INFLAMMATORY CYTOKINES AND PROGRAMMED DEATH-1 CORRELATION IN SUBJECTS DIAGNOSED WITH SYSTEMIC LUPUS ERYTHEMATOSUS IN THE PROVINCE OF BASRA / IRAQ

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Abstract
Background: Several biological indicators have been suggested as potential predictors of subclinical occurrences in SLE. We aimed to examine the correlation between certain inflammatory cytokines and PD-1 in SLE subjects.
Methods: This study had 43 SLE and 53 healthy subjects. Blood samples were collected and subjected to biochemical analysis. Furthermore, the subjects underwent medical history assessments using standardized self-administered questionnaires.
Results: Statistically significant increases ($p<0.01$) were observed in MDA, CRP, ANA, Anti-dsDNA, urea, creatinine, IL-18, IL-37, and PD-1, conversely, statistically significant decreases ($p<0.01$) were observed in C3, C4, CH50, TAC, and GFR in serum levels of SLE subjects compared to controls. This study revealed a positive correlation between IL-18, IL-37, and PD-1, which exhibited significantly positive correlations with MDA, CRP, ANA, anti-dsDNA, urea, and creatinine, and significantly negative correlations with C3, C4, CH50, TAC, and GFR. Moreover, the AUC of ROC curve for IL-18, IL-37, and PD-1 was calculated (0.985, 0.968, and 0.940, respectively).
Conclusion: The potential inflammatory biomarkers for the early development of SLE subjects may include IL-18 and IL-37, which exhibit a positive correlation with PD-1. Additionally, the high positive value of the AUC for IL-18, IL-37, and PD-1 further supports their potential as biomarkers in this context.
Key Words: interleukin-18; interleukin-37; programmed death-1; systemic lupus erythematosus; inflammation; autoimmune disease

Introduction

Systemic lupus erythematosus (SLE) is a combinatorial autoimmune disease of complicated nature, characterized by an overproduction of diverse autoantibodies (1). Dysregulated T-cell-dependent production of autoreactive B-cells is regarded to have an essential part in the progression of SLE. The presence of these autoantibodies is responsible for inducing damage to tissues in multiple organs, such as the joints, kidneys, skin, and central nervous system (CNS) (2).

Interleukin-18 (IL-18) is a multifunctional cytokine that is synthesized by a variety of cells, such as endothelial cells, macrophages, and hematopoietic cells. It plays a crucial role in the controlling of both innate and acquired immunity (3). In more elaboration, IL-18 is an extremely potent stimulator of interferon-gamma (IFN-γ) synthesis, exerting this effect on both natural killer (NK) cells and T-helper 1 (Th1) lymphocytes. Consequently, IL-18 is implicated in the development and progression of autoimmune disorders. The available body of research has provided clarification that IL-18 may possess a significant association with the symptoms of SLE and may contribute to its underlying mechanisms (4).

Interleukin-37 (IL-37), a novel constituent of the IL-1 family, has been acknowledged as an endogenous suppressor of immunological reactions. The amount of IL-37 production exhibits a notable increase in various cell types, including dendritic cells (DCs), peripheral blood mononuclear cells (PBMCs), macrophages, T-cells, and epithelial cells, upon activation by pro-inflammatory cytokines (5). However, it is important to note that IL-37 is not expressed continuously in normal human tissue. The up-regulation of inflammatory cytokine production, including IL-1β, IL-6, and tumor necrosis factor (TNF-α), can be observed when human peripheral blood mononuclear cells (PBMCs) from healthy persons undergo therapy with neutralizing monoclonal anti-IL-37 (6).

Programmed death-1 (PD-1), a constituent of the CD28 family, was initially discovered to play a role in the programmed cell death pathway. Nevertheless, it functions as a negative costimulatory molecular structure and has been observed to be present on the surface of various cell types, including, T cells, activated monocytes, NK cells, myeloid cells, and B cells (7). The programmed cell death protein 1 (PD-1) receptor engages with its ligands, namely PD-L1 and PD-L2, to exert immunosuppressive effects. This interaction leads to the inhibition of lymphocyte activation and the subsequent reduction in cytokine output (8).

Given the available information regarding the different modulatory and inflammatory activities of IL-18, IL-37, and PD-1, as well as their altered levels observed during the early

stages of SLE, our hypothesis posits that these cytokines, specifically IL-18, IL-37, and PD-1, may serve as blood biomarkers for the diagnosis of subjects with SLE. A comprehensive comprehension of these biomarkers is crucial in elucidating their biological function inside the blood serum of subjects (9). Therefore, the current study was undertaken to examine the correlation between the concentrations of these putative blood biomarkers in subjects with SLE and to compare them with a group of subjects who are in healthy condition.

METHODS

Study Design and Subjects Recruitment

A case-control clinical study was conducted, wherein subjects were recruited from Al-Mawany, Al-Sadr, Al-Fayhaa, and Al-Basra teaching hospitals located in the Province of Basra, Iraq. The study was conducted between September 2022 and October 2023.

SLE subjects were diagnosed by clinicians utilizing the diagnostic criteria established by the American College of Rheumatology (ACR) in 1997 (10). The study's protocol (No. 7/54/731 in 11/09/2022) was approved by the Ethics and Behavioral Research Committee (Scientific Committee) of the Department of Chemistry, College of Science, University of Basrah, located in the Province of Basra, Iraq. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki. A comprehensive explanation of the procedures was provided to all subjects, who then signed the informed consent documents.

The current study included individuals with SLE who were between the ages of 27 and 45 and were not taking any drugs for autoimmune diseases. The study excluded subjects with SLE who had coexisting blood-borne contagious infectious diseases such as hepatitis A, B, or C, or HIV, as well as those with cirrhosis, end-stage renal disease, pregnancy, other connective tissue diseases, bacterial infection, diabetes mellitus, uncontrolled hypertension, overlap syndrome, malignant tumors, chronic infections, drug-induced lupus, smoking, alcohol consumption, rheumatic diseases, genetic syndromes, and subjects receiving any steroid or immunosuppressant treatments and those with liver disease (11,12). The healthy control group consisted of individuals between the ages of 27 and 45 who did not have SLE, had no family history of SLE, did not have any acute or chronic autoimmune disorder, and were not taking any medications known to impact immune function. The study required a minimum of 43 people, determined using sample size calculations using a confidence level of 95% and a confidence interval of 10 (13). Each of the subjects exhibited stable clinical conditions for a minimum duration of three months. The collection of demographic data was carried out by means of a structured interview administered during the subjects' visits. The usual self-administered questionnaire was utilized to ascertain information pertaining to age, duration of SLE, SLE Disease Activity Index 2000 (SLEDAI-2K) scores, health behaviors such as smoking, alcohol consumption, and exercise, medical history, and current medication usage.

Samples Collection

During the designated time frame of 09:00 AM and 10:00 AM, samples were collected subsequent to a 30-minute period of relaxation. A volume of 10 mL of recently collected venous blood was extracted from the subjects' veins and thereafter placed into a tube devoid of any anticoagulant. The blood was allowed to coagulate for a duration of 20 minutes at ambient temperature. Subsequently, the sample was subjected to centrifugation with a force of 402 times the acceleration due to gravity (402 x g) for a duration of 20 minutes in order to facilitate the extraction of the serum. The samples were promptly employed in the estimate of variables in this study, while the remaining samples were stored at a temperature of -80°C in deep freeze until their utilization was required.

Measurement of C3, C4, MDA, Urea, and Creatinine with UV-Vis Spectrophotometer

Laboratory kits from Linear Chemicals S.L.U., Barcelona, Spain were used to determining of C3 (Cat. No.: 3170005, sensitivity: 3.42 mA/mg/dL), C4 (Cat. No.: 3171005, sensitivity: 9.34 mA/mg/dL), urea (Cat. No.: 1156015, sensitivity: 8.9 mA/min/mg/dL), and creatinine (Cat. No.: 1123010, sensitivity: 25 mA/mg/dL) while Bioassay Systems, California, USA kit was used to estimate levels of MDA (Cat. No.: DTBA-100, sensitivity: 1-30 µM) by using UV-Vis Spectrophotometer (UV-EMC-LAB, Duisburg, Germany). The samples were subjected to illumination encompassing the ultraviolet (UV) to visible spectral range, utilizing a light source with a wavelength generally ranging from 190 to 900 nm. The determination was made regarding the quantity of light that was absorbed, transported, or scattered at each specific spectrum (14).

