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Study of IL-35 Relation with Systemic Lupus Erythematous (SLE) and Effect Autoantibodies Because Disease

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Annotation: Background; Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of autoantibodies that target self-antigens. Interleukin-35 (IL-35) is a cytokine involved in immune regulation, and its potential role in SLE pathogenesis has gained attention in recent studies. **Aims of the study;** Investigate the relationship between Interleukin-35 (IL-35) levels and the presence of autoantibodies in patients with systemic lupus erythematosus (SLE), and to explore how IL-35 may contribute to the pathogenesis of the disease. **Methodology;** This study involved 176 SLE patients (132 females, 44 males) and 83 healthy controls (78 females, 5 males) from Al-Kafeel Hospital, Karbala, Iraq, from February 2 to October 10, 2024. IL-35 levels were measured using an ELISA kit, while autoantibodies were detected by chemiluminescent immunoassay (CLIA). Blood samples were processed and stored for analysis. Ethical approval and informed consent were obtained. **Result;** The demographic analysis revealed significant differences in gender distribution (P=0.0001) and age (P=0.0001) between patients and controls, with a higher proportion of females and patients over 50 years. Biomarker analysis showed significant increases in SSB-LA, ds-DNA, ANA,

SS-A/Ro, and U1-SnRNP levels in patients compared to controls, with P-values ≤0.01. IL-35 levels showed no significant difference (P=0.124). No significant gender-based differences were observed, except for ANA. Age-related increases in ds-DNA, U1-SnRNP, and IL-35 were noted, with P-values of 0.0102, 0.049, and 0.019, respectively. **Conclusions**; The study highlights significant increases in autoantibodies (SSB-LA, ds-DNA, ANA, SS-A/Ro, and U1-SnRNP) in SLE patients, suggesting a strong immune response in disease pathogenesis. IL-35, however, did not show a distinct role, indicating potential complex immune interactions in SLE.

Keywords: Systemic lupus erythematosus (SLE), Autoantibodies, SSB-LA, ds-DNA, IL-35, Immune dysregulation.

Introduction:

An autoimmune condition known as systemic lupus erythematous (SLE), or lupus, occurs when the body's immune system unintentionally targets healthy tissue throughout the body. Individuals may have mild to severe symptoms. Painful and swollen joints, fever, chest pain, mouth ulcers, enlarged lymph nodes, hair loss, fatigue, and a red rash usually on the face are typical symptoms. If one identical twin has the condition, there is a 24% chance that the other twin will also [1]. SLE is believed to be caused by a confluence of environmental and genetic factors. Smoking and female sex hormones are also thought to raise a person's risk [3]. Autoantibodies' immunological reaction against an individual's own tissues is the mechanism. These antibodies cause inflammation and are most frequently anti-nuclear. A mix of symptoms and laboratory testing are used to make the diagnosis, which can be challenging. SLE has no known cure [1], however there are symptomatic and experimental therapies [4]. NSAIDs, corticosteroids, immunosuppressants, methotrexate, and hydroxychloroquine are possible treatments [5]. The condition has not been demonstrated to be impacted by alternative medicine. The majority of pregnancies among women with lupus are successful, despite their increased risk [1]. Since cardiovascular disease is the leading cause of death, SLE greatly raises the risk of developing it. Approximately nine times as many young women as men are impacted [3]. Individuals with Chinese, African, and Caribbean ancestry are more vulnerable than individuals with European ancestry [2]. When a newborn delivered to a woman with SLE develops symptoms of the disease, it is known as neonatal lupus. This condition typically manifests as a discoid lupus erythematous rash. The majority of the time, neonatal lupus is benign and self-limiting [22]. Fatigue, joint and muscle problems, fever, and malaise are common first and persistent complaints. These signs and symptoms are not included in the diagnostic criteria for SLE since they are frequently observed in conjunction with other illnesses. However, they are regarded as suggestive when they occur in combination with other symptoms and indicators [7]. Although SLE can affect both men and women, it is far more common in women, with a rate of roughly 9 to 1, and the symptoms differ for each sex [6]. Psychiatric symptoms, arthritis, Raynaud syndrome, low white blood cell counts, and relapses are

