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Interleukin-4, and interferon-gamma roles as biomarkers in Systemic Lupus Erythematosus patients.

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Abstract:

Objective: To evaluate interleukin-4 (IL-4), and interferon-gamma (IFN- γ) levels in Systemic lupus erythematosus (SLE) cases in addition to evaluating the correlation of IFN- γ , and IL-4 with disease activity. **Patients and Methods:** This study recruited 60 adult SLE subjects and 60 healthy controls. Subjects underwent complete clinical examination, history taking, as well as evaluation of disease activity in cases with SLE via the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Determination of serum levels of IFN- γ , and IL-4 was done in controls as well as patients. **Results:** IFN- γ , and IL-4 levels were substantially elevated in the SLE cohort than in the controls. Furthermore, the IFN- γ level is significantly positively correlated with SLEDAI, with no link between SLEDAI as well as IL-4. **Conclusion:** Elevated IFN- γ and IL-4 serum levels in cases with SLE play a role in SLE progression and have a role in disease activity. IFN- γ serum levels were positively linked with disease activity and anti-dsDNA, negatively linked with C4, and no significant correlation with C3 and 24 h protein in the urine.

Keywords: Systemic lupus erythematosus, Interleukin-4, Interferon-gamma.

Introduction:

SLE is an autoimmune disorder manifested by the involvement of multisystem immune regulation disruption, driven by deposits of immune complex and autoantibodies^[1].

There is no specific cause of SLE since variables such as sunshine and medicines may exacerbate the disorder, and there is a complicated genetic underpinning^[2]. A number of non-traditional indicators like chemokines, growth factors, as well as cytokines were proven

to be featured by potential clinical implications in SLE^[3]. IFN- γ is known as a homodimeric protein of the type II cytokine secreted by natural killer cells (NK) cells, CD8+T cells, and CD4+ Th1 cells^[4]. IFN- γ stimulates macrophages to attack phagocytosed microorganisms, triggering high microbicidal activities is referred to as the traditional macrophage activation pathway^[5]. Serum IFN- γ was proven to be more elevated in active SLE cases than in

controls and linked to disease activity^[6-7]. Additionally, IL-4 is known to belong to the family of 1 four- α -helical cytokine. Activated eosinophils Th2 cells, basophils, and activated mast cells are IL-4 sources^[8]. IL-4 triggers B cell Ig heavy chain class flipping to the IgE isotype. IgE antibodies aid in eosinophil-mediated defense against some arthropod infections as well as helminthic^[9]. Studies have revealed that IL-4 serum level was more elevated in cases with SLE than in controls^[10-11].

Patients and Methods:

This study was done on 60 participants visiting the outpatient clinic in Sohag University Hospital and 60 healthy controls of both sexes from February 2019 to February 2020. They were categorized into two primary cohorts: Group I (SLE group), including 60 SLE cases identified based on the SLE's classification criteria of the American College of Rheumatology (ACR)^[12]. Group II (controls) comprised 60 healthy individuals of both sexes.

1. Ethical considerations:

This research was authorized by the Faculty of Medicine-Sohag University's Scientific and Ethical Committee. Informed written consent was collected from the controls and patients after discussing the aim of the study and methods.

2. Disease activity:

Evaluation of disease activity was done in cases with SLE via SLEDAI^[13]. The activities were categorized according to the scores of the SLEDAI; very high activity (SLEDAI: 20), high activity (SLEDAI: 11-19), moderate activity (SLEDAI: 6-10), mild activity (SLEDAI: 1-5), as well as no activity (SLEDAI: 0).

3. Sample collection:

Under aseptic conditions, both con-

rols and patients were subjected to 5 ml venous blood withdrawal. They were divided into two portions. Two ml of blood was added to EDTA vacutainer to be used for complete blood count and ESR. Three ml of blood in a sterile plain vacutainer for other investigations through serum separation. Urine samples were taken from both cohorts after 24 hours.