Measurement of CH50, TAC, CRP, ANA, Anti-dsDNA, IL-18, IL-37 and PD-1 with Enzyme Linked Immunoabsorbance Assay (ELISA)

Sandwich human ELISA laboratory kits from BT- Laboratory, Shanghai, China were utilized to measure the levels of serum CRP (Cat. No.: BPE193, sensitivity: 2.92 pg/mL), IL-18 (Cat. No.: E0147Hu, sensitivity: 0.5-100ng/mL), IL-37 (Cat. No.: E1947Hu, sensitivity: 4.56 ng/mL) and PD-1 (Cat. No.: E3331Hu, sensitivity: 0.11 0.2-60ng/ml), while kits from AMSBIO, Milton, UK; Cell Biolabs, California, USA; LS Bio, Washington, USA; and MyBioSource, California, USA were used to determine levels of CH50 (Cat. No.: AMS.E01C0237, sensitivity: 1 IU/mL), TAC (Cat. No.: STA-360, sensitivity: 0.5 mmol/L), ANA (Cat. No.: LS-F67388, sensitivity: 0.938 ng/mL), and Anti-dsDNA (Cat. No.: MBS269122, sensitivity: 1 IU/mL), respectively by enzyme-linked immunosorbent assay (ELISA) (Humareader/Human/Germany). Every procedure occurred in accordance with the guidelines outlined in the kit protocol. The plate was analyzed using a Varioscan® ELISA reader manufactured by Thermo Fisher Scientific, measuring the absorbance at a wavelength of 450 nm. The levels of CH50, TAC, CRP, ANA, Anti-dsDNA, IL-18, IL-37, and PD-1 were quantified by employing a standard curve for measurement (15).

Measurements of BMI, GFR and SLEDAI-2K

Body mass index (BMI) was calculated by the equation: BMI (kg/m^2) = weight (kg)/(height)^2 (m). While glomerular filtration rate (GFR) was calculated by the Modification of Diet in Renal Disease Study (MDRD) equation: GFR (mL/min/1.73 m^2) = 186 × Serum $Cr^{-1.154}$ × $age^{-0.203}$ × 1.212 (if subject is black) × 0.742 (if subject is woman). SLEDAI-2K was measured and evaluated by clinicians as per SLEDAI-2K variables (16).

Statistical Analysis

The statistical analysis was conducted using version 26 of the Statistical Package for the Social Sciences (SPSS), developed

by IBM Corporation, located in Armonk, NY, USA. The data was distributed in a typical manner, and the analysis of variances was employed to compare the groups prior to conducting Dunnett's t-test to ascertain the statistical significance. The correlation coefficient was calculated by means of a scatter plot. The researchers employed binary logistic regression analysis. The sensitivities and specificities, along with the 95% confidence interval, were computed using the receiver operating characteristics (ROC) curve. This curve was constructed by graphing sensitivity on the y-axis against 1-specificity on the x-axis, and the area under the ROC curve (AUC) was determined. The Pearson correlation method was employed to establish the correlations. The values of a particular group frequently exhibited greater (or lesser) magnitudes in compared to the values of the reference group. A significance level of $p<0.05$ was considered to indicate statistical significance, while a significance level of $p<0.01$ was considered to indicate high statistical significance. Additionally, an area under the curve (AUC) value close to 0 or 1 was indicative of a robust diagnostic value.

RESULTS

Subjects Characteristics

A sample size of 56 women subjects was chosen for inclusion in the current research. A total of thirteen subjects were omitted from the study due to their failure to meet the specified inclusion and exclusion criteria. A total of 43 women subjects diagnosed with SLE were included in the study. A total of 53 healthy women controls were selected and paired with the respective subjects for the study. There were no statistically significant differences seen in the mean age (35.44 ± 4.85 vs. 34.29 ± 5.19 years) and BMI (23.92 ± 0.13 vs. 23.99 ± 0.86) between subjects with SLE and the healthy control group. Conversely, the data obtained from Table 1 demonstrates a statistically significant rise ($p<0.01$) in the levels of serum MDA, CRP, ANA, Anti-dsDNA, urea, creatinine, IL-18, IL-37, and PD1 among subjects with SLE. On the other hand, the data obtained from Table 1 reveals that subjects with SLE exhibited a statistically significant reduction ($p<0.01$) in the concentrations of serum C3, C4, CH50, TAC, and GFR. Moreover, the data obtained from the analysis of binary logistic regression demonstrated a significant increase in the levels of C3, C4, CH50, MDA, TAC, CRP, ANA, Anti-dsDNA, urea, creatinine, and GFR ($p = 0.000$, $p = 0.001$, $p = 0.004$, $p = 0.000$, $p = 0.009$, $p = 0.002$, $p = 0.000$, $p = 0.003$, $p = 0.000$, $p = 0.008$, and $p = 0.007$, respectively) when compared to the control group. These findings suggest that these markers may have potential utility in identifying subjects with SLE. Furthermore, the obtained area under the curve (AUC) data indicated that C3, C4, CH50, MDA, TAC, CRP, ANA, Anti-dsDNA, urea, creatinine, and GFR could potentially serve as more precise predictive biomarkers in subjects with SLE (AUC = 0.08, 0.09, 0.09, 0.922, 0.07, 0.894, 0.915, 0.922, 0.919, 0.922, 0.06, respectively).

Table. 1: Serum markers levels of healthy controls and SLE subjects.

The Marker	SLE Patients N = 43 (Women)	Healthy Control N = 53 (Women)	P-Value
	Mean ± SD	Mean ± SD	
C3 (g/L)	0.85 ± 0.39	1.63 ± 0.20	<0.01
C4 (g/L)	0.29 ± 0.12	0.44 ± 0.17	<0.01
CH50 (IU/mL)	51.46 ± 15.24	81.69 ± 8.99	<0.01
MDA (µmol/L)	2.49 ± 0.75	0.87 ± 0.15	<0.01
TAC (pg/mL)	1.62 ± 0.40	2.19 ± 0.46	<0.01
CRP (mg/dL)	10.77 ± 4.83	0.17 ± 0.04	<0.01
ANA (IU/mL)	2.39 ± 0.73	0.98 ± 0.31	<0.01
Anti-dsDNA (IU/mL)	28.22 ± 6.38	15.63 ± 2.47	<0.01
Urea (mg/dL)	54.98 ± 12.60	28.27 ± 7.40	<0.01
Creatinine (mg/dL)	1.07 ± 0.18	0.70 ± 0.10	<0.01
GFR (mL/min/1.73 m²)	61.89 ± 18.66	99.53 ± 18.77	<0.01
IL-18 (pg/mL)	301.61 ± 72.51	92.83 ± 23.78	<0.01
IL-37 (pg/mL)	209.42 ± 59.50	51.28 ± 6.95	<0.01
PD-1 (pg/mL)	779.14 ± 346.89	127.68 ± 24.56	<0.01

The data is displayed as mean ± standard deviation (SD).

IL-18 level and correlation with other parameters

The results obtained from Table 1 indicates that subjects with SLE had a statistically significant ($p<0.01$) elevation in IL-18 levels when compared to the control group of healthy subjects. Furthermore, the results acquired from the analysis of binary logistic regression demonstrated a substantial rise ($p = 0.006$) in IL-18 levels when compared to the control group. This finding suggests that IL-18 could potentially serve as a useful marker in subjects with SLE. In addition, the obtained AUC data suggests that IL-18 may serve as a highly effective predictive biomarker in subjects with SLE (AUC = 0.985), as illustrated in Figure 2. Additionally, the results presented in Figure 3 indicate that there is a strong positive correlation between the levels of IL-18 and the biomarkers MDA, CRP, ANA, Anti-dsDNA, urea, creatinine, IL-37, and PD-1 ($r = 0.923$, $r = 0.974$, $r = 0.871$, $r = 0.901$, $r = 0.924$, $r = 0.908$, 0.672, and $r = 0.543$, respectively). These correlations were found to be highly significant ($p<0.01$), suggesting a strong relationship between IL-18 and these biomarkers. In the present study, it was observed that IL-18 had a strong and statistically significant inverse relationship ($p<0.01$) with the biomarkers C3, C4, CH50, TAC, and GFR, as shown by correlation coefficients of -0.925, -0.581, -0.983, -

0.694, and -0.843, respectively. Conversely, no significant positive associations (p>0.05) were found between IL-18 and BMI.

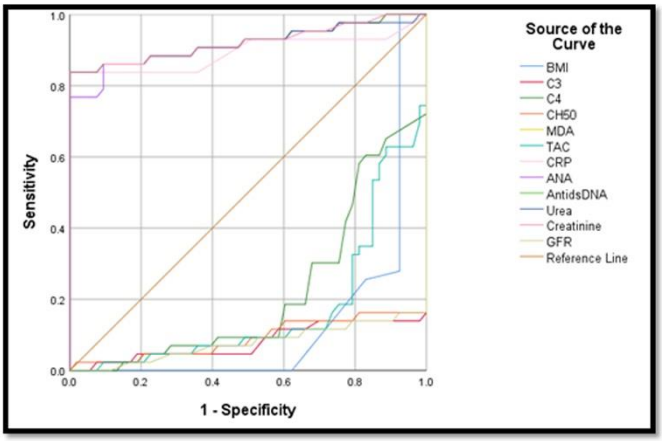


Figure. 1: The ROC curve for BMI and serum C3, C4, CH50, MDA, TAC, CRP, ANA, Anti-dsDNA, urea, creatinine and GFR in SLE and healthy control subjects.