more common in women. Seizures, renal disease, serositis (inflammation of the tissues lining the heart and lungs), skin disorders, and peripheral neuropathy are more common in men [8]. Osteoarticular tuberculosis is especially dangerous for people with SLE [10]. One potential link between SLE and a higher risk of bone fractures in women who are quite young is [11]. Additionally, anemia occurs in approximately 50% of children with SLE [12,13]. Low white blood cell counts (leukopenia) and platelet counts (thrombocytopenia) might result from the illness or as a side effect of medication [14]. Myocarditis, which is an inflammation of the heart muscle, endocarditis, which is an infection of the inner lining of the heart, and pericarditis, which is an inflammation of the outer membrane around the heart, can all be brought on by SLE [15]. The sole obvious indication of renal involvement is frequently the painless passage of blood or protein in the urine. Lupus nephritis can cause acute or chronic renal impairment, which can result in acute or end-stage kidney failure [17]. When SLE impacts the central or peripheral nervous system, neuropsychiatric disorders may develop [18]. Cerebrovascular disease [19], depression, cognitive difficulties, and in severe cases, personality abnormalities [20] are other common neuropsychiatric manifestations of SLE. Patients say that the condition affects their eyes. The most prevalent conditions include retinal detachment, secondary Sjögren's syndrome, and dry eye syndrome [21]. Although there isn't a cure for SLE, there are numerous ways to manage the condition [1]. Between the ages of 45 and 64, the rate is higher among females. Nevertheless, there is insufficient data to draw the conclusion that SLE is less prevalent in some nations than others; environmental variability may be the cause. For instance, sunshine levels vary by nation, and exposure to UV radiation impacts SLE dermatological symptoms [2]. The higher prevalence of SLE in women may be due to hormonal processes. Elevated hydroxylation of estrogen and unusually low levels of androgens in females may be the cause of the start of SLE. Furthermore, it has been demonstrated that variations in GnRH signaling play a role in the development of SLE. Although relapses are more common in women than in men, both sexes experience relapses of the same severity [8]. A blood test that detects antinuclear antibodies in the blood is called an ANA test. The immune system produces proteins called antibodies to combat invading things like germs and viruses. An antinuclear antibody, however, targets your own healthy cells. Because it targets the cell's nucleus, or center, it is referred to as "antinuclear" [23]. A small amount of antinuclear antibodies in the blood is normal. However, a high number could indicate an autoimmune disease. When someone has an autoimmune disease, their immune system unintentionally targets their organs and tissues. Serious health issues may result from these conditions [24]. The most prevalent form of lupus, systemic lupus erythematosus (SLE), can be diagnosed with the aid of an ANA test. Numerous physiological parts, including the joints, skin, heart, lungs, blood vessels, kidneys, and brain, are impacted by lupus, a chronic (long-lasting) illness [25]. Traditionally linked to SLE, SS, subacute cutaneous lupus erythematosus (SCLE), and neonatal lupus erythematosus (NLE), anti-Ro/SSA and anti-La/SSB antibodies are among the most commonly found autoantibodies [26]. Anti-Ro/SSA and anti-La/SSB antibodies are referred to as "anti-Ro antibodies" in honor of the patient who first had them detected. Antibodies to "La," another soluble cytoplasmic RNA protein antigen, were also discovered by the same team [27]. Ro and La were later shown to have the same antigenic makeup as SSA and SSB [28]. Anti-Ro/SSA antibodies are mainly detected in patients with SLE and SS, but they can also occasionally be detected in individuals with other systemic autoimmune disorders, including rheumatoid arthritis (RA), mixed connective tissue disease (MCTD), polymyositis/dermatomyositis (PM/DM), and systemic sclerosis (SSc) [29]. It is common to find anti-Ro antibodies linked to NLE [32], SS/SLE overlap syndrome [31], and SLE [30]. On average, anti-Ro and anti-La antibodies are detected 3.4 years prior to the diagnosis of SLE, which is sooner than other SLE-related autoantibodies including anti-dsDNA, anti-ribonucleoprotein (RNP), and anti-Sm antibodies [33]. According to a different study, anti-Ro antibodies are the type of autoantibody that manifests earliest before symptoms start, with an average age of 6.6 years [34]. The body produces autoantibodies that harm and inflame its own healthy cells, tissues, and/or organs. The time between the start of some autoimmune disorders and their clinical manifestation can be incredibly protracted. The early