4. Routine laboratory investigations:

The rate of erythrocyte sedimentation, complete blood picture, as well as protein in 24 hours collected urine sample, liver function tests, serum creatinine, complement 3, complement 4, Anti-dsDNA, and ANA were among the investigations performed.

5. IL-4 and IFN- γ assay:

IL-4 and IFN- γ were detected utilizing (Luminex[®] 100/200[™] System, Austin, Texas, USA, serial number LXSD 131-07003) and Luminex xponent[®] (one lambda) software version 4.2 for analysis of the results. Luminex[®]-assays utilized a fluorescent bead set, with each bead falling in a different region on the fluorescence. The beads were covered with capture antibodies directed to the analyte of interest^[14]. Normal range of IL-4 (3.5- 6.5 pg/ml), and normal range of IFN- γ (0.1- 2 pg/ml).

6. Statistical analysis:

Coding, tabulation, as well as analysis of data, were performed via the 26th version of [SPSS]. The expression of qualitative data was in the form of percentages as well as numbers, whereas the Chi-square test was utilized in order to verify the correlation between categorical variables. The expression of quantitative data was in the form of standard deviation and mean (Mean \pm SD). Comparison between groups was made utilizing the t-test. Pearson correlation test was adopted to verify the

link between quantitative variables. In addition, a p-value of < 0.05 was deemed statistically significant.

Results:

1. Patients and controls' demographic data:

This study recruited 60 SLE patients, 58 females, and two males, with an age range of (19-48 years) with an average age reaching 31.52 ± 8.30 years. While 60 controls were four males and 56 females, the age range reached (20-50 years) with an average age reaching 32.47 ± 9.19 years (Table 1).

2. Patients and controls' significant laboratory investigations :

A substantial elevation in ESR levels, 24-hour urinary protein, ANA, anti-ds-DNA, IFN- γ , and IL-4 was found ($P < 0.001$), besides elevated serum creatinine levels ($P < 0.05$) in SLE cases than in controls. While there was a marked decline in C4, C3, and PLT levels ($P < 0.001$), as well as diminished WB-Cs, RBCs, and HGB levels ($P < 0.05$) in cases with SLE than in controls (Table 2).

3. SLE patients' disease activity:

As regards SLEDAI activity, 5% of subjects had no activity, 15% had mild activity, 21% had moderate activity, 28% had high activity, and 30 % had very high activity (Table 3).

4. Correlation of IFN- γ with laboratory investigations of SLE patients:

There was substantial positive relation between SLEDAI and IFN- γ ($r = 0.601$, $P < 0.001$) (Fig. 1), as well as anti-ds-DNA and IFN- γ ($r = 0.612$, $P < 0.001$) (Fig. 2). On the contrary, no substantial relationship was detected between IFN- γ with C4 ($r = -0.351$, $P = 0.006$) (Fig. 3). No marked relationship was detected between C3 ($r = -0.180$, $P = 0.169$) (Fig. 4) and with 24 h protein in urine ($r = -0.180$, $P = 0.169$) (Fig. 5).

5. Correlation of IL-4 with laboratory investigations of SLE patients:

There was no marked relation between IL-4 with SLEDAI, anti-dsDNA, and 24 h protein in the urine, C3, and C4 (Table 4).

Table (1): Patients and controls' demographic data.

| Parameters | Patients | Controls | P-value |
|-----------------------------------|---------------------------|---------------------------|------------|
| Gender (male/female) | 58/ 2 | 56/4 | 0.402 (NS) |
| Age, years (mean \pm S.D) range | 31.52 ± 8.30 19-48 | 32.47 ± 9.19 20-50 | 0.554 (NS) |

-NS: Non-significant.

-S.D: standard deviation.

