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Evaluation Role of (Integrin-alpha M Chain (ITGAM), Tumor Necrosis Factor (TNF) Alpha Induced Protein 3 (TNFAIP3) , (Interferon-alpha (IFN- α), and Toll-like Receptor-7 (TLR-7)) for Pathogenesis of Systemic Lupus Erythematosus in Iraqi Patients.

A thesis

Submitted to the Council of the College of Medicine, University of Kerbala, as Partial Fulfillment of the Requirements for the Master Degree of Science in Medical Microbiology

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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صَدَقَ اللهُ العَلِيُّ العَظيم

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We certify that this M.Sc. thesis titled (**Evaluation Role of (Integrin-alpha M Chain (ITGAM), Tumor Necrosis Factor (TNF) Alpha Induced Protein 3 (TNFAIP3) , (Interferon-alpha (IFN- α), and Toll-like Receptor-7 (TLR-7)) for Pathogenesis of Systemic Lupus Erythematosus in Iraqi Patients**) Was prepared under our supervision in the College of Medicine/ University of Karbala, as a partial fulfillment of the requirements for the Degree of Masters of Science in Medical Microbiology

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We, the examiner committee that we've read the M.Sc. thesis entitled: **(Evaluation Role of (Integrin-alpha M Chain (ITGAM), Tumor Necrosis Factor (TNF) Alpha Induced Protein 3 (TNFAIP3) , (Interferon-alpha (IFN- α), and Toll-like Receptor-7 (TLR-7)) for Pathogenesis of Systemic Lupus Erythematosus in Iraqi Patients)** we have examined the student **(Fatimah Abdul Hussein Kadhim)** in its contents. In our opinion it meets the standards of thesis for the master degree in medical microbiology and immunology

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DEDICATION

To ….. The ***holy Prophet Muhammad*** and his progeny (peace be upon them), especially lady of the Women of the Worlds, ***Fatima Al-Zahra, imams Hassan and Hussein ,imam of our time, the awaited Mahdi*** *(may God hasten his honorable release)* .

To ….. The dear ones who gave me their entire lives for my success and excellence my beloved ***father*** *(may God have mercy on him) and my virtuous beloved* ***mother*** .

To ….my support and my share of my joys and sorrows dear loved ones my lovely ***sister*** *and my* ***brothers*** .

To ….. The my ***grandmother*** *(may God have mercy on her).*
To…My teacher, inspiration, Seyed ***Muhammad Baqir Al-Fali*** *(may God have mercy on him).*

To all those who prayed for me with goodness.

Fatimah

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Fatimah

SUMMARY

Systemic lupus erythematosus (SLE) is a chronic multi-system autoimmune disease. The important characterize of this disease represented by the of excess autoantibodies generated when the immune system's tolerance to self-antigen is breakdown. After that, organs damage mediated by the deposition of immune complexes in tissues leads to the activation of complement, accumulation of neutrophils and monocytes, and self-reactive lymphocytes. Despite the difficulties associated with the estimated 134 cases of SLE per 100,000 people worldwide, research had shown that early detection of SLE reduces the risk of flare-ups, prompts use of healthcare services, and lessens the financial burden of the condition. For these reasons, scientists have worked to find possible genetic and secretory indicators of SLE over the years in an effort to help patients who are asymptomatic or subclinical discover the illness early on. Determining the critical signs for the non-invasive early diagnosis of SLE in people who may be at risk is therefore crucial.

The data of serum levels SLE-associated autoantibodies (ANA and anti-dsDNA), complement proteins (C3 and C4) that taken from patient reports to evaluate disease activity .The demographical and clinical features of SLE patients were evaluated . Also, use results of the serum levels of, cytokine IFN-α and TLR-7 receptor as well as expression levels of *ITGAM* and *TNFAIP3* genes were evaluated and comparisons were made between SLE patients and the non-SLE subjects.

Across sectional study was conducted for period of 10 month starting from November /2022 to August / 2023 ; the total number of participants were 120 subjects they were divided into two groups : the first one includes (60) patients with SLE and the second group includes (60) as apparently healthy control .laboratory tests were done by serological techniques of sandwich

ELISA , were tested for specific serum human IFN- α, TLR-7, a whole blood test was performed using PCR technology for gene expression specific ITGAM and TNFAIP3 .

The results obtained indicated the socio-demographical factors such as sex, and family history affects susceptibility to SLE. Results of serum values of ANA and anti-dsDNA autoantibodies , IFN-α and TLR-7 It were used to measure the extent of its relationship to the stages of lupus disease activity. The current statistical study showed that there is no relationship between these immune markers and disease activity, except for C4, which showed a significant relationship with disease activity with a probability value ($p = 0.005$). were also high levels in the SLE patients relative to the control (IFN-α, 344.79±137.98 U/mL SLE vs. 234.88±121.15 U/mL non-SLE ($p = 0.01$) and TLR-7, 786.48±244.68 U/mL SLE vs. 426.66±129.71 U/mL non-SLE ($p = 0.001$)). The expression of *ITGAM* and *TNFAIP3* were higher in the SLE patients compared to the control group by 15.48 and 43.08 folds respectively.

The current study suggest that the evaluation of the SLE disease activity by use SLEDIA score with help of previous results of autoantibodies (ANA, Anti dsDNA) , complement (C3, C4) cytokines (IFN-α and TLR-7) as well as expression levels of ITGAM and TNFAIP3 genes are useful markers for prognosis and management of SLE.

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ABBREVIATIONS

Abbreviations	Terms
ACR	American College of Rheumatology criteria
Anti-CCP	anti-cyclic citrullinated peptide antibodies.
Anti-ENA	Anti-extractable nuclear antigen antibodies
APC	Antigen presenting cells
ANAS	Antinuclear antibodies
CRP	C reactive protein
CD	cluster of differentiation
DC	Dendritic Cells
DNA	Deoxyribonucleic acid
DUB	de-ubiquitinase domain
dsDNA	Double-stranded DNA
GWAS	Gnome-wide association studies
ELISA	Enzyme-linked immunosorbent assay
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate,
EDTA	Ethylene Diamine tetra acetic acid
FcR	Fc receptors
HLA	Human Leukocyte Antigen
HCQ	hydroxychloroquine
IIFA	indirect immunofluorescence assay
ITGAM	Integrin-alpha M Chain
IFN	Interferon
IFN- α	Interferon-alpha
IL	Interleukin
LN	Lupus Nigrites
MHC	major histocompatibility complex
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
PBMCs	peripheral blood mononuclear cells
PCR	Polymerase Chain Reaction
P-Value	Probability Value
PGA	Physicians Global Assessment
qRT	qualitative real-time
SLE	Systemic Lupus Erythematosus

Abbreviations	Terms
SNPs	single-nucleotide polymorphisms
SLICC	Systemic Lupus International Collaborating Clinics
FClq	fibrin clot lysis assay (fluid phase immune assay)
EULAR	The European League Against Rheumatism
SLEDAI	The Systemic Lupus Erythematosus Disease Activity Index .
SClq	Solid-phase quantitative immunoassays
TLR-7	Toll-like Receptor-7
TGF-β	Transforming Growth Factor-β
TNF	Tumour Necrosis Factor
TNFAIP3	Tumour Necrosis Factor Alpha Induced Protein 3
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase

Chapter One

Introduction
and
Literature Review

1.1 Introduction

systemic lupus erythematosus (SLE) is a chronic multi-system autoimmune illness, affects multiple organ systems. It is characterized by the overabundance of autoantibodies produced as a result of the immune system's tolerance to self-antigen being broken. Following this, immunologically mediated tissue damage and the development of circulating immune complexes occur (Dahham *et al.,* 2022).

Ineffective apoptotic clearance, immunological system dysregulation, complement activation, immune complexes, and tissue inflammation are characteristics of SLE. These elements lead to a self-replicating autoimmune phenomenon with diverse clinical manifestations and changing patterns over time, making diagnosis a major problem that depends on clinical competence (Fava and Petri, 2019). According to (Pakpoor *et al* 2018), there is a notable gender imbalance in the condition, with a 9:1 sex ratio favouring females.

Researchers still don't fully understand the precise cause of this sickness. However, there is evidence that both hereditary and environmental variables work together to trigger immunological responses, which then cause B cells to produce autoantibodies and cause cytokine dysregulation, both of which damage tissue and organs. SLE is indicated by the presence of antibodies that target cytoplasmic and nuclear antigens (Karrar and Cunninghame Graham, 2018). The symptoms of SLE can be mild or severe, impact one or more organ systems, and change over time. This makes diagnosing this disease difficult at times. Skin rashes, like the malar "butterfly rash," arthritis, pleurisy, serositis, alopecia, and lupus nephritis are common symptoms of systemic lupus flare-ups. Regretfully, the fact that therapy response can vary and be challenging to predict may frustrate both physicians and patients (Lazar and Kahlenberg, 2023). The illness in question presents with a broad range of clinical manifestations, from mild

skin involvement to significant organ damage, such as kidney failure, pulmonary hypertension, and heart failure. Diagnostic decisions for SLE are based on both clinical and laboratory data. The European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) are presently using the improved classification criteria (Ameer et al., 2022). Within the context of routine clinical practice, the evaluation and tracking of patients with SLE encompass a number of factors, such as: assessing the disease status in terms of activity, damage, and end-organ dysfunction; monitoring adverse drug reactions to immunosuppressive medications and performing hydroxychloroquine (HCQ) eye screenings to ensure medication safety; advocating for preventive health through yearly immunizations; attending to the reproductive health needs of both adults and adolescents; and overseeing the transition of care for child and adolescent patients to adult facilities. These characteristics have been refined into clinical quality indicators by the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) for use in normal care (Thong and Olsen, 2017).

1.2 Significance of the Study

Despite the difficulties associated with the estimated 13–7,713.5 cases of SLE per 100,000 people worldwide, research has shown that early detection of SLE reduces the risk of flare-ups, prompts use of healthcare services, and lessens the financial burden of the condition (Ameer *et al*., 2022). For these reasons, scientists have worked to find putative genetic and secretory indicators of SLE over the years in an effort to help patients who are asymptomatic or subclinical discover the illness early on. Five of these genes are persistently dysregulated in patients with SLE, according to replicated observational studies. These genes are called TNF-alpha-induced protein 3 (*TNFAIP3*) and human integrin alpha M chain (*ITGAM*) (Lingyan, 2018).

Furthermore, cytokine abnormalities lead to immunological failure, initiate inflammation, and cause organ damage in addition to genetic triggers. Interferons IFN-α and IFN-y, which are secreted by immune complexes and cause the overexpression of several inflammatory proteins produced by SLE PBMCs, are the major cytokine signatures involved in the pathogenesis of SLE. Thus, in addition to interferons, other key players in SLE have been shown to be inflammatory cytokines such as interleukin-6 (IL-6), IL-1, and TNF-α, as well as immunomodulatory cytokines like IL-10 and TGF-β. Furthermore, it has recently been determined that IL-21 and IL-17 are relevant in autoimmunity, and new study on IL-2 has refocused attention on this cytokine in SLE studies (Fava and Petri, 2019).

In this study, the expression levels of two SLE-associated genes *ITGAM* (encoding the human integrin alpha M chain, which is essential for mediating cell adhesion to integrin ligands, phagocytosis, and inflammatory cytokine production in macrophages) and *TNFAIP3* (encoding TNF-induced protein 3, a potent suppressor of NF-kB pathways for immune homeostasis) as well as two SLE-associated cytokines, IFN-α and TLR-7 were assessed in patients with SLE in comparison to healthy non-SLE participants. The results of this investigation may point to important indicators for the non-invasive early diagnosis of SLE in those who may be at risk.

1.3 Aim of the Study

The evaluate immune-related gene and cytokine markers of SLE by analysing their expression levels in serum of patients in comparison with non-SLE participants (controls), in correlation with ANA and anti-dsDNA values as well as complement proteins C3 and C4. These goals are achieved through the following objectives :

i. To evaluate the distribution of the study subjects with respect to demographical and clinical characteristics.
ii. To evaluate the disease activity of SLE with titer values of ANA and anti-dsDNA, and complement proteins C3 and C4 in the SLE patients
iii. To measure the levels of IFN-α and TLR-7 in serum of the patients and compare with control by ELIZA .
iv. To determine the expression levels of *ITGAM* and *TNFAIP3* genes in patients with SLE in comparison with controls using qualitative real-time (qRT) PCR.
v. To elucidate the association between expression of genes *ITGAM* and *TNFAIP3* and cytokines IFN-α and TLR-7 in the study subjects.