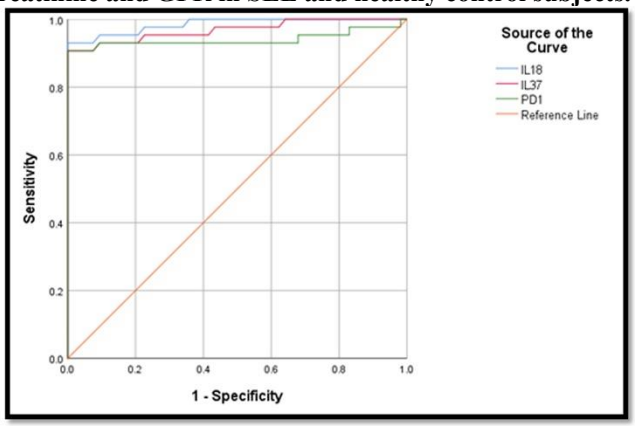
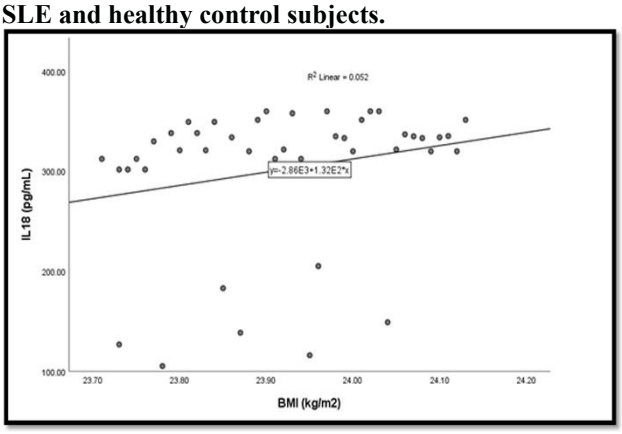
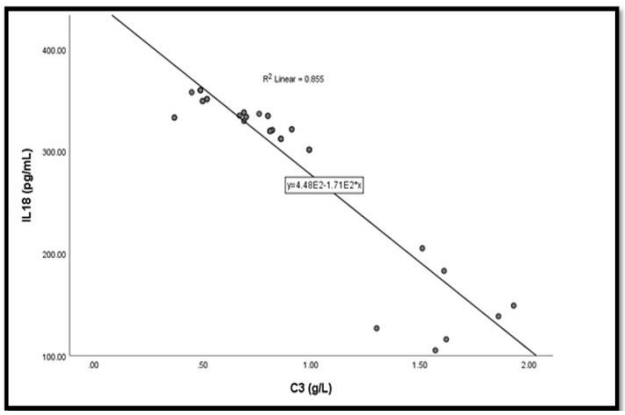


Figure. 2: The ROC curve for serum IL-18, IL-37 and PD-1 in SLE and healthy control subjects.



(A)



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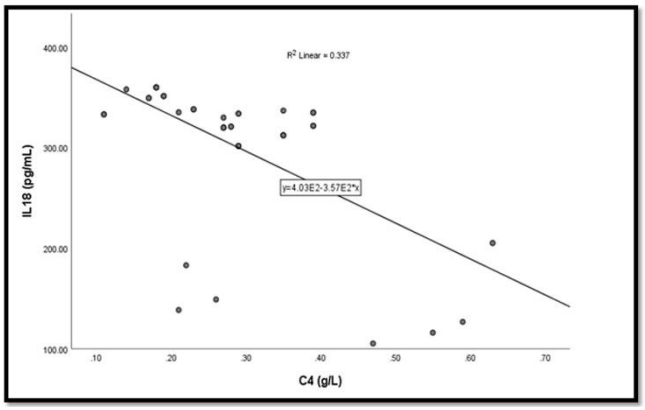

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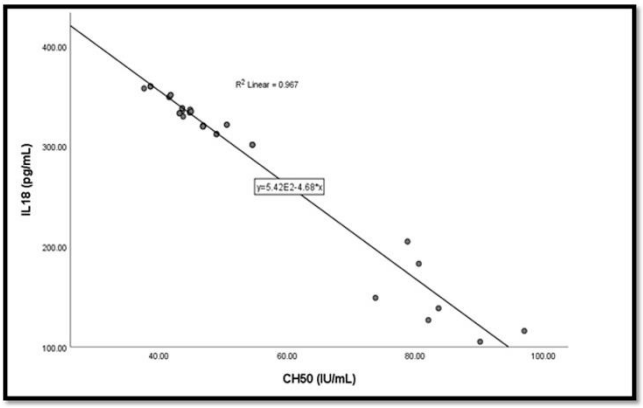

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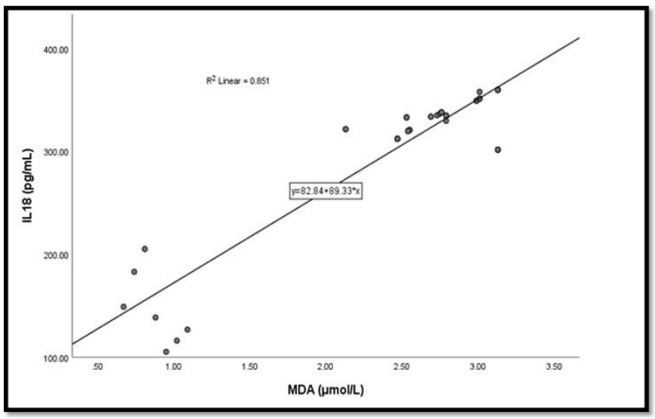

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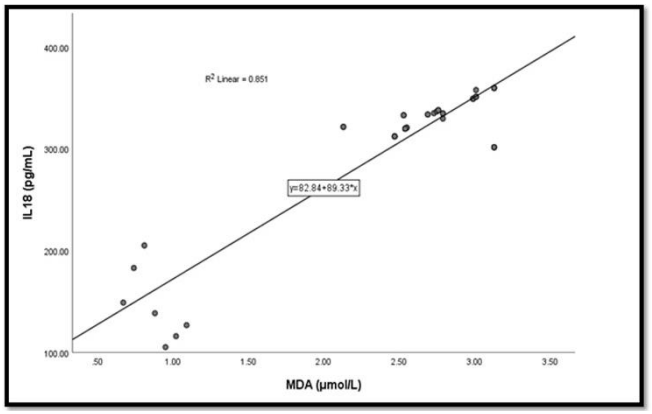
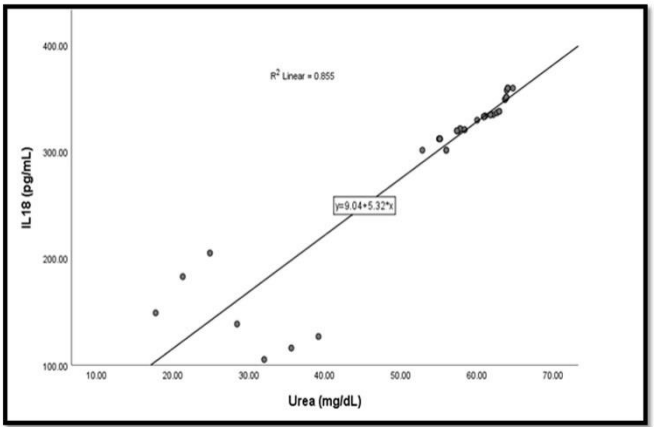
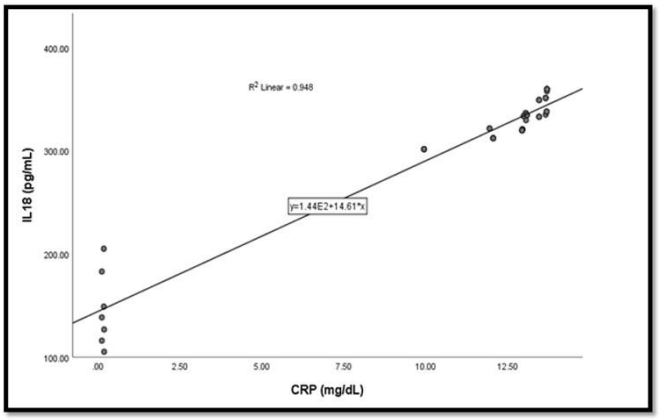

R² Linear = 0.851
y=82.84+89.33*x
IL18 (pg/mL)
MDA (µmol/L)