detection and management of autoimmune disorders are greatly aided by autoantibodies. Anti-double stranded (ds) DNA antibodies are currently utilized as serum indicators to detect SLE (35), but because of their low sensitivity and high specificity (36), they do not support an early diagnosis. Double-stranded DNA is the target antigen of a class of anti-nuclear antibodies (ANA) known as anti-double stranded DNA (Anti-dsDNA) antibodies. Diagnostic labs frequently use blood tests including immunofluorescence and enzyme-linked immunosorbent assay (ELISA) to find anti-dsDNA antibodies. They play a role in the pathophysiology of lupus nephritis and are highly diagnostic of systemic lupus erythematosus (SLE) [37]. These aberrant cells are classified as polymorphonuclear leukocytes with phagocytosed entire nuclei and are seen in the bone marrow of individuals with SLE [38]. One factor contributing to an immunological reaction against dsDNA is extracellular DNA. Studies have shown that anti-dsDNA antibodies are almost 100% specific for SLE, which is why they are employed to diagnose the disease. People without SLE may have lower anti-dsDNA antibody titres, while those with higher titres are more suggestive of the condition. The sensitivity of anti-dsDNA in SLE has been estimated to be between 25 to 85%, which contrasts with the high specificity. Consequently, anti-dsDNA antibodies are suggestive of SLE, but their absence does not rule out the condition. Increases in circulating anti-dsDNA antibody titres can lead to an increase in disease activity. Because of this, physicians track titres over time to evaluate the course of the disease [39]. After autoantibodies attach to autoantigens, immunological complexes (ICs) are formed, which triggers inflammatory reactions [40]. Immune cells communicate with one another through cytokines, which are immune system signaling mediators [41]. In SLE, inflammatory cells secrete inflammatory cytokines, which in turn cause inflammation. There is proof that the pathophysiology of SLE is linked to alterations in cytokine levels [42]. Epstein-Barr virus induced gene 3 (EBI3) and p35 are the two components that make up the cytokine IL-35. The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is the channel via which this heterodimer cytokine communicates [43]. The equilibrium of serum IL-35 levels is a crucial component of health status, given the elevated and lowered levels of IL-35 in cancer and autoimmune illnesses. For instance, in primary and secondary type I diabetes, IL-35 is believed to prevent the immune system from attacking beta cells. Conversely, in some malignancies, immunosuppression is linked to high levels of IL-35 [44]. Peripheral blood IL-35 levels are higher in adult patients with acute myeloid leukemia than in healthy people, confirming IL-35's function as a human leukemia biomarker [44]. Moreover, a new target for cancer treatment is IL-35 in the tumor microenvironment (TME) [45]. Neutralization of IL-35 has been shown to restrict tumor growth in a number of human cancer models [46]. Stated that in genetically vulnerable hosts, environmental variables can cause SLE. Furthermore, elevated apoptosis and abnormalities in the removal of apoptotic bodies are linked to SLE [47]. This family of cytokines can influence immunological responses in both positive and negative ways. The two unique subunits that make up each member of this family are subunit-α and subunit-β. One heterodimeric cytokine with anti-inflammatory qualities is IL-35 [48]. Human U1 spliceosomal RNA has a 5'-trimethylguanosine five-prime cap, is 164 bases long, and generates four stem-loops. During RNA splicing, bases 126 to 133 form the Sm site, which is where the Sm ring is put together, and bases 3 to 10 form a conserved sequence that base pairs with the 5' splice site of introns [50]. The receptors of the IL-12 family members that share subunits should be taken into consideration in order to obtain a better understanding of the IL-35 signaling pathway. IL-35 is an anti-inflammatory cytokine that may drive the development of immunosuppressive cells while inhibiting inflammatory cells [43].