1.4 Literature review

1.4.1 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by overproduction of autoantibody, immune complex deposition inflammation and immune-mediated injury to multiple organ systems, including the hematologic, mucocutaneous, musculoskeletal, and renal systems. (Siegel ,*et al* 2024) . The pathogenesis of this disease is complex and elusive, but it is hypothesized to be associated with

environmental triggers that activate the body's immunological dysfunction and impair immune regulation. The presence of numerous autoantibodies that instigate the formation and deposition of immune complexes, as well as other immunological processes, is implicated in the manifestation of the various symptoms of SLE (Ratib and Saud, 2021).

Although the etiology and pathogenesis of SLE remain unclear, it is believed that genetic susceptibility and environmental factors are contributory The identification of a dependable biomarker for SLE is imperative to ensure an accurate diagnosis (Dahham and Haddad, 2022) .

The autoimmune response can elicit clinical manifestations of a severe nature, with symptoms commonly presenting as rash and renal inflammation which may advance to the point of kidney failure (Qi et al., 2019). The distinguishing feature of systemic lupus erythematosus (SLE) is its heterogeneous presentation, characterized by a progressive accumulation of clinical manifestations over time and an undulating course. Indeed, any organ system may be impacted by this condition. In the nation of Iraq, the initial case was documented in 1971 and the incidence was found to be one case per 1867 members of the population(Sabr, 2020).

1.4.2 Epidemiology of SLE

SLE is a condition that impacts individuals of diverse racial/ethnic and age groups, as well as both sex. The disease exhibits higher prevalence rates among non-Caucasians, women, and adults. The variable course and outcomes of SLE can be attributed to a multitude of factors, including genetic, environmental, sociodemographic, and methodological factors. Non-Caucasians and pediatric-onset cases tend to experience more severe disease and are at a heightened risk of mortality and damage accrual (Pons-Estel *et al*, 2017).

Globally, the prevalence and incidence of SLE exhibit notable variations with respect to sex, age, ethnicity, and time. In Iraq, the prevalence of SLE is estimated to be approximately 1/1867 of the general population, and the first documented case of SLE was reported in the year 1971(Kadhim Abbas *et al*, 2022) .

The occurrence and frequency of SLE exhibit a marked variation across diverse countries and ethnic groups, with comparatively elevated rates in North America as well as African/Arabic populations, and relatively lower rates in Europe, Australia, Hispanic, Asian, and Caucasian populations. This variability may be ascribed to genetic vulnerability, environmental factors, the design of the study, disparities in classification, and diagnostic procedures, and possibly attributed to enhanced diagnostic capability (Frostegard, 2023).

In all of the studies that were examined, it was found that women exhibit a greater prevalence of SLE as compared to men across all age and ethnic demographics. This observation can potentially be attributed to various factors such as estrogen, X chromosome inactivation, Toll-like receptor gene products, as well as microRNA function (Nusbaum *et al*, 2020). The distribution of male to female individuals varied significantly, with ratios ranging from 2 to 1 to as high as 15 to 1 (Rees *et al*, 2017) .

The region of North America exhibited the greatest incidence and prevalence of SLE, whereas Africa, Ukraine, and Northern Australia reported lower rates. Notably, individuals of African ethnicity demonstrated the highest rates, while Caucasians demonstrated the lowest. Additionally, there was a notable upward trend in SLE prevalence over time (Stojan and Petri . 2018) .

The potential correlation between diagnostic enhancement and early disease detection is worth investigating. The study revealed that infection and cardiovascular disease were the primary causes of mortality among SLE

patients, with a notable overall mortality rate two to three times higher than the general population. Moreover, certain racial and ethnic groups were found to have even higher mortality rates (Barber *et al*. 2023).

1.4.3 Etiology of SLE

SLE is a complex disease characterized by multiple organ involvement and an elusive aetiology. Nevertheless, considerable evidence suggests that a combination of genetic, immunological, endocrine, and environmental factors are implicated in the etiopathogenesis of SLE (Justiz Vaillant *et al* . 2023).

Individual genetic risk factors account for a mere one-third of the overall heritability of SLE the remaining risk can be attributed to environmental exposures and gene-environment interactions. The association between SLE risk and environmental factors, such as occupational exposure to crystalline silica, smoking, exogenous oestrogen hormones, dietary intake and sleep, UV radiation, air pollution, solvents, pesticides, vaccines, and medications, infections, and alcohol use has been well-established.

Moreover, gene-environment interactions involving IL-10, ESR1, IL-33, *ITGAM,* and *NAT2* have been observed, further highlighting the intricate interplay between genetic and environmental factors in the development of SLE (Woo *et al* . 2022).

As illustrated in Figure 1.1, the provision of a more extensive elucidation pertaining to the genesis of SLE, along with its various manifestations, might be possible.

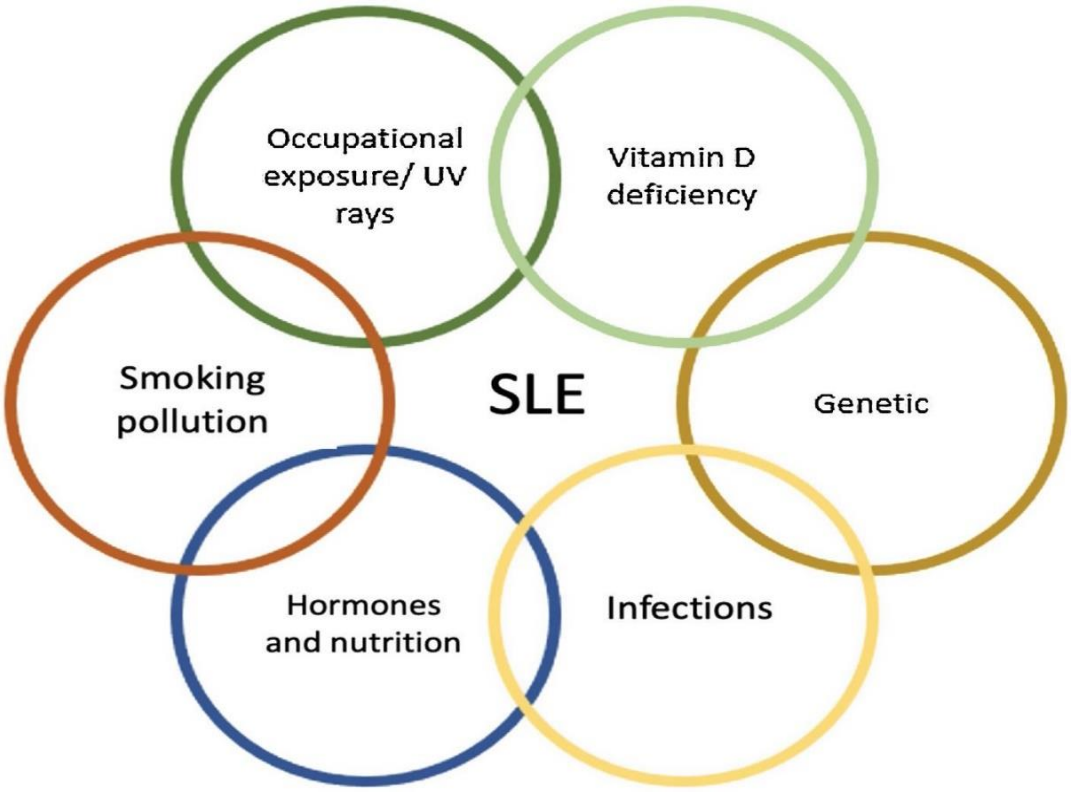

Figure 1.1: The Multifaceted Etiologies of SLE (*Cojocaru, et al . 2011).*

1.4.4 Risk Factors for SLE

1.4.4.1 Genetic Factors

Genome-wide association studies have successfully identified numerous risk loci, exceeding 60 loci, that are associated with susceptibility. However, there is incomplete concordance in monozygotic twin studies, which implies that there are other factors influencing susceptibility, such as epigenetic factors like DNA methylation, microRNA regulation, and histone modification (Maliha F Shaikh 2017).

The heritability of SLE has been estimated to be 66% in twin studies, indicating a significant genetic contribution to its development. Genome-wide association studies (GWAS) have identified approximately 100 SLE susceptibility loci, predominantly in European and Asian populations, which account for up to 30% of the heritability of SLE (Young-Chang Kwon 2019).

The estimated heritability of SLE is notably elevated, signifying a robust genetic predisposition to the ailment and, thus, underscoring the essentiality of genetic investigations (Ha *et al*. 2022); this is illustrated in (Figure 1.2). The majority of these genes are engaged in three different biological processes: the generation of type I interferon, toll-like receptor activity, and immune complex processing. Transduction of immune signals in lymphocytes (Harley *et al*. 2009).

First, lupus development has been linked to abnormalities in antigen-presenting cell-mediated apoptotic cell clearance, processing, and presentation to lymphocytes. Certain loci have identified or proven alleles associated with lupus (e.g., HLA-DR,). The manner that the encoded proteins interact with immune complexes may be influenced by C reactive protein (CRP) and Fc receptors, offering molecular evidence in favor of immune complex processing as a key concept in the pathophysiology of lupus (Nagafuchi *et al*. 2019).

Second, since the 1970s (Hooks *et al*, 1979), interferons have been linked to the pathophysiology of lupus, and numerous more recent investigations have confirmed this. Immune complexes that trigger nucleic acid signaling through toll-like receptors (TLRs) 7 and 9 cause the generation of type I interferon.

Recently, a number of lupus genes (such as *IRAK1, TREX1, IRF5,* and *TNFAIP3*) were discovered through candidate gene and GWAS investigations. These genes encode parts of several pathways that are both upstream and downstream of the generation of type I interferon (Deng and Tsao, 2017).

Since excessive production of interferon may stimulate the release of proinflammatory cytokines and chemokines, the development of dendritic cells, the stimulation of autoreactive B and T cells, the generation of

autoantibodies, and the loss of self-tolerance, it will be crucial to understand how these genes are involved in the etiology of lupus (Wu *et al*. 2022).

Third, it has been discovered that certain lupus susceptibility genes are involved in signal transduction in immune cells, particularly B and T cells. Lack of self-tolerance and autoimmunity results from the activation of B-cells by antigen-mediated cross-linking of the B-cell receptor (surface IgM) and the subsequent interaction of autoreactive B-cell clones with Th2 cells. It has long been established that B- and T-cells play a role in the pathophysiology of lupus, and earlier research has linked these cells to signal transduction pathways. One instance of a causative variation that has been found to increase disease vulnerability is *PTPN22*, a selective phosphatase that regulates signal transduction in T cells (Bolouri *et al*. 2022). Immune-related genes in SLE Replicated genome-wide association study (GWAS) identify five genes consistently dysregulated in SLE. These are *ITGAM, BLK, BANK1, PHRF1, PXK, TNFAIP3*(Goulielmos *et al* . 2018), (Lingyan, 2018) .

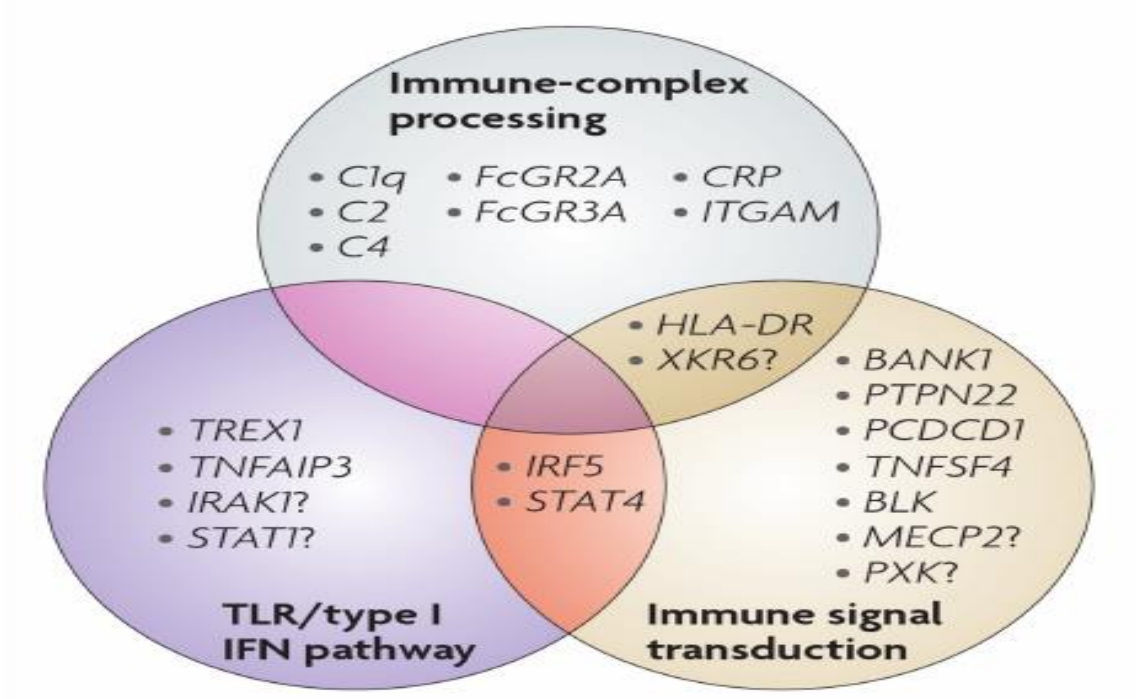

Figure 1.2: Pathways containing established candidate SLE susceptibility genes(Harley IT, *et al* . 2009) .