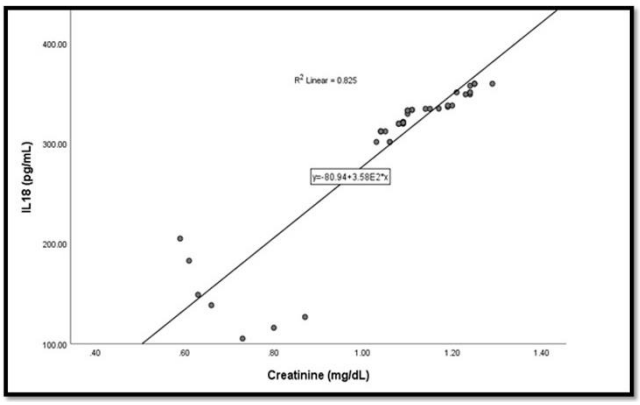
(F)


R² Linear = 0.855
y=9.04+5.32*x
IL18 (pg/mL)
Urea (mg/dL)

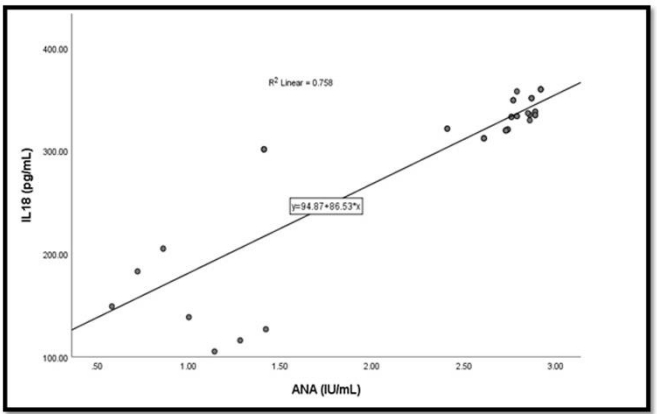
(J)


R² Linear = 0.948
y=1.44E2+14.61*x
IL18 (pg/mL)
CRP (mg/dL)

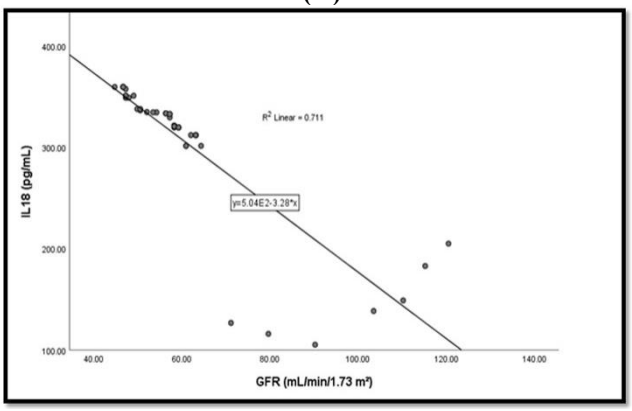
(G)


R² Linear = 0.825
y=-80.94+3.58E2*x
IL18 (pg/mL)
Creatinine (mg/dL)

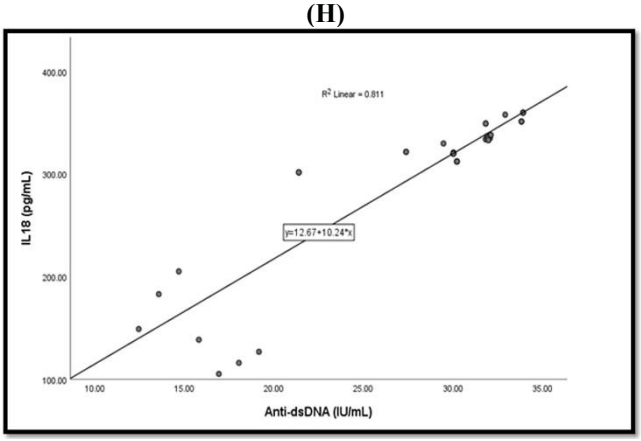
(K)


R² Linear = 0.758
y=94.87+86.53*x
IL18 (pg/mL)
ANA (IU/mL)

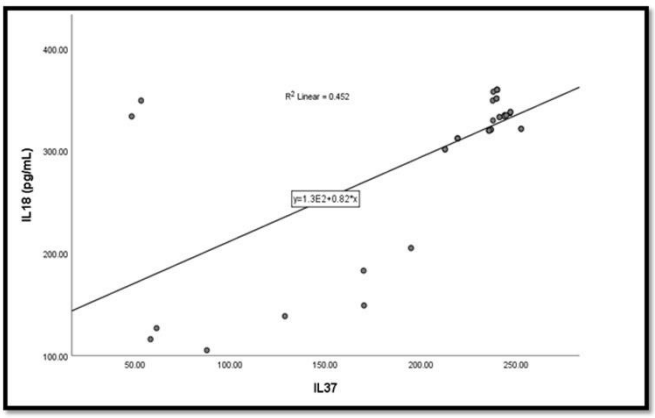
(H)


R² Linear = 0.711
y=5.04E2-3.28*x
IL18 (pg/mL)
GFR (mL/min/1.73 m²)

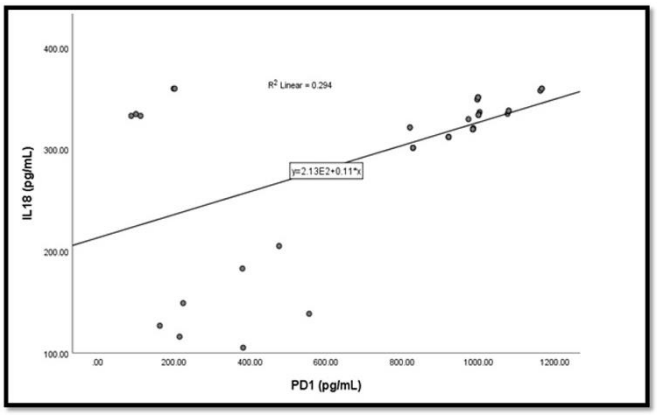
(L)


R² Linear = 0.811
y=12.67+10.24*x
IL18 (pg/mL)
Anti-dsDNA (IU/mL)

(I)

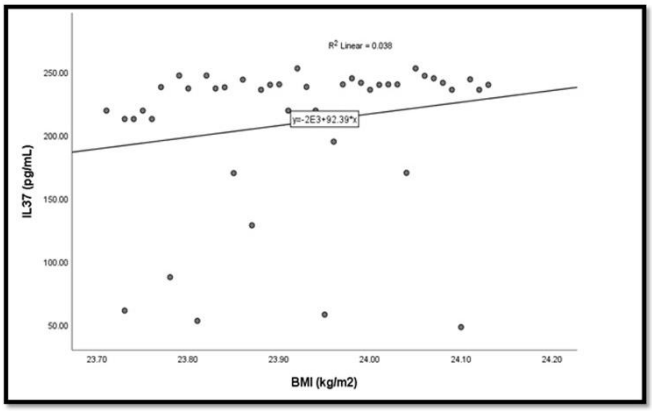

R² Linear = 0.452
y=1.3E2+0.82*x
IL18 (pg/mL)
IL37

(M)



(N)

Figure. 3: The scatter plot for IL-18 against (A) BMI, (B) C3, (C) C4, (D) CH50, (E) MDA, (F) TAC, (G) CRP, (H) ANA, (I) Anti-dsDNA, (J) urea, (K) creatinine, (L) GFR, (M) IL-37 and (N) PD-1 in SLE subjects.



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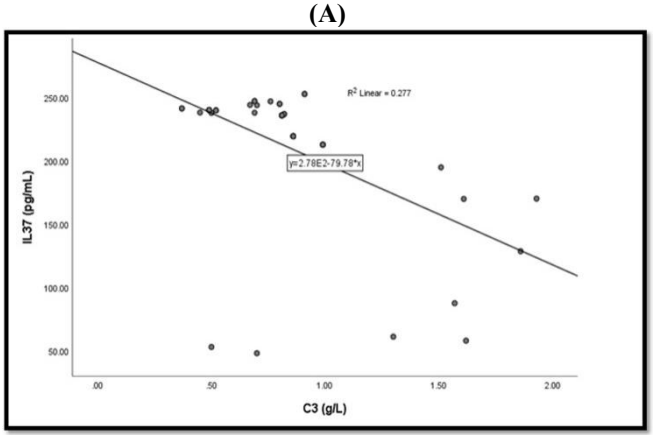

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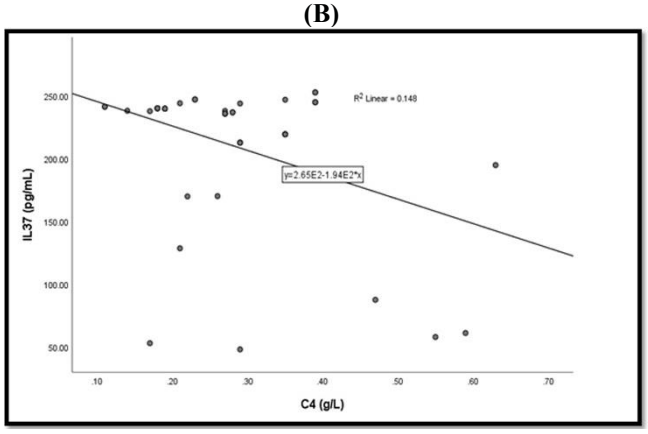