Table (2): laboratory investigations of patients and controls.

| Parameters (mean±S.D) | Patients | Controls | P-value |
|-----------------------------|---------------|--------------|---------|
| ESR | 41.47±34.94 | 11.78±3.36 | <0.001 |
| WBCs | 6.13±2.85 | 7.02±2.01 | 0.01 |
| RBCs | 4.43±0.49 | 4.94±0.28 | 0.02 |
| HGB | 12.03±1.38 | 13.51±1.05 | 0.01 |
| PLT | 218.77±93.94 | 241.65±72.29 | <0.001 |
| serum creatinine | 1.83±1.13 | 0.89±0.22 | 0.03 |
| 24 h urinary protein | 316.17±229.99 | 92.33±32.49 | <0.001 |
| C3 | 59.17±46.54 | 111.85±26.56 | <0.001 |
| C4 | 10.20±9.33 | 20.95±8.96 | <0.001 |
| ANA | 4.85±3.11 | 0.72±0.34 | <0.001 |
| anti-dsDNA | 189.57±109.03 | 9.78±5.07 | <0.001 |
| IL-4 | 8.85±3.39 | 4.99±0.99 | <0.001 |
| IFN-γ | 5.85±2.85 | 0.83±0.42 | <0.001 |

-S.D: standard deviation. -ESR=erythrocyte sedimentation rate. -ANA: antinuclear antibodies.
 -Anti-dsDNA: anti-double-stranded DNA. -C3: complement 3. -C4: complement 4.
 -WBCs: white blood cells. -RBCs: red blood cells. -PLT: platelet -HGB: heamoglobin.
 -IFN-γ: interferon gamma. -IL-4: interleukin 4.

Table (3): SLE Disease Activity Index (SLEDAI) in SLE subjects.

| | | No | Percent |
|---------------|---------------------------|----|---------|
| SLEDAI | No activity | 3 | 5.0 |
| | Mild activity | 9 | 15.0 |
| | Moderate activity | 13 | 21.7 |
| | High activity | 17 | 28.3 |
| | Very high activity | 18 | 30.0 |
| | Total | 60 | 100.0 |

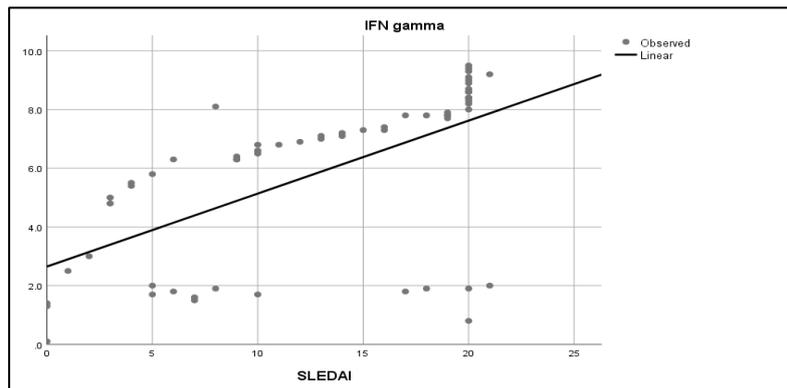


Fig (1): Correlation between SLEDAI and IFN-γ.

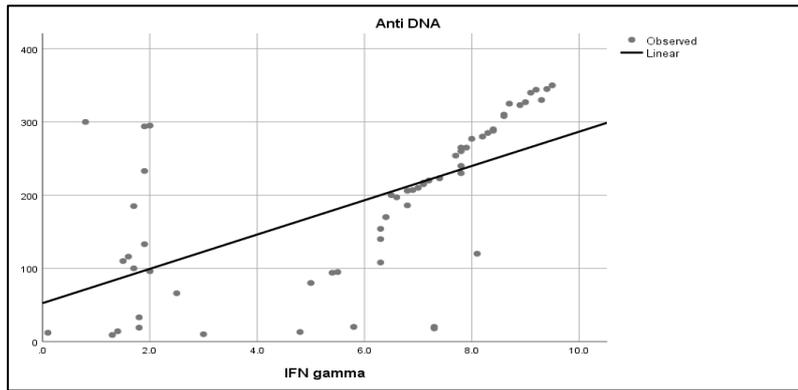


Fig. (2): Association between anti-dsDNA and IFN- γ .