1.4.4.1.1 The MHC Region

Genetic investigations of SLE began with the revelation that the major histocompatibility complex (MHC) area carries a risk. Unfortunately, efforts to identify the variations causing the significant risk that this region confers in SLE have been hampered by the extraordinarily complicated linkage disequilibrium structure of this locus, which spans an astounding 7.2 Mb spanning >400 genes in subjects originated from Europe. The class II alleles (HLA-DR3 and DR2) in European populations had the most persistent HLA connections with SLE, according to a recent meta-analysis of study findings spanning the previous 30 years (Fernando and Vyse, 2021).

Nonetheless, the MSH5 gene, in the class III region, showed the strongest correlation with SLE in the recently released GWAS results from women of European descent (Catalina *, et al*. 2020). Whether MSH5 or one of its close neighbors is an independent risk factor for SLE separate from the HLA-DR genes that have been so often linked to lupus calls for more research. Different populations have different MHC haplotype patterns, and analysis of non-Europeans has shown that additional alleles (such as HLA-DR4) increase the chance of developing lupus in these communities (Hanscombe et al., 2018, Harley *et al*. 2008).

Despite being one of the human genome's most investigated regions, it is still unknown how exactly this region contributes to the total hereditary risk of SLE. Consequently, research conducted in much bigger cohorts that assess the complete MHC locus rather than particular areas and that include non-Europeans has the potential to significantly advance our knowledge of the pathophysiology of lupus (Bakela *et al* . 2018).

1.4.4.1.2 The Integrin-alpha M Chain (*ITGAM*) gene

The gene known as *ITGAM* is responsible for encoding integrin alpha-M, which is a vital component in the phagocytosis of C3b-coated particles by immune cells. In cases of systemic lupus erythematosus (SLE), a mutation in *ITGAM* can lead to a decrease in its capacity to effectively clear immune complexes (Putra , *et al* 2021).

The *ITGAM* gene has been identified as a susceptibility gene for SLE, which is quite surprising given that previous expression studies failed to provide convincing evidence for its involvement in the disease. The *ITGAM* gene encodes the integrin αM protein, which is responsible for the formation of the cell surface receptor CR3 and is expressed in neutrophils, macrophages, and dendritic cells. It is possible that a functional mutation in *ITGAM* may affect the trafficking of leukocytes or the uptake of apoptotic cells or immune complexes (Rhodes and Vyse, 2008).

Genetic variations within the *ITGAM* gene have been found to heighten the susceptibility to lupus nephritis. This is accomplished through the disruption of CD11b functionality, resulting in impaired ligand binding, cell adhesion, phagocytosis, catch-bond formation, and reduced production of inflammatory cytokines. Nevertheless, it is plausible to mitigate these functional deficiencies through the use of pharmacologic activation of CD11b, as well as the application of small molecule agonists.(Khan et al., 2018) .The genetics of lupus a functional perspective show in (figure 4) (Song, *et al* . 2020).

Variations occurring at the *ITGAM* gene, which encodes for the CD11b chain of the Mac-1 (alphaMbeta2; CD11b/CD18; complement receptor-3) integrin, have been identified as one of the most potent genetic risk factors for systemic lupus erythematosus (SLE). Specifically, a genetic variant causing an amino acid substitution in the Mac-1 integrin exhibits a strong

association with SLE and is likely to contribute to the pathogenesis of this disease by impairing the immunological functions of the integrin(Fagerholm et al., 2013).

The integrin grouping of proteins is comprised of a total of 24 adhesion receptors which perform noteworthy functions in biological systems through the facilitation of cellular adhesion as well as signalling interactions with extracellular matrix proteins and other cellular entities.(Mould and Humphries, 2004) .

The proper regulation of the conformation of β2 integrins on the leukocyte surface is of paramount importance for the efficient facilitation of immune cell adhesion and extravasation into lymph nodes and tissues. This process is vital for the initiation of a successful immune response (Schittenhelm, et al 2017). Until the activation of inside-out and outside-in communication mechanisms, integrin-mediated adhesion and extravasation into the tissue are not initiated (Figure 1.3).

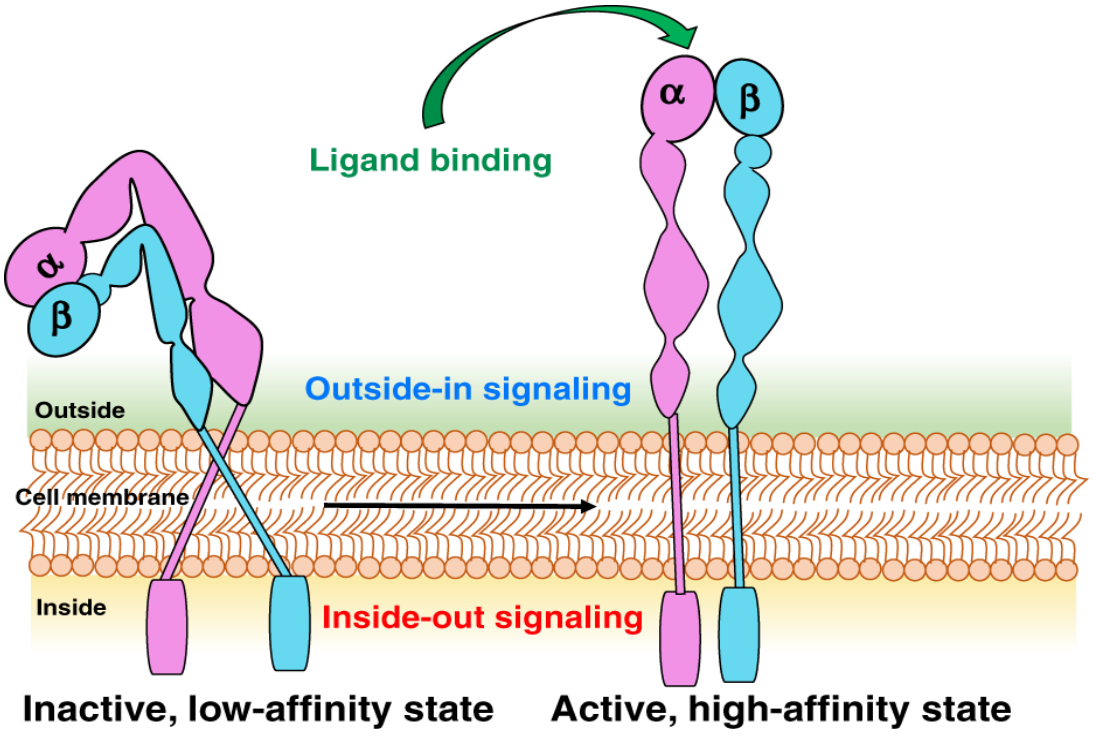

Figure 1.3: Schematic of integrin structure and activation (Mezu-Ndubuisi,*et al*.2021).

Additionally, Signalling has the ability to alter cellular interactions through its impact on receptor affinity and avidity. Within the immune system, β2 integrins, which are exclusively found on leukocytes, are essential for facilitating leukocyte recruitment, cellular interactions, and intracellular signalling cascades.CD18 and one of four α-subunits make up β2 integrins, which can lead to either pro-inflammatory or anti-inflammatory outcomes (Figure 1.4) The α-subunit involved plays a significant role in determining the function and expression of β2 integrins (Schittenhelm et al., 2017).

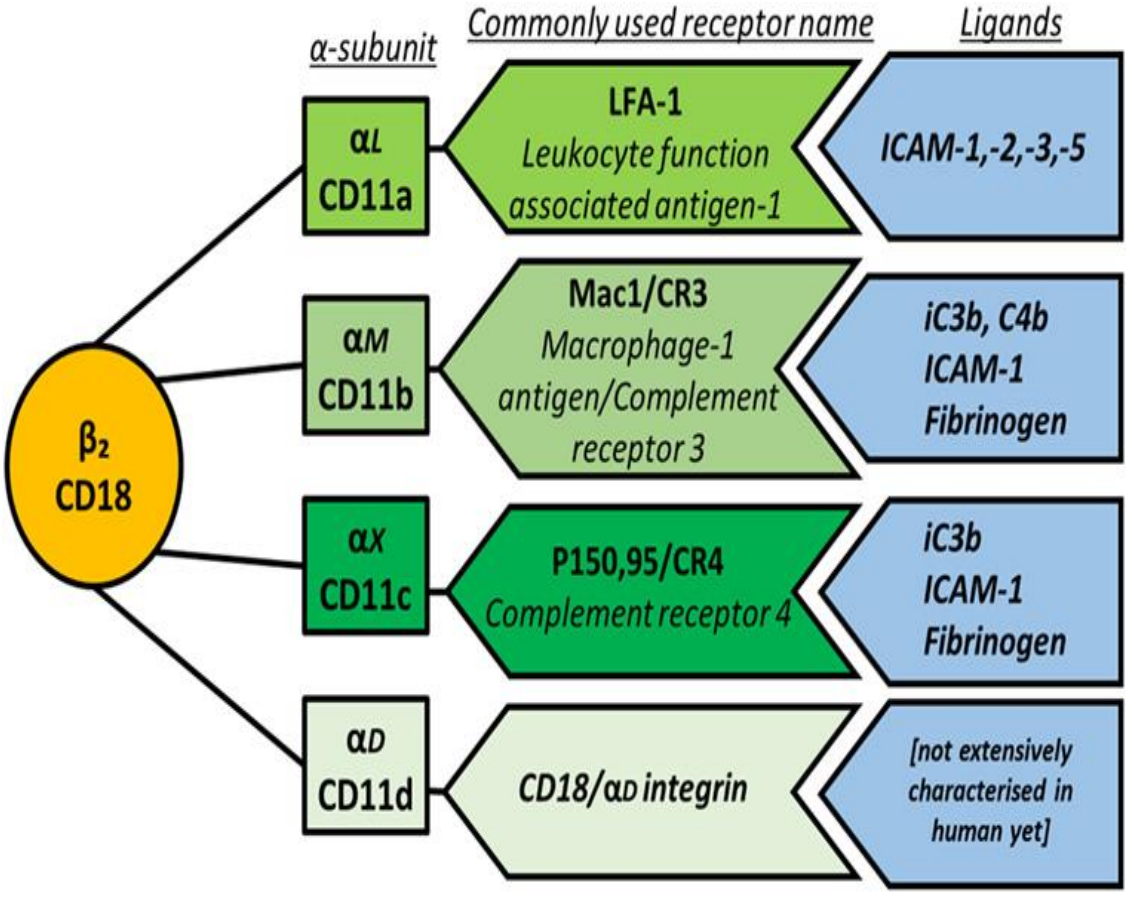

Figure 1.4: Schematic representation of β2 integrin subunit pairing, depicting the β-subunit CD18 as the common subunit non-covalently associating with one of four α-subunits (Schittenhelm et al., 2017)

1.4.4.1.3 The Tumour Necrosis Factor, Alpha-induced Protein 3 (*TNFAIP3*)

A20, otherwise referred to as *TNFAIP3*, is a cytoplasmic protein that holds significant importance in the regulation of inflammation and immunity. It acts as a negative regulator to prevent hyperactivation which could potentially result in autoimmune disorders and systemic inflammation. Conversely, excessive activation may trigger fatal systemic inflammation or autoimmunity. Moreover, A20 also functions as a tumour suppressor in specific B cell lymphomas (Wu, *et al* 2020).