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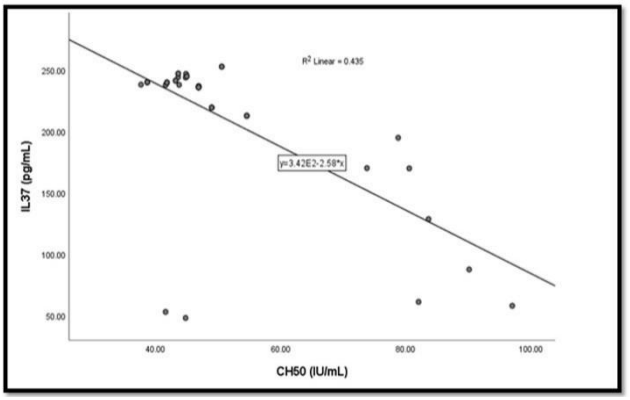

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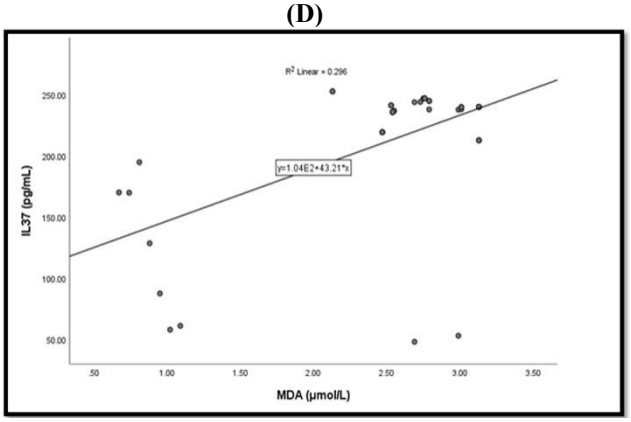

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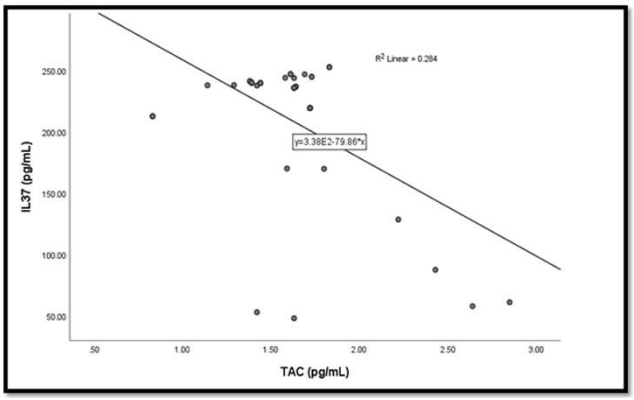

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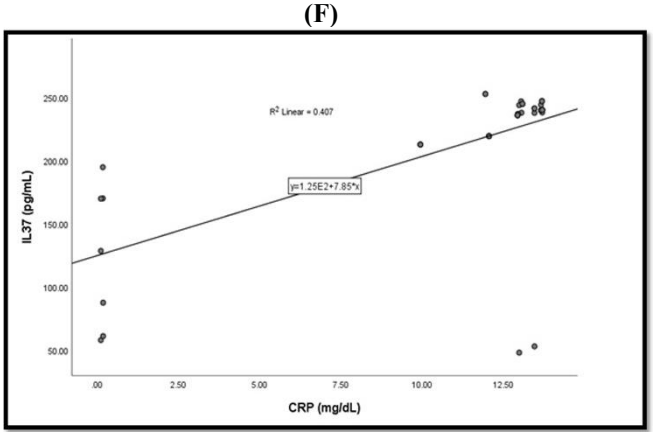

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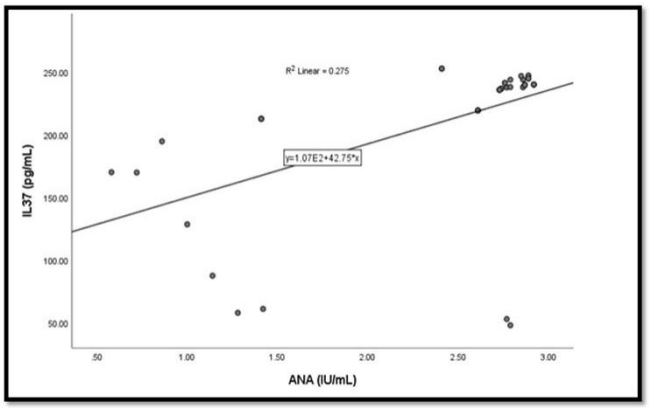
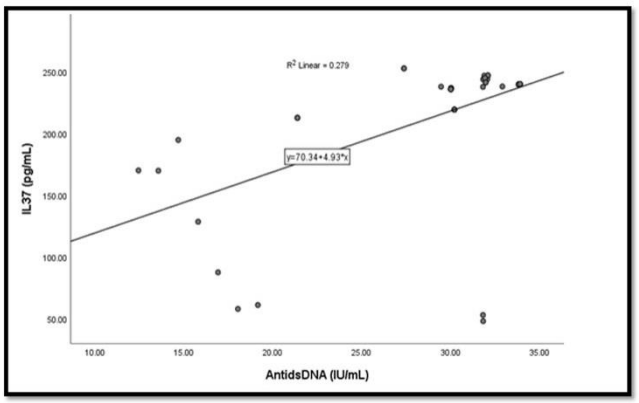
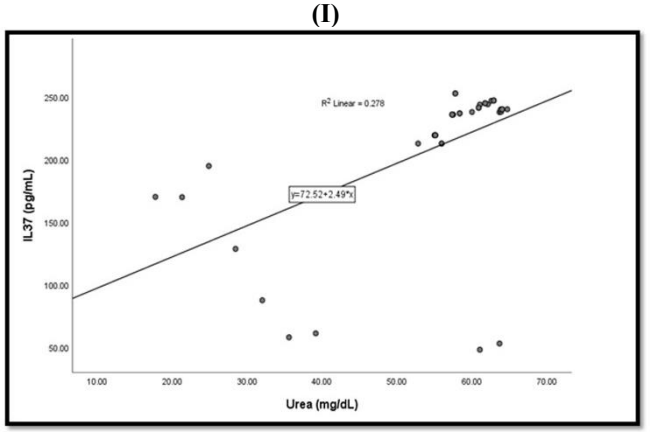


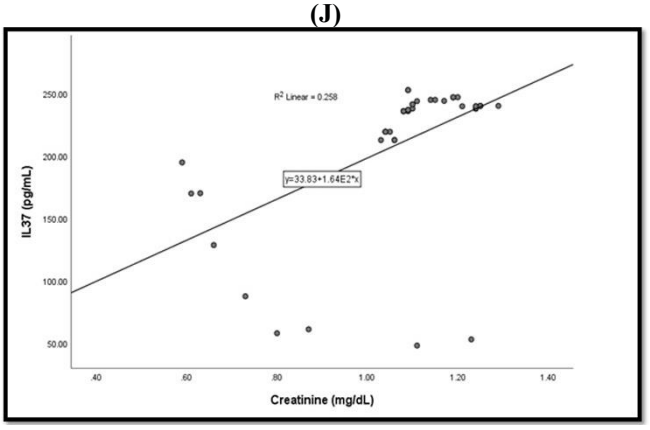
(H)



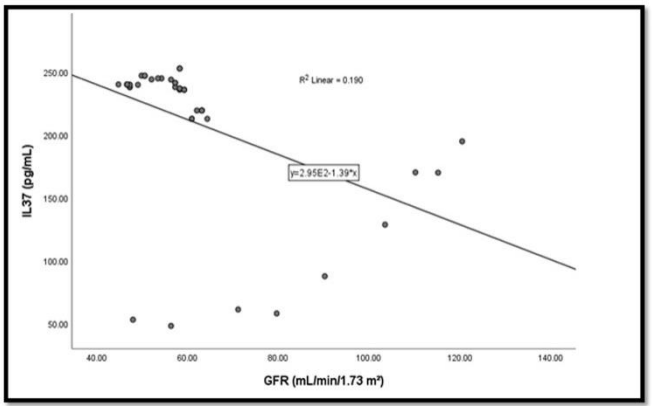
(I)