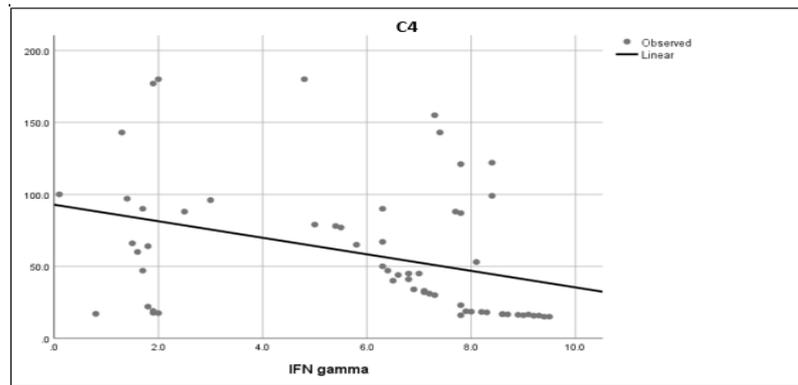


Fig. (3): Association between C4 and IFN- γ .

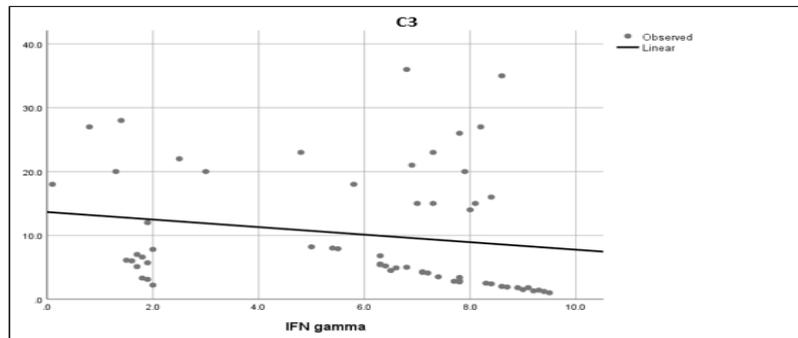


Fig. (4): Correlation between C3 and IFN- γ .

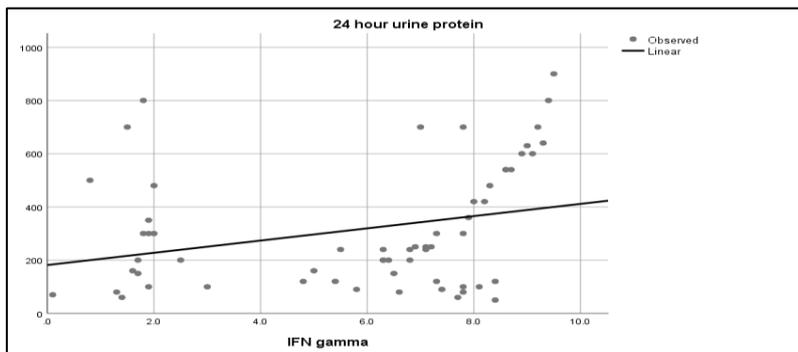


Fig. (5): Correlation between 24h urine protein and IFN- γ .

Table (4): Correlation of IL-4 with laboratory investigations of SLE patients.

| | | IL-4 |
|--------------------------|----------------------------|-------------|
| SLEDAI | Pearson Correlation | -0.195 |
| | P-value | 0.136 |
| anti-dsDNA | Pearson Correlation | -0.199 |
| | P-value | 0.127 |
| 24h urine protein | Pearson Correlation | -0.014 |
| | P-value | 0.918 |
| C3 | Pearson Correlation | -0.007 |
| | P-value | 0.959 |
| C4 | Pearson Correlation | 0.153 |
| | P value | 0.244 |

-IL-4: interleukin 4.

- C3: complement 3.

-Anti-dsDNA: anti-double-stranded DNA.

-SLEDAI : SLE Disease Activity Index.