Association of the *TNFAIP3* region has subsequently been replicated in RA and was also reported in SLE by two groups using GWAS. These observations implicated that *TNFAIP3* may be a common susceptibility gene to multiple autoimmune diseases. (Liu, *et al* 2018).

The *TNFAIP3* region has been found to be associated with rheumatoid arthritis (RA) and SLE, suggesting it may be a common susceptibility gene for multiple autoimmune diseases. A meta-analysis was conducted to examine the association between *TNFAIP3* gene polymorphisms and SLE susceptibility in different populations. The meta-analysis found a significant association between SLE and *TNFAIP3* polymorphisms (rs2230926, rs5029937, rs5029939, and rs3757173), with different associations observed in Europeans and Asians,(Zhang et al., 2016).

TNFAIP3/A20 is a prominent autoimmune disease risk locus that is correlated with hypomorphic *TNFAIP3* expression and exhibits complex chromatin architecture with over 30 predicted enhancers(Pasula et al., 2022) (Figure 1.5) . A20 is a potent anti-inflammatory protein that inhibits NF-κB signaling and inflammatory gene expression, preventing cell death. Mutations in the A20/*TNFAIP3* gene are associated with various inflammatory and autoimmune diseases in humans and mice.

A20 acts as a "Ub-editing" enzyme, interfering with the ubiquitination status of NF-κB signaling proteins, and also inhibits cell death in multiple cell types. (Martens and van Loo, 2020) . A coding variant in the de-ubiquitinase domain (DUB) of A20 (Phe127Cys) is associated with SLE susceptibility and has an NF-κB independent role. The Phe127Cys risk allele is associated with increased PADI4 mRNA expression and protein levels, histone H3 citrullination, NET formation, and presence of anti-cyclic citrullinated peptide autoantibodies in SLE patients. The effects of the TNFAIP3 risk variants are summarized in (Figure 1.5) (Hagberg et al., 2020b).

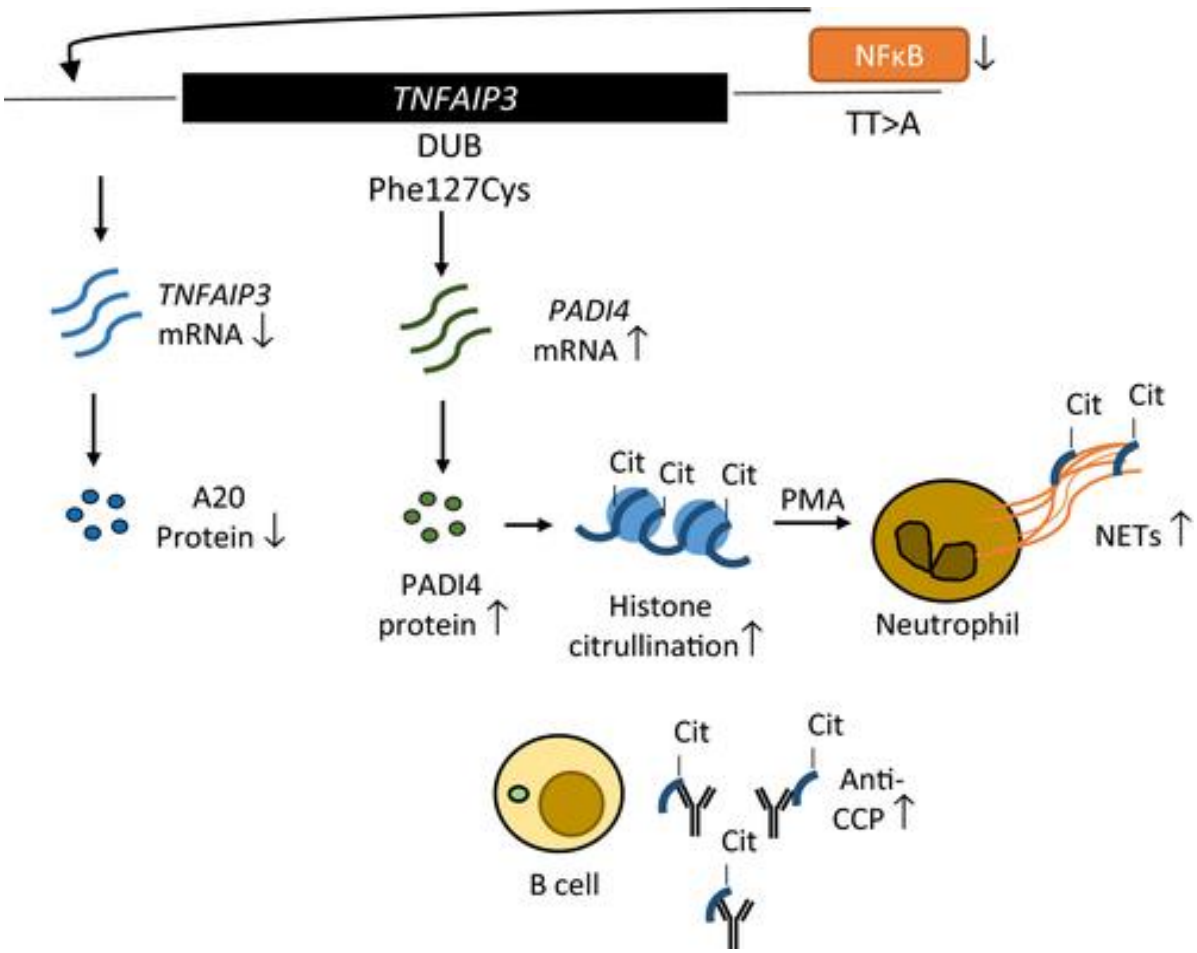

Figure 1.5: Effects of *TNFAIP3* SLE risk variants (Hagberg, *et al* , *L. 2020a).*

1.4.4.2 Immunological factors

Breakdown of self-tolerance is the main pathogenesis of SLE, the innate and adaptive immune networks are interlinked with each other through cytokines, complements, immune complexes and kinases of the intracellular machinery(Pan et al., 2020).

SLE patients exhibit various abnormalities in their immune system, with autoantibodies to nuclear antigens and an activated type I interferon (IFN) system being characteristic. Deficiencies in complement cascade components (C1q, C2, and C4) are strongly associated with SLE susceptibility, leading to impaired clearance of apoptotic material and an excess of nuclear antigens accessible to the immune system.Type I IFN, triggered by nucleic acid-containing immune complexes and neutrophil extracellular traps (Figure 1.6) plays a pathogenic role in SLE by activating multiple arms of the immune system (Hagberg et al., 2020a).

The main autoantibodies present in the serum of SLE patients are directed against nuclear components [double-stranded DNA (dsDNA), ribonucleoproteins, histones and others nuclear components, leading to tissue damage through inflammation, immune complex deposition, and infiltration within damaged organs.(Herrada et al., 2019). T-cells also contribute to autoimmunity in SLE with defects associated with CD8+ and T-regulatory cell function occurring along with an expanded CD3 +C D4 – CD8 – T-cell lineage (Maliha Shaikh 2017).

1.4.4.2.1 Anti-nuclear antibodies (ANAs)

The ANAs are a class of antibodies that bind to cellular in the nucleus, including proteins, DNA, RNA, and nucleic acid-protein complexes. ANA identification has been the foundation of diagnosis for autoimmune connective tissue disease, including systemic lupus erythematosus (SLE), Sjogren's syndrome, and

polymyositis/dermatomyositis. increased titers are characteristic of individuals with connective tissue disorders. So, the sensitivity and specificity used to detect ANAs are important to diagnosis. (Nosal, *et al* 2022).

Anti-nuclear antibodies (ANAs) can be found in healthy individuals and patients with other autoimmune and no autoimmune diseases, not exclusive to SLE. ANAs are typically classified into two groups, antibodies to histones , DNA and antibodies to nuclear material. (Accapezzato et al., 2023a). ANA is commonly found in SLE and can be used for screening, diagnosis, and prognosis, but it has a high sensitivity (95-97%) and low specificity (20%), meaning that a positive ANA does not confirm SLE, while a negative ANA makes it less likely (Pisetsky et al., 2019). The indirect immunofluorescence assay (IIFA) is the reference method for ANA screening, but it is being replaced by newer assays based on ELISA and automated high throughput multiplex assays, raising concerns about their accuracy and sensitivity(van der Pol et al., 2018).

1.4.4.2.2 Anti-double stranded DNA (dsDNA)

Anti-dsDNA antibodies are the most studied antibodies of the lupus-related autoantibodies. The dogma is that these are the most important autoantibodies in systemic lupus erythematosus (Fu et al., 2015)As one of the most distinct ANA types Anti-double-stranded deoxyribonucleic acid (dsDNA) antibodies have more than 95% specificity for SLE but are found in only about 60% to 70% of SLE patients(Justiz Vaillant et al., 2023). Anti-double-stranded DNA AAb positivity is one criterion present in both ACR and SLICC classifications(Didier et al., 2018). The presence of anti-dsDNA antibodies has been correlated with renal involvement anti-dsDNA antibodies contribute to the pathogenesis of lupus nephritis through binding, either directly or indirectly, to cross-reactive antigens or chromatin materials, respectively, to resident renal cells and/or extracellular matrix

components, thereby triggering downstream cellular activation and proliferation as well as inflammatory and fibrotic processes(Yung and Chan, 2015). Among the affected organs, renal involvement indicates major internal damage in SLE patients. Anti-dsDNA antibodies are present in serum of patients with LN , Anti-dsDNA antibodies directly or indirectly interact with renal antigens, thus producing immune complexes Nevertheless, renal damage is not initiated solely by the complexes of a chromatin fragment and IgG depositing in the mesangial matrix or glomerular basement membranes (Wang and Xia, 2019).

SLEDAI score along with anti-dsDNA levels disease activity were found to have a significant positive connection, however their levels can change over time. As a result, especially in cases with active nephritis, levels may be undetectable throughout treatment and rise during a flare. The diagnostic sensitivity of anti-dsDNA antibodies is limited (52% to 70%) because of their fleeting presence (Ameer et al., 2022).

1.4.4.3 Inflammatory Factors

Cytokines play a crucial role in regulating immune response and their dysregulation is a key factor in autoimmune diseases like SLE. In SLE, aberrant expression of cytokines such as type I interferon, IL-6, and IL-17 contributes to autoimmunity, abnormal immune responses, and tissue damage. Reduced levels of important cytokines like IL-2 lead to defective regulatory T cell responses and promote autoimmunity in SLE (Katsuyama and Moulton, 2021).Interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-17 (IL-17), and interferon-gamma (IFN-γ) are among the cytokines that have a major role in SLE pathogenesis. Specific genes involved in interferon production and signaling are linked to SLE susceptibility. Cytokines, such as interferon and interleukins, play a significant part in immune cell function and inflammation in SLE.IFN-α, IL-6, IL-8, IL-17, and IL-18 serum cytokines are correlated with the severity

of SLE disease as determined by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). It has been suggested that circulating IL-18 levels can predict harm to the renal system, which may serve as a marker to indicate damage to the kidneys and identify those who are at risk of kidney failure (Ruchakorn et al., 2019).

Cytokine imbalances, particularly interferon alpha, contribute to immune dysfunction, inflammation, and organ damage in systemic lupus erythematosus (SLE) by inducing the secretion of inflammatory proteins.(Ohl and Tenbrock, 2011).

Cytokines have an important role in the pathogenesis of autoimmune diseases like systemic lupus erythematosus (SLE) and psoriasis (PS). Due to their crucial roles in the immune cells' development, differentiation and regulation; any dysregulation in their production and/or action can lead to the development of autoimmune diseases. (Farid et al., 2022).

Cytokines and inflammatory mediators play a crucial role in the development of systemic lupus erythematosus (SLE), and their profiles could potentially serve as biomarkers for monitoring and treating the disease, although further research is needed to understand their complex interactions with other biological variables (Manuel Rojas, 2018).