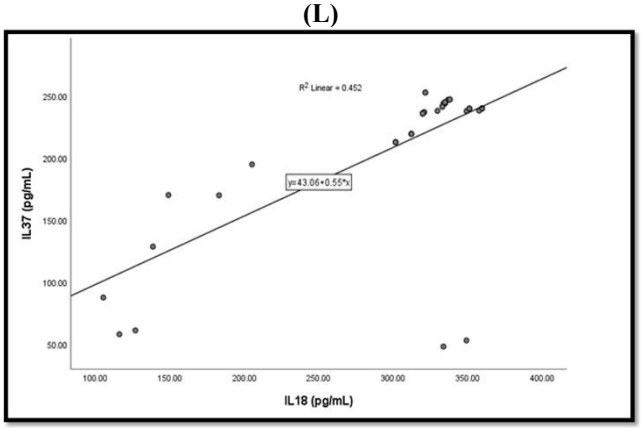
(J)



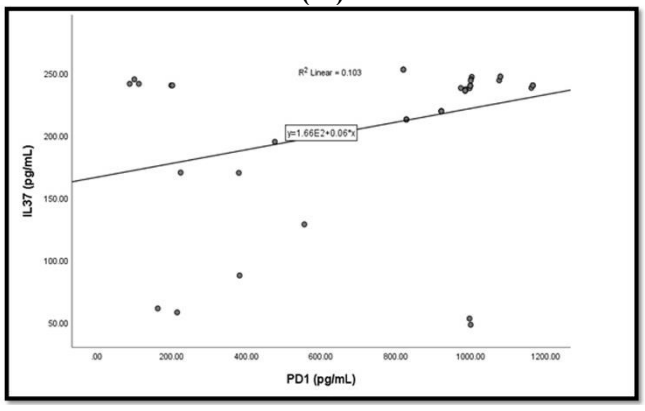
(K)



(L)

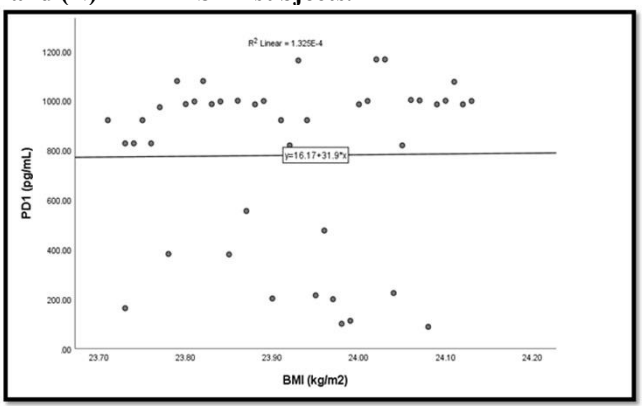


(M)

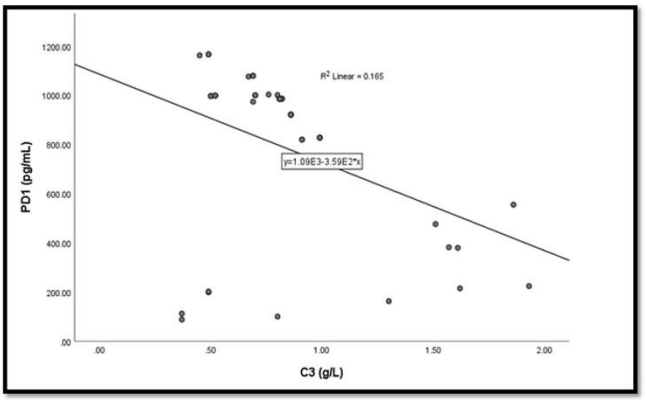


(N)

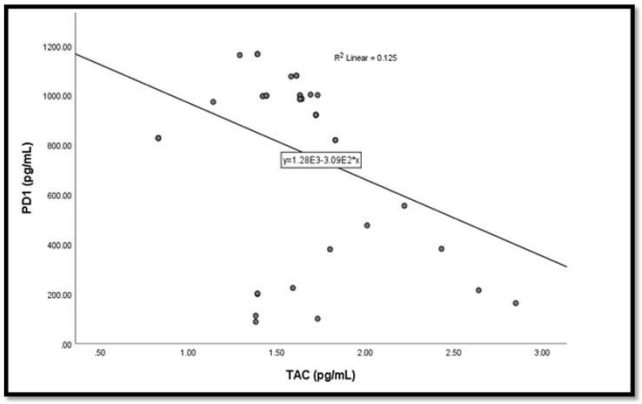
Figure. 4: The scatter plot for IL-37 against (A) BMI, (B) C3, (C) C4, (D) CH50, (E) MDA, (F) TAC, (G) CRP, (H) ANA, (I) Anti-dsDNA, (J) urea, (K) creatinine, (L) GFR, (M) IL-18 and (N) PD-1 in SLE subjects.



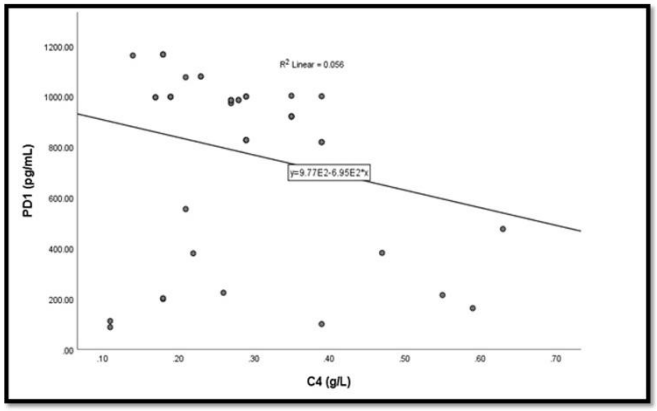
(A)


R² Linear = 0.165
y=1.09E3-3.59E2*x
PD1 (pg/mL)
C3 (g/L)

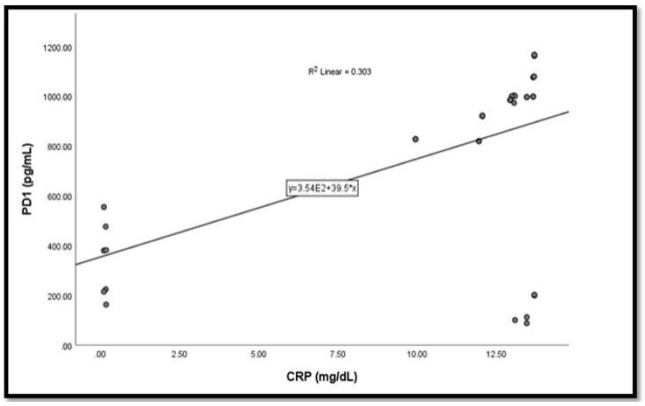
(B)


R² Linear = 0.125
y=1.28E3-3.09E2*x
PD1 (pg/mL)
TAC (pg/mL)

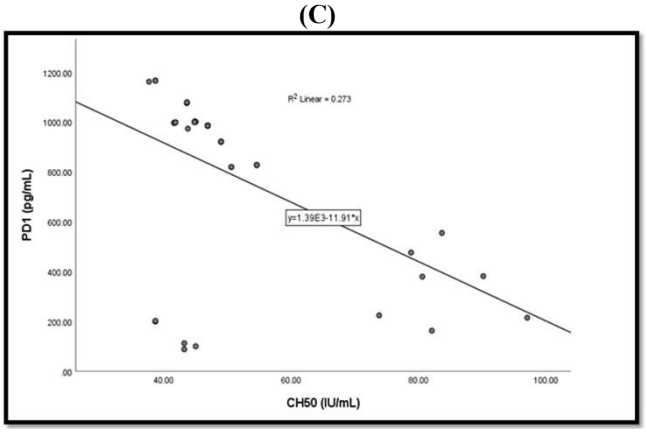
(F)


R² Linear = 0.056
y=9.77E2-6.95E2*x
PD1 (pg/mL)
C4 (g/L)

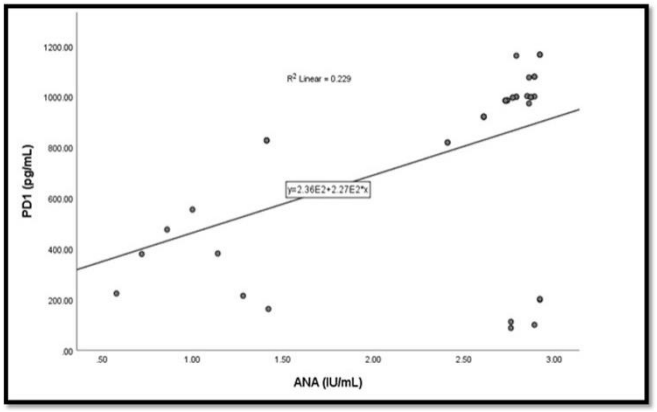
(C)


R² Linear = 0.303
y=3.54E2+39.5*x
PD1 (pg/mL)
CRP (mg/dL)