Methodology:

Apparatus and Reagents:

This study utilized various laboratory instruments, including a bench centrifuge (VEB, Germany), an ELISA reader (Calabasas, USA), and chemiluminescent immunoassay (CLIA) and immunodiffusion chromatography (IDC) systems from England. IL-35 levels were assessed using Bioworld Technology kits (USA).

Study Design and Participants:

A total of 176 systemic lupus erythematosus (SLE) patients (132 females, 44 males) and 83 healthy controls (78 females, 5 males) were recruited from Al-Kafeel Hospital, Karbala, Iraq, between February 2, 2024, and October 10, 2024. Ethical approval was obtained, and informed consent was secured from all participants. Data were collected through direct interviews and medical records.

Sample Collection and Preparation:

Venous blood (5 mL) was drawn from each participant, allowed to clot, and centrifuged at 1000 x g for 15 minutes. The obtained serum (3 mL) was stored at -20°C for subsequent IL-35 and autoantibody measurements.

Serological Analysis:

IL-35 levels were quantified using an ELISA kit following the manufacturer's protocol. Autoantibodies, including dsDNA, ANA, SS-A/Ro, SS-B/La, and U1-snRNP, were detected using the IDS ANA Screen chemiluminescent immunoassay (CLIA).

Assay Procedure:

The ELISA assay involved serial dilution of IL-35 standards (39–2500 pg/mL), incubation of samples with biotin-labeled detection antibodies, streptavidin-HRP, and substrate reaction at 37°C. Optical density (O.D.) was read at 450 nm, and concentrations were extrapolated from the standard curve. The CLIA method employed an automated IDS-iSYS system for antibody quantification using antigen-coated magnetic particles and chemiluminescence detection.

Statistical analysis:

Data were analyzed using appropriate statistical software. Mean values, standard deviations, and significance levels ($p<0.05$) were determined to evaluate differences between groups.**Ethical approval:**

The study was approved by the human ethics committee of Al-Imamain Alkadhimain Medical City, Everyone who took part in the study was told about it and asked to sign a consent form. The patient was also guaranteed that his information would be kept private.

Results

Demographic Characteristics of Patients and Control Group

The analysis of demographic characteristics revealed a significant difference in gender distribution between patients and controls (P=0.0001). The majority of patients were female (75.00%) compared to (93.98%) in the control group. Additionally, age distribution showed a statistically significant difference (P=0.0001), with a higher proportion of patients aged over 50 years (46.59%) compared to only (14.46%) in the control group. The mean age of patients (46.19 ± 0.97 years) was significantly higher than that of the control group (36.61 ± 1.60 years) (P = 0.0002).

Table 1: Comparison of Gender and Age Distribution Between Patients and Control Group

Factor		Patients (No=176)	Control (No= 83)	P-value
Gender	Male	44 (25.00%)	5 (6.02%)	0.0001 **
	Female	132 (75.00%)	78 (93.98%)	
Age groups (year)	<40 yr.	40 (%)	47 (56.63%)	0.0001 **
	40-50 yr.	54 (%)	24 (28.92%)	

	>50 yr.	82 (%)	12 (14.46%)	
Age	Mean ±SE	46.19 ±0.97	36.61 ±1.60	0.0002 **
** (P≤0.01).				

Comparison of SSB-LA Levels Between Patients and Control Group

The statistical analysis showed a highly significant increase in SSB-LA levels among patients (221.63 ± 18.91) compared to the control group (74.05 ± 20.31) (P=0.0001). The T-test value (60.814) further confirms the significant difference (P≤0.01), indicating a strong association between elevated SSB-LA levels and the patient group.