-C4: complement 4.

Discussion:

SLE is an autoimmune impairment defined by the development of several polyclonal autoantibodies, as well as the existence of many molecular and cellular abnormalities in the immune system, inducing inflammation in addition to damaging various organs^[1]. SLE is a chronic disorder that may be fatal if vital organs are compromised but usually causes persistent debilitating sickness^[2].

The pathophysiology of SLE is unknown. Nevertheless, it is hypothesized to be the consequence of a complex combination of different environmental, hormonal, and genetic factors^[15]. IFN- γ has just one member and is generated by multiple cells like T cells as well as NK cells^[16]. Th2 cells, mast cells, basophils, and NKT cells all generate IL-4, which has a key part in the regulation of various antigen-triggered naïve T cells to be developed into IL-4 generating Th2 via signaling of IL-4R^[17]. This study attempted to determine the contributions of IFN- γ , and IL-4 as biomarkers in cases with SLE.

In this study, substantially elevated IFN- γ serum levels were found in cases with SLE in comparison with controls, and this was in agreement with many studies.^[18-19-20]

IFN- γ stimulates classes I and II MHC molecule transcription and thus the subsequent severity and development of SLE^[21]. IFN- γ contributes to the autoimmune process by stimulating the production of IgG antibodies that promote the inflammation of tissues, activate macrophages, as well as trigger complement^[22]. IFN- γ participates in the progression of cutaneous manifestations such as malar rash, alopecia, inflammatory arthritis, and LN^[23].

Yu and Wang.^[24] found that the level of IFN- γ was diminished in the SLE group compared to controls. They attributed their findings to the fact that Th2 or Th cells might be characterized by varying functions in disease phases, and it can also be attributable to the small number of subjects in their research, reaching 20 subjects.

In this study, substantially elevated IL-4 serum levels were found in SLE cases than in controls, and this result was consistent with different studies^[25-26]. IL-4 contributes to the rescue of B cells from apoptosis, promoting the survival of autoreactive B lymphocytes, inducing the switching of antibody isotype class, resulting in elevated affinity and pathogenic autoantibodies,

treatment of IL-4 activated the secretion of IgG anti-dsDNA antibodies^[27].

Other studies documented that IL-4 levels were lower in the SLE cohort than in controls^[18-28-29]. Many studies reported a similar level of IL-4 in SLE cases compared with controls^[30-31].

A substantial positive relationship was detected between SLEDAI and IFN- γ , as confirmed by other studies^[18-32], along with substantial differences in IFN- γ serum concentrations between mild and highly elevated activity groups proven to be substantially elevated in the very high activity cohort. Other studies reported no relation between SLEDAI and IFN- γ , which was due to various sample sizes, the diversity of laboratory as well as clinical symptoms in SLE, and variations in the ELISA test's sensitivity^[31]. This study revealed no marked relation between SLEDAI and IL-4, which is compatible with prior studies.^[18-31]

In this study, there was a substantial negative correlation between C4 with IFN- γ . Moreover, the association of the three-two cytokines' levels with C3 demonstrated no negative link between C3 and IL-4 and IFN- γ , and these results are confirmed by several studies^[18-33].

It was found that there was a marked positive association between IFN- γ and anti-dsDNA, which is compatible with different studies^[34-35]. In contrast, no significant relation was detected between anti-dsDNA and IL-4 and SLE cases, which agrees with different studies^[18-36]. This research revealed no substantial association between IFN- γ and IL-4 with 24-hour protein in the urine, which aligns with other studies^[18].

Conclusion:

IFN- γ and IL-4 serum levels were more elevated in SLE cases than in controls and were proven to contribute to SLE pathogenesis. IFN- γ serum levels

were positively linked with disease activity and anti-dsDNA, negatively linked with C4, and no significant correlation with C3 and 24 h protein in the urine. In addition, no marked relation was detected between 24 h protein in the urine, anti-dsDNA, IL-4 and SLEDAI, C3, and C4.

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