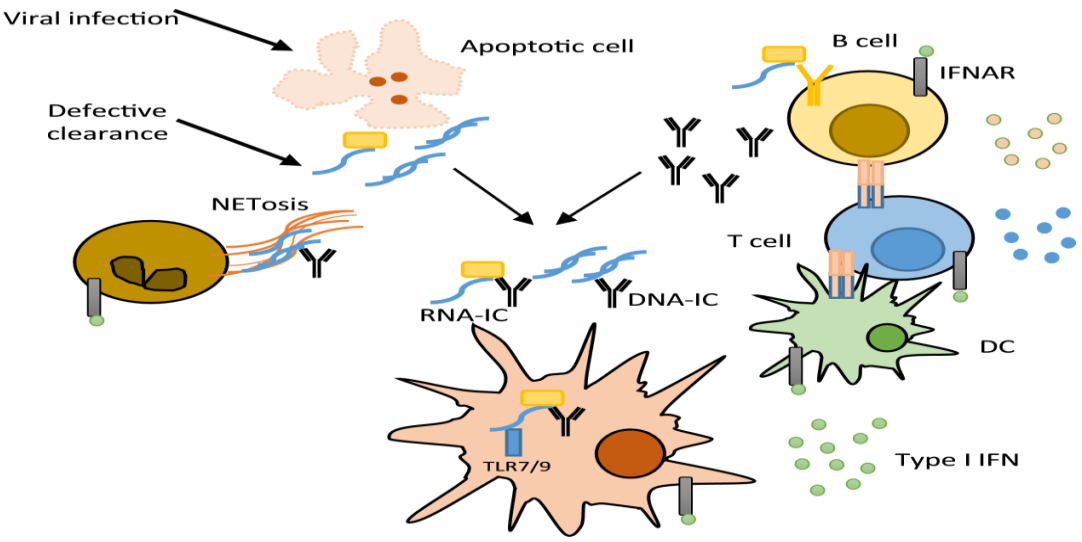

Figure 1.6: Immunologic aberrations contributing to the pathogenesis of SLE .(***Hagberg, et al. 2020a)*** **.**

1.4.4.3.1 The Interferons alpha (IFN- α)

There are three distinct types of interferons (IFNs), which are cytokines involved in viral immune responses. It has been demonstrated that disease activity and autoantibody levels, primarily anti-dsDNA and anti-Ro/SSA, are correlated with Type-IIFN system activation. Type I interferons, including interferon-α (IFN-α), play a key role in the pathophysiology of systemic lupus erythematosus (SLE). This cytokine, which is mostly produced by plasmacytoid dendritic cells, facilitates T- and B-cell differentiation, proliferation, and survival, acting as a bridge between the innate and adaptive immune systems (Lambers et al., 2021) .Interferon (IFN-I) cytokines, specifically IFNα, play a significant role in the pathogenesis of SLE by affecting both innate and adaptive immunity. Elevated IFN-I expression in SLE leads to dysregulation of immune function, resulting in increased disease severity, hyperinflammation, skin rash, multiorgan damage, and elevated autoantibody production, The IFN-I signature in SLE also promotes CD8 T-cell exhaustion, CD4 proliferation, and the formation of B-cell germinal centre and Th17 cells (figure 7)

contributing to autoimmunity and organ damage,(Demers-Mathieu, 2023) . Plasmacytoid dendritic cells (pDCs) are believed to be the main source of IFN-α in systemic lupus erythematosus (SLE), and their deficiency has been shown to improve lupus symptoms in mice models. Genetic factors, such as polymorphisms in IRF5 and IRF7 genes, are associated with increased levels of type I IFN in SLE patients**.**(Postal et al., 2020) .

Patients with SLE and high IFN-1 activity have higher disease activity scores and a greater risk of relapse in remission. Dysfunctional pDCs and other immune cells contribute to IFN-1 dysregulation in SLE. UVB exposure can trigger IFN-1 responses through the cGAS/STING DNA-sensing pathway, potentially causing SLE flares in susceptible patients (Fernandez-Ruiz and Niewold, 2022). A study in Iraq assessed the serum level of interferon-alpha (IFN-α) in female patients with SLE. The average SLEDAI score was 8.53 ± 3.42, and proteinuria, ESR, creatinine, and AST were significantly higher in SLE patients compared to controls, Serum levels of IFN-α were increased in SLE patients, but there was no significant difference compared to controls. IFN-α showed a negative correlation with SLEDAI in both active and inactive groups. There was no significant correlation between IFN-α serum levels and clinical manifestations or laboratory investigations, except for age and disease duration, which were

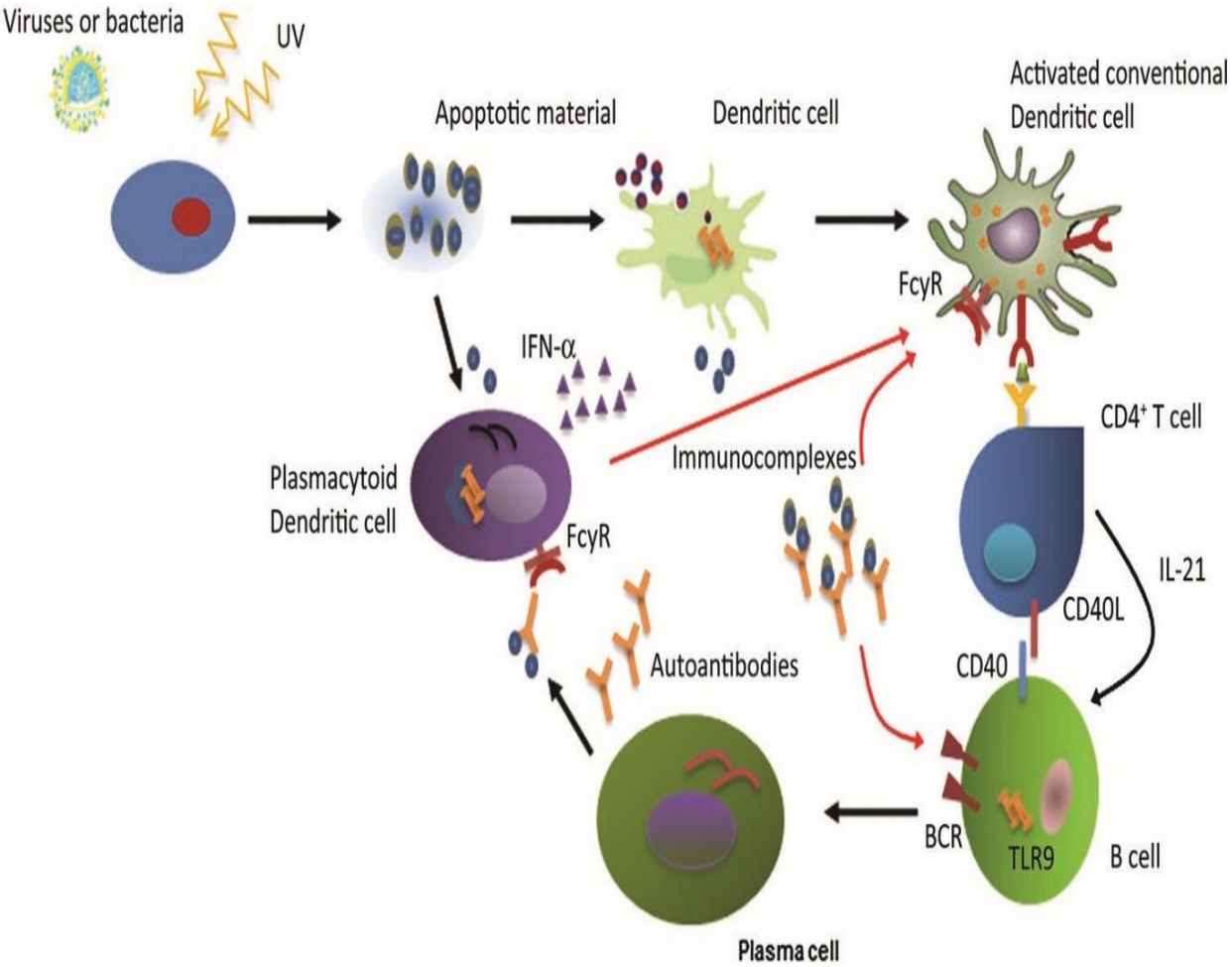

Figure 1.7: Clinical consequences of type I interferon signature in SLE *(Bertsias et al 2010).*

identified as risk factors for increasing IFN-α serum levels (Abdulridha et al., 2022). The increase of IFN-I gene scores, associated with elevation of IFN-α and IFN-γ serum levels were found to be 44%, 45%, and 14% respectively, with a strong positive correlation between IFN-α levels and IFN-I gene scores , there is a study conducted on patients ,the majority of whom are woman and Caucasians population(Chasset, F and Trendelenburg, 2022).

1.4.4.3.2 Toll-Like Receptor 7 (TLR-7)

Type I IFN production is mainly triggered by the activation of nucleic acid-binding pattern recognition receptors, including the endosomal toll-like receptors (TLR) 3 4, 7 and 9, the cytosolic sensor cyclic GMP-AMP

synthase (cGAS), and the RNA-sensor RIG-I–like receptors (RLRs)-MAVS(Crowl et al., 2017).

Toll-like receptor (TLR) 7 and TLR8 are transmembrane receptors that recognize single-stranded RNA. Activation of these receptors results in immune cell stimulation and inflammatory cytokine production, which is normally a protective host response. However, aberrant activation of TLR7/8 is potentially pathogenic and linked to progression of certain autoimmune diseases such as lupus. (Vlach et al., 2021) .

TLR-7 is consistently overexpressed in PBMCs of different ethnic SLE populations and is associated with a type I interferon transcript signature. TLR-7 gene variants, such as rs179008 and rs3853839, are linked to an increased risk of SLE in African and Asian populations.

The ITGAM gene and intrinsic IgE also play a role in regulating TLR7 signalling and affecting the risk of SLE in humans.(Devarapu and Anders, 2018).

Genome-wide association studies (GWAS) have revealed that TLR7 single-nucleotide polymorphisms (SNPs) influence SLE risk, clinical phenotype, and autoantibody production. TLR-7 promotes the development of SLE at the genetic and molecular levels, making it a potential target for therapy. It contributes to the loss of germinal centre tolerance and drives the extrafollicular B-cell response in SLE.(Wen et al., 2023). TLR activation, particularly through TLR7, TLR8, and TLR9, has been implicated in the development of systemic lupus erythematosus (SLE) as patient sera contain self-RNA and self-DNA that can act as ligands for these receptors, contributing to the aberrant immune response seen in SLE. Mouse models suggest a pathogenic role for TLR7 signalling and a protective role for TLR9 signalling in SLE (Mohammad Hosseini et al., 2015) .

1.4.5 Diagnosis and Classification of SLE

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with variable clinical features,. Clinical and serological heterogeneity are critical features in SLE, making it difficult to define in its diagnosis. Antinuclear antibodies (ANA) are the important serological marker in more than 95% of SLE patients. The improved set of European Alliance of Associations for Rheumatology (EULAR) classification enabled accurate diagnosis of SLE. The treatment focuses on remission, preventing organ damage, and improving the overall quality ofClassification criteria are important for identifying homogeneous groups of patients for research studies and trials, and the 1982 revised American College of Rheumatology ACR SLE classification criteria have been widely used. (Ameer, *et al* .2022).

The 2012 SLICC classification criteria for SLE included new manifestations, antibody tests, and refined definitions, emphasizing the importance of immunological criteria and histology-proven nephritis. The new EULAR/ACR classification criteria for SLE aim to maintain high specificity while achieving a sensitivity close to the SLICC criteria, with positive ANA as an entry criterion and weighted items based on severity. The new criteria have a specificity of 93% and a sensitivity of 96%, with a cut-off of 10 for classification, primarily based on class III/IV lupus nephritis. (Aringer, *et al* 2019).

1.4.5.1. The 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for SLE

The 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for SLE (Figure 1.8) are more sensitive than the 1997 ACR criteria and more specific than the 2012 SLICC criteria, performing well in patients with early disease and different ethnicities. The EULAR/ACR classification criteria score can be used to predict disease

activity and organ damage in SLE patients, with a score ≥20 indicating higher disease activity and a score <10 associated with less damage (Whittall-Garcia et al., 2022).