Table 2: Mean ± SE of SSB-LA in Patients and Control Group

Group	Mean ±SE of SSB-LA
Patients	221.63 ±18.91
Control	74.05 ±20.31
T-test	60.814 **
P-value	0.0001
** (P≤0.01).	

Comparison of ds-DNA Levels Between Patients and Control Group

The analysis revealed a highly significant increase in ds-DNA levels among patients (281.59 ± 16.34) compared to the control group (9.10 ± 0.63) (P=0.0001). The T-test value (46.916) further supports this significant difference (P≤0.01), indicating a strong correlation between elevated ds-DNA levels and the patient group.

Table 3: Mean ± SE of ds-DNA in Patients and Control Group

Group	Mean ±SE of ds-DNA
Patients	281.59 ±16.34
Control	9.10 ±0.63
T-test	46.916 **
P-value	0.0001
** (P≤0.01).	

Comparison of ANA Levels Between Patients and Control Group

The findings indicate a significantly higher ANA level in patients (6.72 ± 0.68) compared to the control group (1.79 ± 0.32) (P=0.0001). The T-test value (1.997) confirms this statistical significance (P≤0.01), suggesting a strong association between elevated ANA levels and the patient group.

Table 4: Mean ± SE of ANA in Patients and Control Group

Group	Mean ±SE of ANA
Patients	6.72 ±0.68
Control	1.79 ±0.32
T-test	1.997 **
P-value	0.0001
** (P≤0.01).	

Comparison of SS-A/Ro Levels Between Patients and Control Group

The results demonstrate a significantly higher SS-A/Ro level in patients (337.38 ± 20.58)

compared to the control group (126.48 ± 21.22) (P=0.0001). The T-test value (5.658) confirms the statistical significance (P≤0.01), highlighting a strong association between increased SS-A/Ro levels and the patient group.

Table 5: Mean ± SE of SS-A/Ro in Patients and Control Group

Group	Mean ±SE of SS-A\RO
Patients	337.38 ±20.58
Control	126.48 ±21.22
T-test	5.658 **
P-value	0.0001
** (P≤0.01).	

Comparison of U1-SnRNP Levels Between Patients and Control Group

The findings indicate a significantly elevated U1-SnRNP level in patients (28.88 ± 2.23) compared to the control group (9.65 ± 0.92) (P=0.0001). The T-test value (6.514) confirms the statistical significance (P≤0.01), suggesting a strong correlation between increased U1-SnRNP levels and the patient group.

Table 6: Mean ± SE of U1-SnRNP in Patients and Control Group

Group	Mean ±SE of U1-SnRNP
Patients	28.88 ±2.23
Control	9.65 ±0.92
T-test	6.514 **
P-value	0.0001
** (P≤0.01).	

Comparison of IL-35 Levels Between Patients and Control Group

The results show no significant difference in IL-35 levels between patients (141.94 ± 2.98 ng/dl) and the control group (150.31 ± 4.72 ng/dl) (P=0.124). The T-test value (12.667) indicates that the difference is statistically non-significant (NS), suggesting that IL-35 may not play a distinguishing role between the two groups.

Table 7: Mean ± SE of IL-35 (ng/dl) in Patients and Control Group

Group	Mean ±SE of IL-35 (ng/dl)
Patients	141.94 ±2.98
Control	150.31 ±4.72
T-test	12.667 NS
P-value	0.124
NS: Non-Significant.	

Gender-Based Comparison of Autoantibodies and IL-35 Levels in Patients

The comparison of biomarker levels between male and female patients showed no statistically significant differences in SSB-LA (P=0.211), ds-DNA (P=0.129), SS-A/RO (P=0.337), U1-SnRNP (P=0.576), and IL-35 (P=0.119). However, ANA levels were significantly higher in females (7.59 ± 0.82) compared to males (4.10 ± 1.02, P=0.0258), indicating a possible gender-related variation in ANA expression.