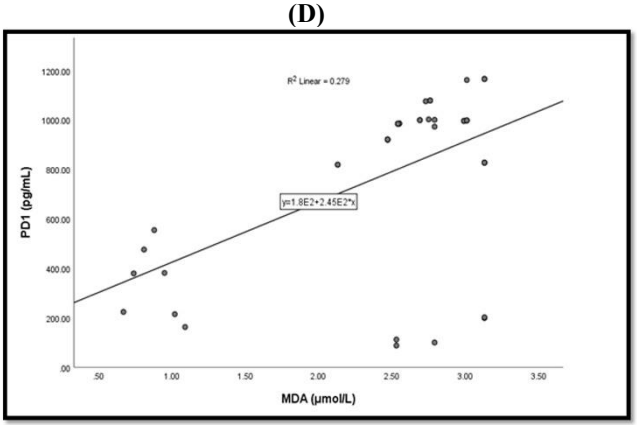
(G)


R² Linear = 0.273
y=1.39E3-11.91*x
PD1 (pg/mL)
CH50 (IU/mL)

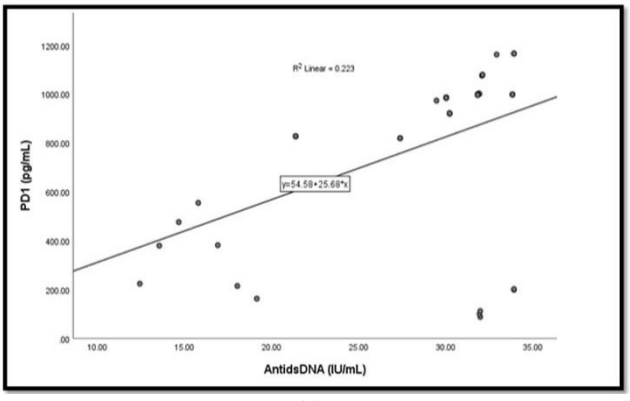
(D)


R² Linear = 0.229
y=2.36E2+2.27E2*x
PD1 (pg/mL)
ANA (IU/mL)

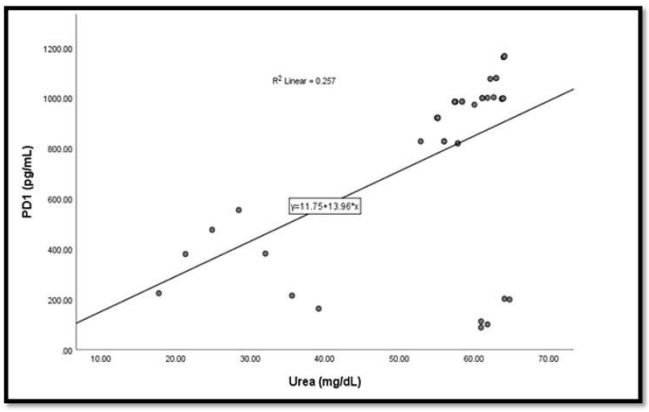
(H)


R² Linear = 0.279
y=1.8E2+2.45E2*x
PD1 (pg/mL)
MDA (µmol/L)

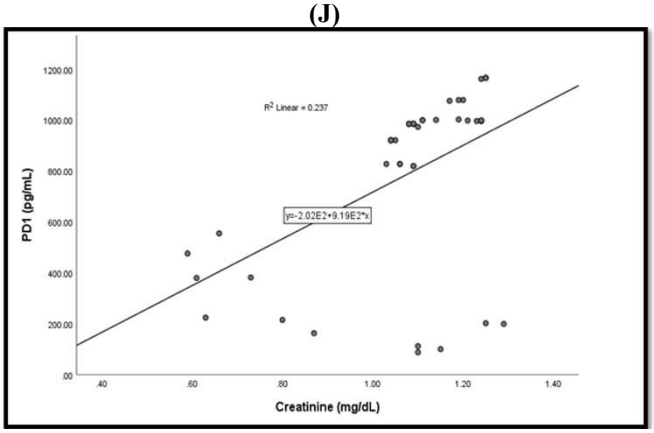
(E)


R² Linear = 0.223
y=54.58+25.68*x
PD1 (pg/mL)
AntidsDNA (IU/mL)

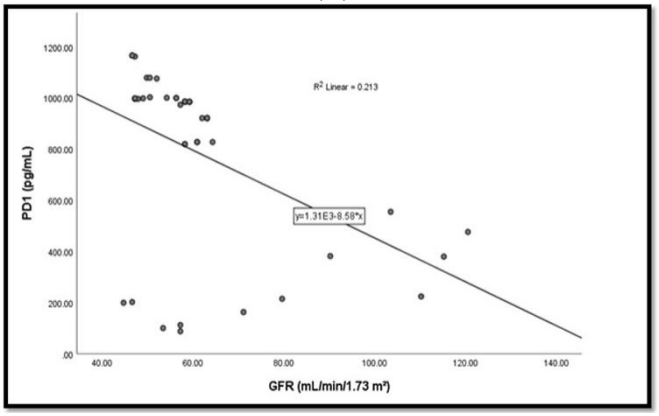
(I)



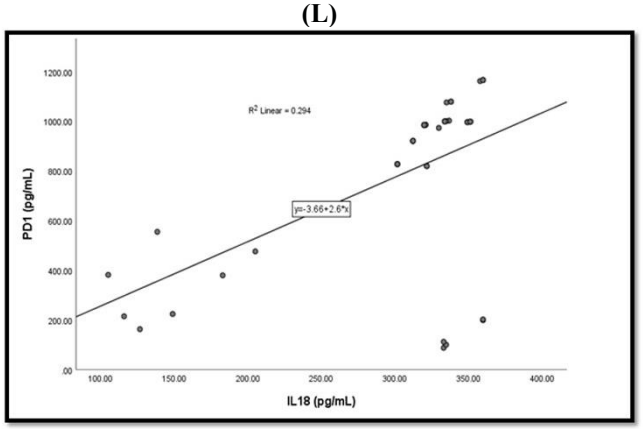
(J)



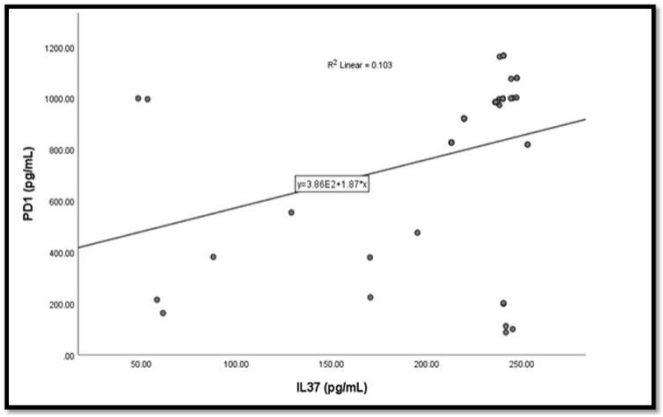
(K)



(L)



(M)



(N)

Figure. 5: The scatter plot for PD-1 against (A) BMI, (B) C3, (C) C4, (D) CH50, (E) MDA, (F) TAC, (G) CRP, (H) ANA, (I) Anti-dsDNA, (J) urea, (K) creatinine, (L) GFR, (M) IL-18 and (N) IL-37 in SLE subjects.

IL-37 level and correlation with other parameters

The findings presented in Table 1 demonstrates a statistically significant increase ($p<0.01$) in serum IL-37 levels among subjects with SLE as compared to the control group of healthy subjects. Additionally, the data collected from the analysis of binary logistic regression indicated a statistically significant rise in IL-37 levels ($p = 0.009$) when compared to the control group. This finding suggests that IL-37 could potentially serve as a useful marker in subjects with SLE. In addition, the obtained AUC data indicates that IL-37 has the potential to serve as a highly predictive biomarker in subjects with SLE (AUC = 0.968), as illustrated in Figure 2. Furthermore, the results indicate a strong and statistically significant positive relationship ($p<0.01$) between IL-37 and several biomarkers, including MDA, CRP, ANA, Anti-dsDNA, urea, creatinine, IL-18, and PD-1 ($r = 0.544$, $r = 0.638$, $r = 0.524$, $r = 0.528$, $r = 0.527$, $r = 0.508$, $r = 0.672$, and $r = 0.502$ respectively). Furthermore, IL-37 exhibited strong and statistically significant negative correlations ($p<0.01$) with C3, C4, CH50, TAC, and GFR ($r = -0.526$, $r = -0.501$, $r = -0.660$, $r = -0.533$, and $r = -0.500$). Conversely, there were no significant positive correlations ($p>0.05$) observed between IL-37 and BMI, as depicted in Figure 4.

