an IL-23 specific antibody. Standards or samples are placed into the appropriate Microelisa plate wells, and the specific antibody is then added. Next, an antibody specific for IL-23 that has been coupled with horseradish peroxidase (HRP) is added to each Microelisa plate well. Parts that aren't combined are eliminated. Only the wells containing IL-23 and the HRP-conjugated IL-23 antibody will initially appear blue after adding TMB substrate solution to each well. These wells will later become yellow upon the addition of stop solution. At 450 nm in wavelength, the optical density (OD) is determined spectrophotometrically. The OD value and IL-23 concentration have a linear relationship. The content of IL-23 in the samples was ascertained by comparing their optical density (OD) to the standard curve.

Statistical Analysis

The statistical analysis was performed by IBM SPSS Statistics for Windows, version 23 (IBM SPSS, IBM Corp., Armonk, NY). Shapiro–Wilk test was utilized to assess normal value distribution. Mean ± standard deviation (minimum–maximum) were used to present quantitative variables, while frequencies (number of cases) and relative frequencies (%) were used for categorical data. Statistical comparisons were made for independent parametric parameters by Mann Whitney test when data were not normally distributed and unpaired Student's t-test for normally distributed parametric parameters. Pearson Chi square ($\chi 2$) test was used for comparing categorical data. Correlations were done between quantitative variables using Spearman correlation coefficient. P-values were statistically significant if less than 0.05.

Results

The demographic criteria of both patients and controls are presented in Table 1. Where P-value is (0.329, 0.097) in age and sex respectively is non-significant. Study group included 100 (82%) females and 18 (18%) males

with mean age 31.75±7.04 years. The duration of the disease ranged from 1–17 years. The age at disease onset ranged from (17–61) years with the mean 26.96±6.92. The clinical and laboratory data within the patients group are presented in Table 2. Anti-ds-DNA antibodies were present in 49% of patients. The comorbidities were hypertension in 37% patients and diabetes mellitus in 8% of patients (10%). While IL-23 in patients is 21.78±29.19 (0.1–117.4) compared with control 3.67±4.08 (0.1–16.3), where P value= 0.001*

Discussion

SLE is an inflammatory multisystem autoimmune disease with a complex origin and pathophysiology that affects several organs. The pathophysiology of SLE involves both innate and adaptive immune dysfunction. In the inflammatory pathways of SLE, autoantibodies and the ensuing development of immune complexes play a critical role. Immune complexes induce a large number of cytokines (10). The specific pathogenetic mechanisms underlying SLE remain unclear, thus researchers have been examining a number of pro- and anti-inflammatory cytokines in the hopes of elucidating their possible function. These cytokines include interferon-gamma (IFNγ), IL-6, IL-10, and tumor necrosis factor (TNF), which have been shown to be useful in evaluating SLE clinical activity and serological results (11). Study involved 100 patients diagnosed as SLE, and 96 gender and age matched persons served as healthy controls. All participants were tested for IL-23 serum level, and it was correlated with disease parameters within the SLE group.

Our study's findings showed that SLE patients' serum levels of IL-23 were noticeably higher than those of healthy controls. These findings corroborated numerous other studies that shown higher IL-23 serum levels in SLE patients relative to healthy, normal controls (12).

These findings suggested that IL-23 may be linked to clinical parameters and may play a role in the pathophysiology of SLE.

The IL-23/IL-17 axis, which stimulates autoimmunity and chronic inflammation, is correlated with both IL23 and IL17, both of which play critical roles in inflammation (13).

Furthermore, greater SLE disease activity has been linked to raise IL-17

serum levels in SLE patients, suggesting a role for the IL-23/IL-17 axis in SLE etiology and disease activity (14). It was discovered that in mice prone to lupus, a loss of IL-23 receptor results in a reduction in IL-17 production, protecting the animals from contracting the illness (15).

Since IL-23 has been shown to play a function in the pathophysiology of synovitis in RA, its significance in the development of arthritis is clear. It has been established how important the IL-23/IL-17 axis is to the pathogenesis of RA, which involves immune cells, osteoclasts, and synoviocytes. In contrast to their increased levels in RA patients' blood and synovial fluid, both IL-17 and IL-23 were absent from healthy joints. This suggests that the IL-23/IL-17 axis plays a role in the pathophysiology of RA (13).

Furthermore, the link between hypocomplementemia and SLE disease activity, particularly in lupus nephritis, may account for the correlation with C3 and C4 intake. These findings suggest that IL-23 may have an impact on SLE activity, particularly lupus nephritis. However, given that it is a single-center study with a small cohort size and a lengthy disease duration, our work has certain limitations. Notably, patients' pre-quantification medication confounding impact cannot be disregarded; yet, after treatment, IL-23 serum level is significantly higher than in normal populations and is linked to disease activity. Hence, more studies, preferably multi-centric and on a larger scale are required to support our results.

Conclusion

According to the results of the current study supported by results of the previous studies in the same field, we can conclude that IL-23 has higher levels in serum of SLE patients, and is correlated to activity of SLE. As a pro-inflammatory cytokine included in many inflammatory pathways, it could play an important role in SLE pathogenesis and disease activity. This role makes IL-23 a potential biomarker or therapeutic target in SLE, so more studies are required to get benefits from these results in the management strategies of SLE.

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