Table 8: Mean ± SE of SSB-LA, ds-DNA, ANA, SS-A/RO, U1-SnRNP, and IL-35 (ng/dl) in Male and Female Patients

Gender	Mean ±SE					
	SSB-LA	ds-DNA	ANA	SS-A\RO	U1-SnRNP	IL-35 (ng/dl)
Male	180.47 ±33.31	239.21 ±27.98	4.10 ±1.02	303.22 ±41.03	26.73 ±4.43	133.89 ±6.91
Female	235.35 ±22.58	295.72 ±19.59	7.59 ±0.82	348.77 ±23.79	29.59 ±2.58	144.62 ±3.22
P-value	0.211 NS	0.129 NS	0.0258 *	0.337 NS	0.576 NS	0.119 NS
* (P≤0.05), NS: Non-Significant.						

Age-Based Variations in Autoantibody and IL-35 Levels in Patients

The analysis of biomarker levels across age groups showed a significant increase in ds-DNA levels (P=0.0102) and U1-SnRNP levels (P=0.049), with the highest ds-DNA levels observed in patients older than 50 years (318.33 ± 27.05) compared to those under 40 (222.53 ± 29.65). IL-35 levels were also significantly different (P=0.019), showing an increasing trend with age. However, differences in SSB-LA (P=0.582), ANA (P=0.098), and SS-A/RO (P=0.136) were not statistically significant.

Table 9: Mean ± SE of SSB-LA, ds-DNA, ANA, SS-A/RO, U1-SnRNP, and IL-35 (ng/dl) Across Different Age Groups

Age (year)	Mean ±SE					
	SSB-LA	ds-DNA	ANA	SS-A\RO	U1-SnRNP	IL-35 (ng/dl)
<40	267.0 ±44.07	222.53 ±29.65	5.33 ±1.16	271.17 ±41.02	36.52 ±4.58	131.68 ±5.37
40-50	12681 ±23.38	269.56 ±24.61	5.85 ±1.03	355.23 ±38.34	28.93 ±4.35	139.08 ±5.12
>50	261.95 ±29.34	318.33 ±27.05	7.96 ±1.14	357.92 ±30.02	25.11 ±3.05	148.81 ±4.66
P-value	0.582 NS	0.0102 **	0.098 NS	0.136 NS	0.049 *	0.019 *
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.						

Discussion:

The autoimmune condition known as systemic lupus erythematous (SLE) is typified by activation of the type I interferon system, dysregulated autoantibody synthesis, and involvement of several organs [55]. With a female to male ratio of 9–10:1 [57], SLE is one of the most prevalent chronic rheumatic disorders throughout the spectrum in women [56]. Although it is well acknowledged that SLE and the majority of rheumatic disorders are characterized by a female preponderance, the pathophysiological processes behind the sexual dimorphism remain unknown. Numerous theories have been proposed to explain this sex bias, including the gut microbiota [63], sex-dependent environmental factors [62], sex hormones [59], sex chromosomes [60], sex differences in gene regulation [61], and intrinsic sex differences of the immune system [58]. In my study in table (11) difference between patient and control in gender parameter is non-significant, significant and very significant according parameters. One of the most prevalent SLE serum autoantibodies is the anti-SSB antibody, which is generated before SLE symptoms appear (64). According to earlier research, the anti-SSB antibody plays a major role in the pathophysiology of SLE and remains largely stable throughout the disease. The fact that the anti-SSB antibody in this study is unaffected by the SLEDAI score lends credence to this. Among SLE patients, the anti-SSB antibody positivity rate was 25.7%. Variations in detecting techniques could be the cause of the