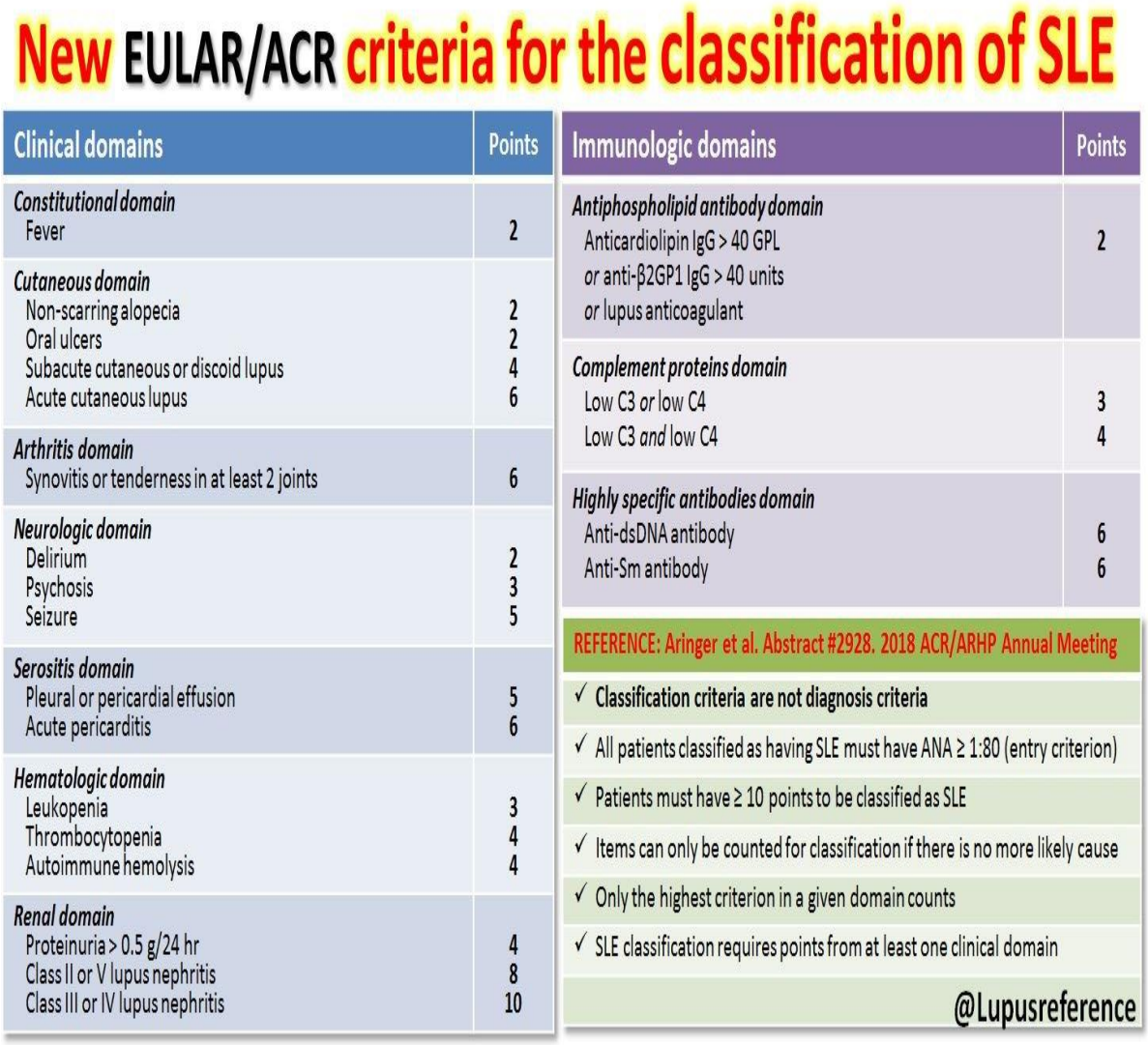

Figure 1.8: The EULAR/ACR SLE classification criteria (Whittall ,et al . 2022).

The Systemic Lupus International Collaborating Clinics (SLICC) group revised and validated the American College of Rheumatology (ACR) systemic lupus erythematosus (SLE) classification criteria to improve clinical relevance and incorporate new knowledge about SLE immunology. The EULAR/ACR-2019 criteria showed similar sensitivity to the SLICC-2012 criteria and maintained specificity at ACR-1997 criteria values. ANA status is included as a mandatory criterion in the EULAR/ACR-2019

criteria, but excluding ANA negative patients may disproportionately affect certain subgroups of SLE patients. The number of criteria required for positive classification in children and young people may need to be adjusted to optimize sensitivity and specificity in JSLE. Classification criteria for SLE aim to define homogenous groups for clinical trials, but the heterogeneity of the disease and individual factors pose challenges in creating criteria that are both specific and inclusive (Lythgoe et al., 2022; Huang et al., 2022). Figure 1.9 show the timeline of SLE classification criteria modifcations.

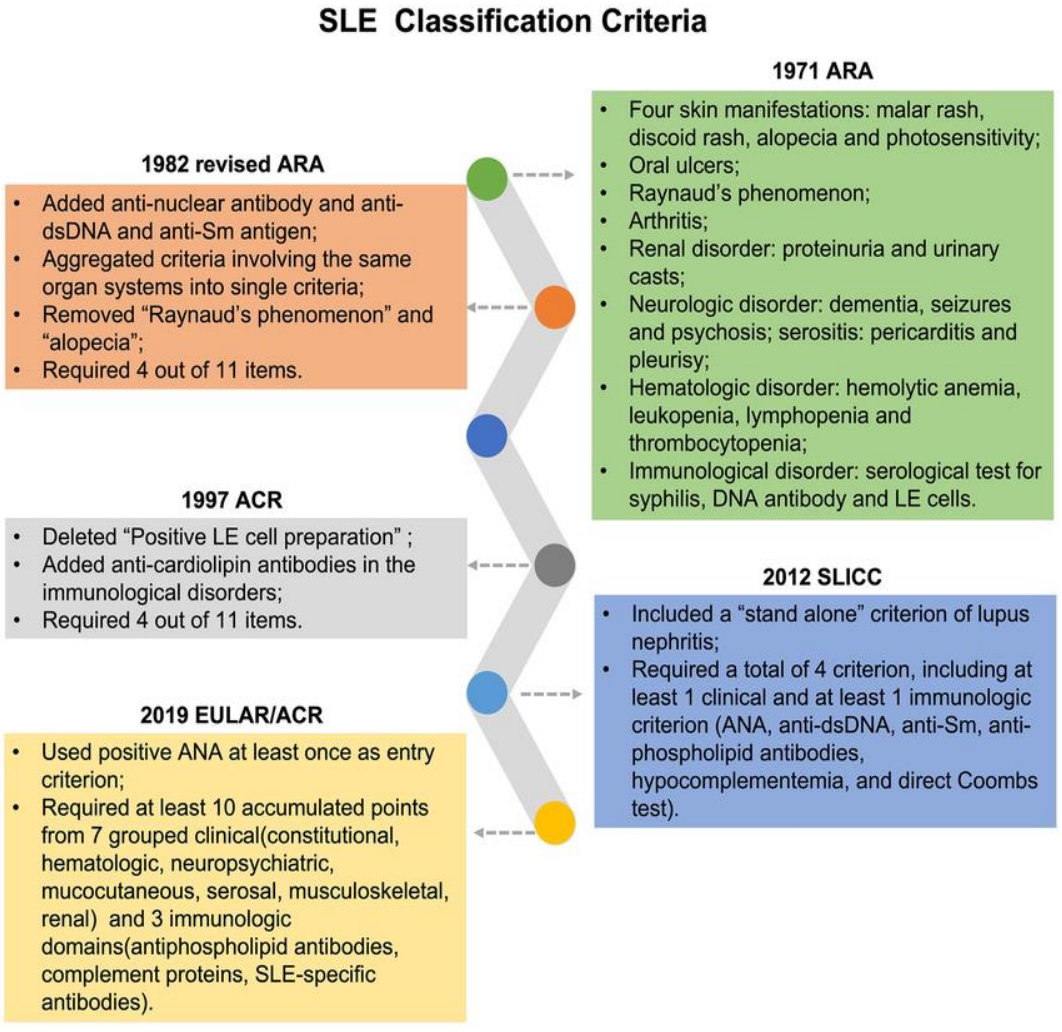

Figure 1.9: Timeline of SLE Diagnosis and Classification Criteria (Huang, et al. 2022).

1.4.5.2 The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)

SLEDAI is a global index developed in 1985 to assess lupus disease activity, consisting of 24 weighted clinical and laboratory variables across nine organ systems, with scores ranging from 1 to 8 and a total possible score of 105; SLEDAI-2000 was introduced in 2002 as a measure of global disease activity, SLEDAI-2 K is a modified version of the original SLEDAI that allows for the documentation of persistent disease activity in specific descriptors. It has been validated and proven to be sensitive to change over time, and is a strong predictor of mortality in SLE. The total score falls between 0 and 105, with a score of 6 considered clinically important (Mikdashi and Nived, 2015).

The SLE Disease Activity Index (SLEDAI) and the British Isles Lupus Assessment Group Index 2004 (BILAG 2004) are widely used measures to assess disease activity in systemic lupus erythematosus (SLE), with modifications made to allow for scoring of persistently active disease and proteinuria.(Thanou et al., 2021) . There are improved versions of SLEDAI such as SLEDAI-2K and SELENA-SLEDAI, which are commonly used in clinical trials. The original SLEDAI, SLEDAI-2K, and SELENA-SLEDAI have the same weighting for items and organ damage but have different definitions for each item. Proteinuria and skin rash/stomatitis/hair loss have different scoring criteria in the original SLEDAI compared to SLEDAI-2K and SELENA-SLEDAI. SLEDAI is simple to score and can be used for retrospective studies, but it only scores the presence of each item and does not evaluate the severity. SELENA-SLEDAI defines severe flare based on specific criteria, including changes in SELENA-SLEDAI score, development or deterioration of certain conditions, and increase in physician's global assessment score (Ohmura, 2021) .

The most widely used disease activity measures in international, multicentre trials are the SLE Disease Activity Index (SLEDAI) and the British Isles Lupus Assessment Group Index (BILAG) Beyond their individual strengths and weaknesses , both instruments were developed through a consensus approach to derive thresholds for changes in disease activity (Thanou et al., 2019) A SLEDAI score of 3 or higher and a 1-point increase in PGA is considered a mild/moderate flare, while a SLEDAI score greater than or equal of a 12 and higher than or equal of a 2.5-point increase in PGA is considered a severe flare; treatment criteria differ based on the severity of the flare.(Arora et al., 2020) For the doctor, determining the level of disease activity in patients with SLE is essential because it guides treatment choices. Distinguishing between disease activity and damage is crucial for determining the right course of treatment and long-term prognosis. SLEDAI (Table 1.1) are more practical for everyday use (George Bertsias, 2012).

Table 1.1: The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (George et al ,2012).

Descriptor	Definition	Score
Seizure	Recent onset. Exclude metabolic, infectious or drug-related causes	8
Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Includes hallucinations; incoherence; marked loose associations; impoverished thought content; marked illogical thinking; bizarre disorganised or catatonic behaviour. Exclude the presence of uraemia and off ending drugs	8
Organic brain syndrome	Altered mental function with impaired orientation or impaired memory or other intellectual function, with rapid onset and fluctuating clinical features. Includes a clouding of consciousness with a reduced capacity to focus and an inability to sustain attention on environment and at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, increased or decreased psychomotor activity. Exclude metabolic infectious and drug-related causes .	8

Cranial nerve	New onset of a sensory or motor neuropathy involving a cranial nerve	8
Lupus headache	Severe, persistent headache; may be migrainous	8
Cerebrovascular	New syndrome. Exclude arteriosclerosis	8
Vasculitis	Ulceration, gangrene, tender fi nger nodules, periungal infarction, splinter haemorrhages. Vasculitis confi rmed by biopsy or angiogram	8
Arthritis	More than two joints with pain and signs of inflammation	4
Myositis	Proximal muscle aching or weakness associated with elevated creatine phosphokinase/aldolase levels, electromyographic changes, or a biopsy showing myositis	4
Casts	Heme, granular or erythrocyte	4
Haematuria	More than 5 erythrocytes per high power fi eld. Exclude other causes	4
Proteinuria	More than 0.5 g of urinary protein excreted per 24 h. New onset or recent increase of more than 0.5 g per 24 h	4
Pyuria	More than 5 leucocytes per high power fi eld. Exclude infection	4
New malar rash	New onset or recurrence of an inflammatory type of rash	4
Alopecia	New or recurrent. A patch of abnormal, diff use hair loss	4
Mucous membrane	New onset or recurrence of oral or nasal ulceration	4
Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening	4
Pericarditis	Pericardial pain with at least one of rub or effusion. Confirmation by ECG or echocardiography	4
Low complement	A decrease in CH50, C3 or C4 levels (to less than the lower limit of the laboratory determined normal range)	2
Increased DNA binding	More than 25% binding by Farr assay (to more than the upper limit of the laboratory determined normal range, e.g., 25%)	2
Fever	More than 38ºC aft er the exclusion of infection	1
Thrombocytopenia	Fewer than 100 000 platelets	1
Leucopenia	Leucocyte count <3000/mm^3 (not due to drugs)	1

1.4.6 Symptoms and Clinical Features of SLE

A wide range of manifestations, from minor symptoms to severe, life-threatening diseases, are seen in SLE. Compared to patients identified beyond 50 years of age, adults diagnosed prior to this age group typically had better 10-year survival rates, more immunosuppressive drug usage, and cutaneous signs such as malar rash and renal abnormalities such lupus nephritis (Merola et al., 2014) .