PD-1 level and correlation with other parameters

The findings presented in Table 1 demonstrates a statistically significant increase ($p<0.01$) in serum PD-1 levels among subjects with SLE as compared to the control group of healthy subjects. In addition, the data obtained from the study of binary logistic regression demonstrated a statistically significant rise in PD-1 level ($p = 0.0002$) when compared to the control group. This finding suggests that PD-1 level may serve as a promising marker in subjects with SLE. In addition, the obtained AUC data indicated that PD-1 may serve as a possibly more effective predictive biomarker in subjects with SLE, as evidenced by an AUC value of 0.940, as depicted in Figure 2. Furthermore, the PD-1 marker exhibited a strong and statistically significant positive association ($p<0.01$) with several other biomarkers, including MDA, CRP, ANA, Anti-dsDNA, urea, creatinine, IL-18, and IL-37 ($r = 0.528$, $r = 0.550$, $r = 0.513$, $r = 0.509$, $r = 0.507$, $r = 0.532$, $r = 0.543$, and $r = 0.502$ respectively).

Furthermore, PD-1 exhibited strong and statistically significant negative correlations ($p<0.01$) with C3, C4, CH50, TAC, and GFR ($r = -0.500$, $r = -0.501$, $r = -0.523$, $r = -0.504$, and $r = -0.517$) in addition to non-significant positive correlations ($p>0.05$) with BMI, as depicted in Figure 5.

DISCUSSION

Systemic lupus erythematosus (SLE) is classified as an autoimmune disorder characterized by the breakdown of self-tolerance and the appearance of autoantibodies targeting several organs within the body (17).

The findings of our study revealed a statistically significant positive correlation between the level of IL-18 and several markers including MDA, CRP, ANA, Anti-dsDNA, urea, creatinine, IL-37, and PD-1 in subjects with SLE when compared to the control group. Additionally, a statistically significant negative correlation was seen between IL-18 and markers such as C3, C4, CH50, TAC, and GFR in SLE subjects as compared to controls. The findings of our study align with previous research that has identified a link between IL-18 and these markers in subjects with SLE (18,19). The observed phenomenon could potentially be attributed to the inflammatory response in SLE. Elevated levels of IL-18 may arise as a result of the pro-inflammatory condition observed in SLE, given that immune cells release IL-18 in response to inflammatory stimuli (20). Furthermore, subjects diagnosed with SLE may experience an imbalance in the synthesis and functionality of interleukins, such as IL-18. The dysregulation of IL-18 production can result in an overproduction of this cytokine, hence leading to the development of inflammation and malfunction in the immune system (21). Furthermore, it is worth noting that SLE frequently presents with kidney involvement, which is clinically referred to as lupus nephritis (LN). Increased levels of IL-18 may arise as a consequence of renal tissue injury, given that the kidneys are a significant site of IL-18 synthesis (22). In addition, it is important to note that endothelial cells, which form a lining along the inner walls of blood arteries, possess a significant impact on the overall well-being of the vascular system. SLE has the potential to induce endothelial dysfunction, resulting in the initiation of an inflammatory response and subsequent impairment of vascular integrity. The aforementioned malfunction has the potential to induce the secretion of IL-18 and various other cytokines (23). Additionally, it is worth noting that immune complexes, which are comprised of a combination of antibodies and antigens, have the ability to develop and accumulate within different tissues, such as blood vessels and organs. The activation of immune cells by these immunological complexes can result in the synthesis of IL-18 and various other inflammatory mediators (24). In addition, subjects diagnosed with SLE exhibit heightened vulnerability to infections owing to the impaired state of their immune system resulting from the condition. Specific infections have the ability to induce the secretion of IL-18 as a component of the immunological reaction to the infection (25).

The analysis of our data indicates a statistically significant positive correlation between IL-37 and MDA, CRP, ANA, Anti-dsDNA, urea, creatinine, IL-18, and PD-1 in subjects with SLE when compared to the control group. Conversely, there is a very significant negative correlation between IL-37 and C3, C4, CH50, TAC, and GFR in SLE subjects as compared to the controls. This finding aligns with several studies that have shown a correlation between IL-37 and these biomarkers in subjects with SLE (26,27). The observed phenomenon may be ascribed to the presence of inflammation, as IL-37 potentially serves as a compensation strategy employed by the organism to mitigate the heightened inflammation and immunological response characteristic of the ailment. IL-37 functions as an endogenous anti-inflammatory mediator, and elevated concentrations of this cytokine may serve as a regulatory mechanism to moderate the inflammatory response (28). Autoimmune pathogenesis in SLE, wherein the immune system engages in the detrimental activity of targeting and damaging healthy tissues. This aberrant immune response subsequently elicits inflammatory reactions (29). Prior research has demonstrated that the expression of IL-37 is significantly increased in various cells upon exposure to pro-inflammatory cytokines, including IL-18, IFN-γ, IL-1β, and tumor necrosis factor (TNF). Conversely, IL-37 expression is either lower or not consistently present in steady-state target cells and normal human tissues (30). IL-37 is potentially synthesized as a response to the occurrence of autoimmunity, with the aim of alleviating the autoimmune assault on the organism (31). In addition, IL-37 has the ability to induce immunomodulatory effects through the inhibition of multiple immune cell types, such as T cells and DCs. Elevated levels of IL-37 may serve as a regulatory mechanism to mitigate immunological dysregulation in SLE (32). Moreover, the occurrence of endothelial dysfunction can result in inflammation and subsequent impairment of blood vessel integrity. IL-37 has the potential to be generated as a defensive mechanism in response to the occurrence of endothelial dysfunction. The activation of inflammatory reactions in SLE, which can result in detrimental effects on many tissues, such as the skin, joints, and internal organs (33). IL-37 is potentially secreted in response to tissue damage as a component of the organism's endeavors to restrict further inflammation and facilitate the restoration of harmed tissues. Furthermore, there was a dysregulation of several cytokines and chemokines in SLE. The presence of these imbalances has the potential to impact the synthesis and functionality of IL-37 and several other cytokines, hence leading to an increase in IL-37 levels (34).

The analysis of our data indicates a statistically significant positive correlation between PD-1 and MDA, CRP, ANA, Anti-dsDNA, urea, creatinine, IL-18, and IL-37 in subjects with SLE as compared to the control group. Conversely, a very significant negative correlation was observed between PD-1 and C3, C4, CH50, TAC, and GFR in SLE subjects as compared to controls. This finding aligns with numerous studies that have shown a correlation between PD-1 and inflammatory cytokines in subjects with SLE (12,14). The observed elevation of serum PD-1 levels in subjects with SLE may be attributed to the presence of persistent inflammation and an exaggerated immune response. The persistent inflammatory condition can result in the activation of immunological checkpoints such as PD-1, which serves as a regulatory mechanism to mitigate exaggerated immune reactions (35). Elevated levels of PD-1 may potentially serve as a compensation strategy employed by the immune system to mitigate the inflammatory responses and inflammation that are typically associated with SLE. The human body may upregulate the expression of PD-1 as a mechanism of

self-regulation, aiming to mitigate and impede more tissue damage (36). Moreover, PD-1 serves as a distinguishing marker for T cell exhaustion, a condition characterized by diminished responsiveness of T cells to antigenic stimulation (37). SLE may result in T cell malfunction and exhaustion, which might manifest as heightened PD-1 expression on T cell surfaces due to compromised functionality (38). In addition, by the coexistence of autoantibodies and immune complexes in SLE, which are comprised of self-antigens. Prolonged exposure to these endogenous antigens can induce T cells to upregulate PD-1 expression during their interaction with autoantigens, in an effort to attenuate the immune response directed towards self-antigens (39). Furthermore, subjects diagnosed with SLE exhibit a heightened susceptibility to infections as a result of impaired immune system functionality. Infections have the potential to trigger immunological responses and activate immune checkpoints, such as PD-1 (40).

The current study employed a cross-sectional and retrospective design, prioritizing descriptive rather than causal findings. Furthermore, it should be noted that the data pertaining to both subjects with SLE and the controls of the Province of Basra, Iraq, may not provide an accurate depiction of the prevailing circumstances. This limitation arises from the relatively small sample size employed in the study. Nevertheless, the outcomes of this study can be utilized in the advancement of therapeutic approaches for the early identification, alleviation, or control of subjects with SLE in the Province of Basra, Iraq. Further investigation using a more expansive and heterogeneous sample size may be imperative in order to substantiate the significance of IL-18, IL-37, and PD-1 in SLE.

CONCLUSION

The strong correlation shown between IL-18, IL-37, and PD-1 with C3, C4, CH50, MDA, TAC, CRP, ANA, Anti-dsDNA, urea, creatinine and GFR suggests that these inflammatory biomarkers may have significant involvement in the immune response and inflammatory processes associated with SLE. Additionally, the utilization of biochemical analysis may contribute to the comprehension of the underlying pathogenic mechanisms that can lead to renal dysfunction and kidney disease in subjects with SLE.

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AUTHOR CONTRIBUTION

SAA and AJMA were involved in the conception and planning of the research. SAA performed the data acquisition/collection, calculated the experimental data, performed the analysis, drafted the manuscript, and designed the figures. SAA and AJMA aided in interpreting the results and took part in the critical revision of the manuscript.

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