discrepancy between our results and those of earlier research. According to studies, the specificity of anti-SSB antibodies for the diagnosis of SLE can reach 96.7% when rheumatoid arthritis (RA) and primary Sjögren's syndrome (pSS) are ruled out. Sometimes a diagnosis cannot be made right away, and in these situations, patients should be continuously monitored to prevent misdiagnosis and treatment delays. Increased interest in the topic has been sparked by studies that have demonstrated the role of the anti-SSB antibody in the development of an idiotype-anti-idiotype network in the pathophysiology of SLE (65). In my study in table (5) and figure 1, difference between patient and control in SSB-LA parameter is very significant $P \leq 0.01$. Among the most commonly found autoantibodies against ENA are anti-Ro/SSA and anti-La/SSB antibodies, which have historically been linked to SLE, SS, subacute cutaneous lupus erythematosus (SCLE), and neonatal lupus erythematosus (NLE) [66]. Systemic sclerosis (SSc), polymyositis/dermatomyositis (PM/DM), mixed connective tissue disease (MCTD), and rheumatoid arthritis (RA) are among the other systemic autoimmune disorders that might occasionally exhibit anti-Ro/SSA antibodies, while SLE and SS patients are the main ones [67]. There are theories suggesting that anti-Ro antibodies may directly cause tissue damage, even though the pathogenic significance of autoantibodies in autoimmune illness is still unclear. The cytoplasm and nucleus of keratinocytes both undergo de novo production of the Ro antigens in response to UV exposure. Additionally, the expression of antigens on the cell surface is increased by UV irradiation [68]. In my study in table (8) and figure (4) difference between patient and control in SSA\RO parameter is very significant $P \leq 0.01$. In a Chinese population, studies found that single nucleotide polymorphisms (SNPs) in the IL-35 gene were linked to genetic vulnerability to rheumatoid arthritis and SLE (71). Furthermore, in a Chinese SLE population, EBI3 subunit polymorphisms were linked to renal disease, namely rs4740, which influences both genotype frequency and allele frequency (70). In an Egyptian group, however, this rs4740 polymorphism was not linked to an increased risk of SLE (72). Therefore, the rs4740 SNP might be the key element that preferentially speeds up the evolution of SLE in the Chinese population but not in the Egyptian one. For the first time, this meta-analysis demonstrated that IL-35 levels were considerably lower in Chinese SLE patients—including those with active disease and those with dormant SLE—than in healthy controls. Compared to those in remission, Chinese patients with active SLE had lower levels of IL-35. Nonetheless, there was no statistically significant difference between those with active SLE and healthy controls in the pooled standardized mean difference of IL-35 levels across all nations. Furthermore, in the analysis, the drug had no effect on the levels of circulating IL-35 in the entire group of SLE patients. SLE is a complicated autoimmune illness that causes organ failure (73), and in order to treat and avoid complications, it must be detected early (74). Another important element that speeds up the onset of autoimmune diseases is an imbalance of pro-inflammatory and anti-inflammatory cytokines (69). Autoimmune illnesses, including SLE, have been linked to lower serum levels of anti-inflammatory cytokines such as IL-10, TGF-β, and IL-35. According to earlier research, IL-35 gene variants linked to the Chinese Han population's risk of SLE are connected to the blood level of IL-35. Furthermore, in Chinese SLE patients, IL-35 mutation, specifically the EBI3 polymorphism rs4740, is strongly linked to renal and haematological disorders, which are clinical manifestations of active SLE. IL-35 levels were lower in Chinese patients with active SLE than in Egyptian patients, according to a subgroup analysis comparing active and inactive SLE. Consequently, this outcome corroborated the earlier study's findings that IL-35 polymorphisms that impact SLE disease activity in the Chinese population may have an impact on IL-35 levels. This finding suggested that the Chinese population with SLE may have a reduced capacity for IL-35 production. This could be related to genetic polymorphism in IL-35 (rs4740), which was found to be common in Chinese SLE patients (70) but not in Egyptian SLE patients (72). Variations in IL-35 levels and clinical phenotype can have a big impact on how well a medication works. The management of SLE patients in various nations is made extremely difficult by this heterogeneity, which highlights the possibility of creating individualized treatment plans like customized medicine (75). In my study in table (10) and figure (5), difference between patient and control in IL-35 parameter is non-significant $P = 0.124$. A higher titer of anti-dsDNA