The most common signs and symptoms of systemic lupus erythematosus were antinuclear antibody (ANA), hematological and immunological diseases, arthritis, and skin manifestations. Consequently, a sizable fraction of preclinical lupus (PL) (about 10% to 20%) frequently progresses to SLE. Steroids and other immunosuppressive medications including methotrexate and azathioprine are used to treat the majority of individuals with preclinical lupus (Ameer, *et al* 2022).

Multiple autoantibody development is frequently accompanied with organ inflammation. Frequent flare-ups of inflammatory disease activity caused damage to accumulate, which raises morbidity and mortality in the end. Long-term comorbidities arise, including accelerated atherosclerosis brought on by conventional and non-traditional causes. The clinical manifestations are outlined below in Table 1.2 (Shaikh et al., 2017).

Table 1.2: Clinical Presentation of SLE (Shaikh, et al, 2017) .

System	Clinical manifestations
Constitutional	Fever, raised inflammatory markers, lymphadenopathy, fatigue, weight loss
Dermatological	Mouth ulcers, malar rash, photosensitivity, discoid rash, subacute cutaneous lupus, alopecia, livedo reticularis, purpura, vasculitis, urticarial
Musculoskeletal	Arthritis, myositis, arthralgia, myalgia
Renal	Glomerulonephritis, proteinuria, haematuria, uraemia
Cardiac	Libman-Sacks endocarditis, pericarditis, myocarditis, accelerated atherosclerosis, hypertension, dyslipidaemia
Respiratory	Pleurisy, pleural effusions, interstitial lung disease, pulmonary hypertension, pulmonary haemorrhage, pulmonary fibrosis
Gastroenterological	Abdominal pain, ascites, hepatitis, hepatosplenomegaly, peritonitis, clitis
Neurological	Seizures, psychosis, headaches, mononeuritis multiplex, peripheral and cranial neuropathy, cerebrovascular accident, chorea
Haematological	Anaemia, thrombocytopenia, leucopenia
Vascular	Raynaud's phenomenon, vasculitis, thrombosis

Chapter Two

Materials
and
Methods

2. Subject and Study Design

In this cross-sectional study, patients with systemic lupus erythematosus (SLE) were studied at the University of Kerbala in Iraq's Department of Medical Microbiology at the College of Medicine. The participants in this study included (120) were divided into two groups: healthy control group consists (60) person, and the SLE patients' group consists (60). The patients ranged in age from 16 to 65 years old and were of both sexes. The study was conducted between November 2022 and August 2023.

Patients from the rheumatology and nephrology clinics in patient at AL-Imam Husain Medical City in Kerbala Governorate and Marjan Medical City in Babylon Governorate participated in this study.

All patients fulfilled the American College of Rheumatology (ACR) criteria for classification of SLE, SLE disease activity index (SLEDAI) score seemingly healthy control subjects—57 female and three male—were included for comparison; these subjects were normal, healthy, and had no family history of SLE or other obvious medical conditions. Their sex and age ranges correspond with those of the patients.

Ethical approval was obtained from Karbala Medical College Ethical Cmmittee . Also , verbal approbal was taken from all the patients and healthy participants before taking on the sample , During sample collection , health measures and safety was taken through an interview conducted with patients and/or their parents using a questionnaire, demographic and clinical data (such as name, age, sex, medical history, laboratory analysis such as ANA, anti-ds DNA , C3, and C4) were gathered (Appendix A). Patient whom diagnosed with>10 EULAR/ACR points for SLE and>1:80 ANA titer will be inclusion in the study, then the patients were divided into three categories according to the disease activity score using software application with supervision of a rheumatologists , the SLEDIA score consists form

24 items across nine organ systems, with a possible score range of (0 to 105). This categories being mild for patients who scored from (1-5) points , moderate (6-10), and severe activity from (11-19) points . Excluded from the study were participants with a history of connective tissue diseases, SLE, autoimmune diseases, inflammation, pregnancy, malignant tumours, and neurological disorders

Figure 2.1 provides a schematic overview of the study's methodology, approaches, and procedures.

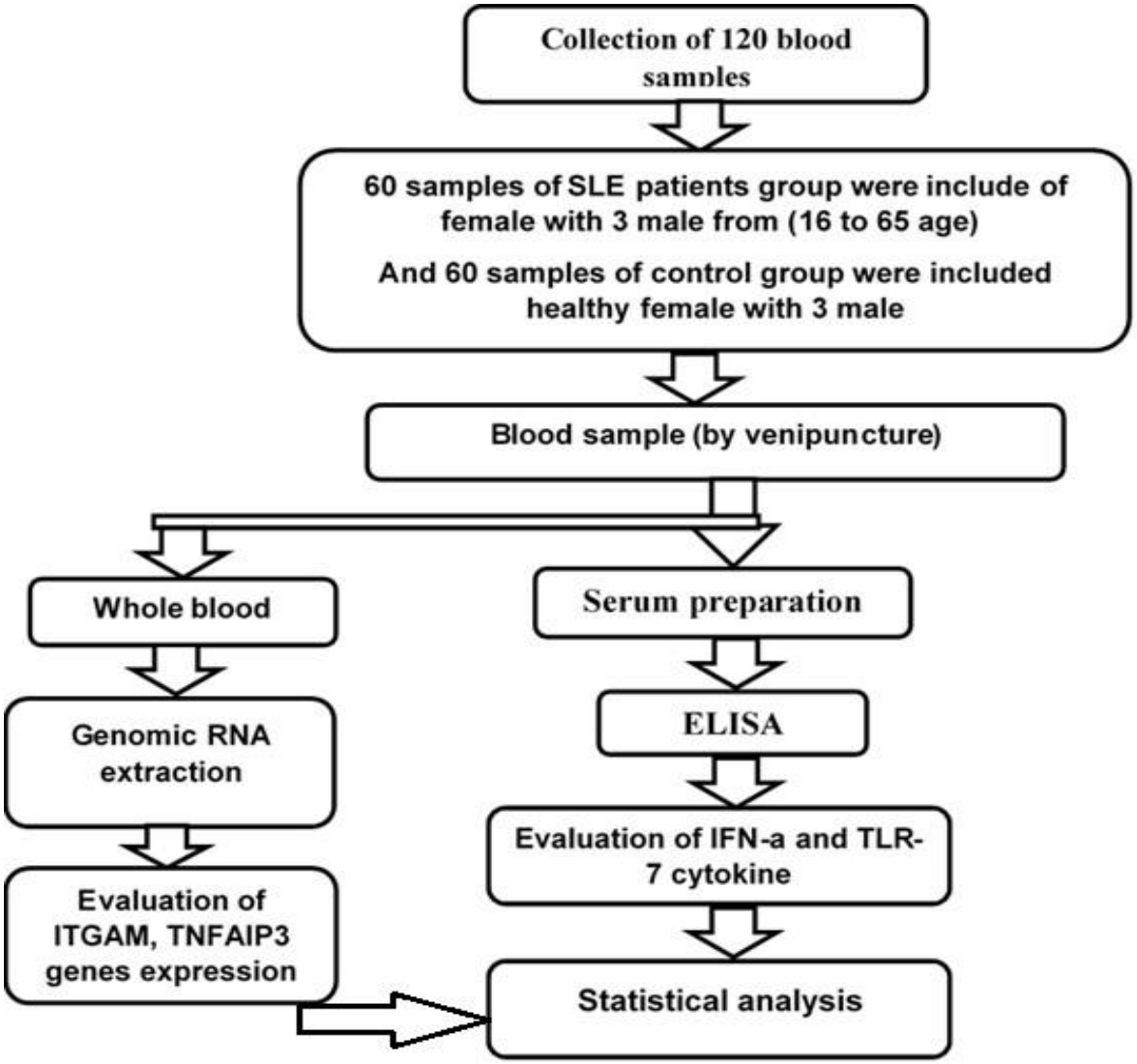

Figure 2.1: Schematic Presentation of the Study Design as well as Techniques and Procedure.

2.1 Materials

2.1.1 Equipment and Instruments

In the present study, the following Equipment and Instruments were used (Table 2.1).

Table 2:1 Equipment and instruments with their and contrary of origin

Equipment and Instrument	Manufacturing Company	Country
Disposable syringe (5ml)	Medeco	Germany
EDTA tube	Trust Lab	China
Gel tube	Trust Lab	China
Micropipettes (size)	Trust Lab	China
Plastic rack	Trust Lab	China
Centrifuge	Hattich	Germany
Deep freezer	Hettich	Germany
ELISA reader and printer	Human	Germany
Microcentrifuge	Thermo Fisher Scientific	USA
ELISA automated washer	Indiamart	India
Vortex	Scientific industrial	USA
Incubator	Selecta	Germany
Gloves	ALS	China
Eppendorf tubes (2 ml)	Trust Lab	China
Filter Tips for PCR (100MlAnd 200Ml)	ABD	China
Tips (Yellow and blue)	Chinas	China
Cold medical box	VB	China
Water bath	Polyscience	USA
Incubator	Memmert	Germany
PCR Tubes flat cup (Sterile) - 0.2ml	Bio basic	Canada
Micro spin Centrifuge	My Fugene	China
Micropipette 1.0-10ul	NEXTY-10	Japan
Micropipette 10 – 100ul	Slamed	Japan
Micropipette 100-1000ul	Slamed	Japan

Micropipette 50 – 200ul	Slamed	Japan
Tips (Blue 1000 µl/ yellow 200 µl /White 10 µl)	Bioland	USA
Nanodrop Spectrophotometer	Thermo Fisher Scientific	USA
Real time PCR	QIAGEN Rotor gene Q	Germany
Thermocycler (PCR)	G-storm	US
Vortex	Stuart Scientific	UK

Table 2.2: Chemicals and Biological Materials Used in the Study

Chemical	Sources	Country
Absolute ethanol	SIGMA	USA
Chloroform	SIGMA	USA
Oligonucleotide (Primers)	Alpha DNA	Canada

2.1.2 Kits and Reagents

2.1.2.1 The Gene Expression Analysis Kits

All kits included in this investigation as well as their contents are documented in Tables 2.3 – 2.6, along with their manufacturing company and sources of origin(Appendix B) .

Table 2.3: The Study Kits Company and Sources .

Biological materials	Source
TransZol Up Plus RNA Kit	China
EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix	China
TransStart® Top Green qPCR Super Mix	China

Table 2.4: The TransZol Up Plus RNA Kit's Components, (TransGen, biotech. ER501-01).

Components	(100 rxns)
TransZol Up	100 ml
Clean Buffer 9 (CB9)	110 m1
Wash Buffer 9 (WB9)	24 ml
RNase-free Water	40 ml
RNA Spin Column with Collection tubes	100 each
RNase-free Tube (1.5 ml)	100 each

Table 2.5: The *EasyScript*® One-Step gDNA Removal and cDNA Synthesis SuperMix Components.

Component	(50 rxns)
EasyScript RT/RI Enzyme Mix	50 µl
gDNA Remover	50 µl
2xES Reaction Mix	500 µl
Random Primer (N9) (0.1 µg / µl)	50 µl
Anchored Oligo(dT)18 Primer (0.5 µg / µl)	50 µl
RNase-free Water	500 µl

Table 2.6: The Reaction components of *2xEasyTaq*® PCR SuperMix (25 µL) .

Component	Final Volume reaction
Template	4 µl
Forward Primer (10 µM)	1 µl
Reverse Primer (10 µM)	1 µl
2xEasyTaq® PCR SuperMix	12.5 µl
Nuclease-free Water	6.5 µl
Total volume	25 µl

2.1.2.2 Primers using in this study

The primers were designed using the Primer 3plus, V4, and double checked by the University Code of Student Conduct (UCSC) programs, and with their reference sequences in the National Center for Biotechnology Information (NCBI) database. They were synthesized and lyophilized by Alpha DNA Ltd. (Canada). Table (2.7) displays all primer sequences utilized in this study's assays .