antibodies is seen in SLE patients with active diffuse neuropsychiatric complications as opposed to patients with focal neuropsychiatric or non-inflammatory central nervous system disease, even though an elevated serum level of anti-dsDNA antibodies is not always associated with neurological dysfunctions or neuropsychiatric activity [76]. In my study in table (6) and figure 2, difference between patient and control in dsDNA parameter is very significant P≤0.01. Despite the low overall frequency, ANA-negative SLE was seen in the great majority of studies, irrespective of ethnicity or geography [78]. The SLICC classification criteria for ANA-negative SLE necessitate a diagnosis [77]. However, the SLICC classification criteria are not diagnostic criteria; they are merely a categorization criteria for SLE. This certainly makes diagnosing SLE, especially ANA-negative SLE, more challenging. A significant obstacle to diagnosing ANA-negative SLE is that rheumatologists and other medical professionals are likely to overlook it because of its low prevalence and difficulty in identifying it. Studies on ANA-negative SLE are currently scarce, and the impact of glucocorticoids on ANA detection is not considered. The substrate for ANA detection changes, which is one of the most significant causes [80]. In the Hep-2 cell era, antinuclear antibody-negative SLE is quite uncommon. Recent cohort studies have demonstrated that ANA has a high positive rate in SLE, with various studies reporting a range of 96.8 to 99.8% [81]. To put it another way, ANA-negative SLE is extremely uncommon but does exist, which makes early diagnosis and therapy extremely difficult. In patients whose ANA are negative for IIF, anti-dsDNA may be found using alternative techniques. Numerous research have also proven this. CIA found that 11.3% of patients with anti-cellular antibody-negative SLE and 28.4% of patients with ANA had elevated anti-dsDNA. Therefore, it is crucial to detect anti-dsDNA using a single analytical test, even in circumstances when the ANA is negative. The test aids in risk assessment for clinical consequences and helps identify additional SLE patients [82]. In my study in table (7) and figure (3), difference between patient and control in ANA parameter is very significant P≤0.01. Small nuclear ribonucleoprotein particles (snRNPs), RNA polymerases, SS-A/Ro, SS-B/La, tRNA synthetases, signal recognition particles (SRP), and others are examples of RNA-associated molecules, a class of autoantigens (83). Systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), inflammatory myositis, and systemic sclerosis (SS) are among the rheumatic disorders that have been linked to autoantibodies that identify snRNPs. Indeed, snRNPs were first discovered to bind to autoantibodies in the sera of patients with MCTD and SLE (84). The percentage of anti-RNP antibodies was shown to differ significantly between SLE patients with biopsy-proven myositis and those without myositis (80% and 21%, respectively) in one study (85). Clinical characteristics of the SLE/myositis patients were erosive joint disease (60%) at initial presentation, interstitial lung disease (ILD) (100%), and Raynaud's phenomenon (100%) (86). In my study in table (9) and table (5), difference between patient and control in U1-SnRNP parameter is very significant P≤0.01. The survey's main finding is that only roughly 25% of respondents had SLE diagnosed within a year of their first symptoms, with a median reported diagnosis delay of 2 years (IQR: 0–6). The median age at SLE diagnosis of 30 years and the proportion of childhood-onset SLE were consistent with the majority of epidemiological studies in Europe. This is a lot less than what was previously stated in a sizable UK. patient survey (16). In my study in table (12) difference between patient and control in age parameter is non-significant, significant and very significant according parameters.

Conclusion:

The study demonstrates significant elevations in various autoantibodies (SSB-LA, ds-DNA, ANA, SS-A/Ro, and U1-SnRNP) in patients with systemic lupus erythematosus (SLE), highlighting their role in immune dysregulation and disease progression. The higher autoantibody levels reflect an abnormal immune response targeting self-antigens. However, IL-35 levels did not differ significantly between patients and controls, suggesting that IL-35 may not play a crucial role in distinguishing SLE from healthy individuals. These findings emphasize the complex immune mechanisms involved in SLE pathogenesis.

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