Table 2.7: The Study's Designed Primers .

Primer	**Sequence (5′→3′ direction)**	**primer size bp**	**Product size bp**	**Ta** °C
ITGAM (Gene Expression)				
Forward	GCTGATGCCCAATAAAGATG	20	139	58
Reverse	TGTACACTTGAATGCCTTGT	20		
***GAPDH*- Glyceraldehyde 3-phosphate dehydrogenase**				
Forward	GAAATCCCATCACCATCTTCCAGG	24	160	58
Reverse	GAGCCCCAGCCTTCTCCATG	20		
TNFAIP3(Gene Expression)				
Forward	GGTTGCTGTCATATTTGCTC	20	194	58
Reverse	TCGTCTGGGAAAAACTTAGG	20		

2.1.2.3 ELISA kits (Appendix C)

Table 2.8: Elisa kit for detection serum (Human Interferon α ELISA kit).

Reagent	**Quantity**
Standard Solution (1280pg/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Reagent	**Quantity**
Stop Solution	6ml x1
Substrate Solution A	6ml x1

Substrate Solution B	6ml x1
Wash Buffer Concentrate (25X)	20ml x1
Biotinylated human IFN-α Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Table 2.9: ELISA Kit for Detection Serum Human TLR-7 (Toll Like Receptor 7)

Reagents	**Quantity**		**Storage Condition**
	48T	**96T**	
Pre-Coated Microplate	6 strips x 8 wells	12 strips x 8 wells	-20°C (6 months)
Standard (Lyophilized)	1 vial	2 vials	-20°C (6 months)
Biotinylated Antibody (100×)	60 µL	120 µL	-20°C (6 months)
Streptavidin-HRP (100×)	60 µL	120 µL	-20°C (6 months)
Standard/Sample Diluent Buffer	10 mL	20 mL	4°C
Biotinylated Antibody Diluent	6 mL	12 mL	4°C
HRP Diluent	6 mL	12 mL	4°C
Wash Buffer (25×)	10 mL	20 mL	4°C
TMB Substrate Solution	6 mL	9 mL	4°C (store in dark)
Stop Reagent	3 mL	6 mL	4°C
Plate Covers	1 piece	2 pieces	4°C

2.2 Methods

2.2.1 sample collection

Blood samples were taken by venipuncture after the antecubital fossa was cleaned with 70% ethanol. Five milliliters of blood were extracted and put into Eppendorf tubes with 1,20 milliliters of Trizol reagent, and four milliliters of blood were taken into a gel tube for the manufacture of serum, respectively. The serum was produced by centrifuging the mixture for 15 minutes at 3000 rpm. The supernatants were then transferred into Eppendorf tubes and kept cold until needed

2.2.2 Determination TLR-7 Cytokine using ELISA

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). Human TLR7 antibody has been pre-coated on the plate. When TLR7 from the sample is added, it binds to the coated antibodies on the wells. The human TLR7 antibody that has been biotinylated is then added, and it binds to TLR7 in the sample. Next, the biotinylated TLR7 antibody binds to streptavidin-HRP. During a washing phase, unbound streptavidin-HRP is removed following incubation. After adding the substrate solution, color changes in response to the concentration of human TLR7. The addition of an acidic stop solution ends the process, and the absorbance is measured at 450 nm.

2.2.2.1 Assay Procedure

i. As directed, prepared all reagents, standard solutions, and samples. Before using, prepared all of the reagents to room temperature. The experiment was carried out at room temperature.
ii. The standard well has been filled with 50µl of standard solution .
iii. The sample wells were filled with 40µl of material, followed by 10µl of anti-TLR7 antibody, 50µl of streptavidin-HRP, and standard wells

(not the blank control well). It was Blended thoroughly. Then applied a sealant to the plate. At 37°C, And incubate for 60 minutes .

iv. The sealer were Take off and use wash buffer to wash the plate five times.For every wash, The were soaked wells in a minimum of 0.35 ml of wash buffer for 30 to 60 seconds. Every were Aspirated well in the automated washing system, then each well were filled to the brim with wash buffer and repeat the process five times. The plate Blotted onto absorbent material such as paper towels.

v. Each well was Filled with 50µl of substrate solution A, followed by 50µl of substrate solution B. Plate was incubated for 10 minutes at 37°C in the dark with a fresh sealer covering it.

vi. After adding 50µl of Stop Solution to each well, the blue hue instantly turn yellow.

vii. Within ten minutes of adding the stop solution, the optical density (OD value) of each well were measured using a microplate reader set to 450 nm.

2.2.3 Determination of IFN-α Cytokine using ELISA

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). Human IFN-α antibody has been pre-coated on the plate. When IFN-α from the sample is added, it binds to the coated antibodies on the wells. The human IFN-α antibody that has been biotinylated is then added, and it binds to IFN-α in the sample. Next, the biotinylated IFN-α antibody binds to streptavidin-HRP. During a washing phase, unbound streptavidin-HRP is removed following incubation. After adding the substrate solution, color changes in response to the concentration of human IFN-α. The addition of an acidic stop solution ends the process, and the absorbance is measured at 450 nm.

2.2.3.1 Assay Procedure

i. The standard well was Filled with 50µl of the standard solution

ii. The sample wells were Filled with 40µl of material, followed by 10µl of anti- IFN-α antibody, 50µl of streptavidin-HRP, and standard wells (not the blank control well). Then Blended thoroughly. And Applied a sealant to the plate. At 37°C, then incubated for 60 minutes.

iii. The sealer was Take off and used wash buffer to wash the plate five times. For every wash, The wells were soaked in a minimum of 0.35 ml of wash buffer for 30 to 60 seconds. Every well was Aspirated in the automated washing system, then filled each well to the brim with wash buffer and repeat the process five times. The plate Blotted onto absorbent material such as paper towels.

iv. Each well was Filled with 50µl of substrate solution A, followed by 50µl of substrate solution B. The plate incubated for 10 minutes at 37°C in the dark with a fresh sealer covering it.

v. After adding 50µl of Stop Solution to each well, the blue hue instantly turn yellow.

vi. Within ten minutes of adding the stop solution, The optical density (OD value) was measured of each well using a microplate reader set to 450 nm.

2.2.4 Gene Expression Analysis

2.2.4.1 RNA Extraction from the Blood Sample

i. Total RNA was extracted from all samples using the TransZol Up Plus RNA Kit Reagent according to the manufacturer's instructions. As follows:

ii. 250 ml of the blood was centrifuged (1 minute/12,000 rpm), the supernatant was discarded, and the pellet was resuspended in 750 µl TransZol Up, Overnight, the samples were kept at-23ºC.

iii. For each ml of TransZol Up Reagent, added 200 µl of chloroform. the tube was vortexed gently for 30 seconds and incubated for 3 minutes at room temperature.

iv. The tube was spun up for 15 minutes at 2–8 oC at 10,000 rpm. Three phases were identified in the mixture: an interphase, a lower pink organic phase, and an upper colorless aqueous phase that contained the RNA. In TransZol, the aqueous upper phase makes up between 50 and 60 percent of the total volume. Up.

v. The colorless upper phase containing RNA was transferred to a fresh RNase-free tube (some aqueous phase can be retained to prevent DNA contamination from interphase). added a volume corresponding to 96%–100% ethanol (this phase may show precipitates). Gently stir by turning the tube around.

vi. The produced solution and the precipitates were combined and moved to a spin column. centrifuged for 30 seconds at ambient temperature at 12,000 rpm. removed the flow through (this procedure is repeated if the amount of lysate exceeds the capacity of the spin column).

vii. The spin column was filled with 500 µl of CB9. centrifuged for 30 seconds at ambient temperature at 12,000 rpm. threw away the flow through.

viii. Through step 6 were gone once more

ix. Added 500 µl of WB9 to the spin column, making sure to include ethanol. centrifuged for 30 seconds at ambient temperature at 12,000 rpm. threw away the flow through. Step 8 was carried out once more.

x. The centrifuged the column matrix was done for two minutes at ambient temperature at 12,000 rpm to remove any remaining ethanol. After that, Then leave it to air dry for a few minutes.

xi. After cleaning, the spin column was put into a 1.5 mL RNase-free tube. After adding 50–200 µl of RNase-free water, the mixture was incubated for one minute at room temperature.

xii. Centrifuging for a minute at 12,000 rpm was used to elute the RNA. (It is advised to repeat Steps 11 and 12 to complete the second elution in order to increase the yield.)

xiii. The isolated RNA was kept in a -20°C storage.

2.2.4.2 RNA Concentration and Purity Assessment

The 2000c Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) was used to evaluate the concentration and purity of extracted RNA in order to determine the quality of samples for subsequent analysis in RT-qPCR. The samples ranged in RNA concentration from 73-147 ng/µl, while the absorbance of the samples was measured at two distinct wavelengths to determine RNA purity (260 and 280nm). The presence of an A260/A280 ratio of around 2.0 suggested that the RNA sample was pure

2.2.4.3 Synthesis the cDNA form mRNA and miRNA

2.2.4.3.1 First Strand cDNA Synthesis, Reaction Component

Using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit, total RNA was reverse-transcribed to complementary DNA (cDNA). According to the manufacturer's instructions, the operation was performed in a reaction volume of 20 µl. (4µl) of total RNA had to be reversely transcribed.

Table 2.10: cDNA Synthesis Reaction Component

Component	**volume reaction**
mRNA/miRNA	4 µl
Anchored Oligo(dT)18 Primer (0.5 µg / µl)	1 µl
Random Primer (0.1 µg / µl)	1 µl
GSP	1 µl/ 10 pmol
2xES Reaction Mix	10 µl
EasyScript ® RT/RI Enzyme Mix	1µl

gDNA Remover	1µl
RNase-free Water	1µl
Total volume	20µl

Incubated a random primer for 10 minutes at 25°C. For qPCR, incubated an anchored oligo (dT) 18 primer and GSP for 15 minutes at 42°C. In order to inactivate enzymes, they incubated for 5 seconds at 85°C. As shown in Table 2.11.

Table 2.11: Thermal Cycler Steps for cDNA Reverse Transcription Conditions

	Step 1	**Step 2**	**Step 3**
Temperature	25 ºC	42°C	85 ºC
Time	10 min	15 min	5 Sec.

2.2.4.4 Quantitative Real Time PCR (qRT–PCR)

Using the sensitive reverse transcription-quantitative polymerase chain reaction (qRT-PCR) approach, which measures steady-state mRNA levels, the expression levels of the *ITGAM* and *TNFAIP3* genes were estimated. The target gene's expression was verified using a quantitative real-time qRT-PCR SYBR Green test. Alpha DNA Ltd. (Canada) designed and synthesized primer sequences for the *ITGAM* and *TNFAIP3* genes, then lyophilized and stored at -20°C.

The endogenous control gene *GAPDH*'s mRNA levels were amplified and utilized to normalize the *ITGAM* and *TNFAIP3* gene's mRNA levels.

Table 2.12 shows the primer sequences for *GAPDH, ITGAM* and *TNFAIP3* genes. The Quantitative Real Time PCR (qRT–PCR) was carried out using the QIAGEN Rotor Gene Q Real-time PCR System (Germany).

The expression levels and fold changes of the *ITGAM, TNFAIP3 and GAPDH* genes were assessed using the *TransStart*® Top Green qPCR Super Mix kit and measuring the threshold cycle (Ct). Every reaction was

performed twice. The needed volume of each component was determined using Table 2.12.

Table 2.12: The Components of Quantitative Real-Time PCR were Employed in the *GAPDH, ITGAM and TNFAIP3* Gene Expression Experiments

Components	**20 µl rxn**
2xTransStart® Top Green qPCR Super Mix	10
Nuclease free water	6
Forward Primer (10 µM)	1
Reverse Primer (10 µM)	1
cDNA	2

The cycling protocol was programmed for the following optimized cycles and according to the thermal profile shown in Table 2.13 while the thermal profile of *GAPDH, ITGAM* and *TNFAIP3* gene expression is presented in Figure 2.2.

Table 2.13: The Thermal Profile of *GAPDH, ITGAM and TNFAIP3* Gene Expression

Step	**Temperature (**ºC)	**Time (sec.)**	**Cycles**
Enzyme activation	94	30	1
Denaturation	94	5	40
Annealing	58	15	
Extension	72	20	
Dissociation	55 ºC-95 ºC		1

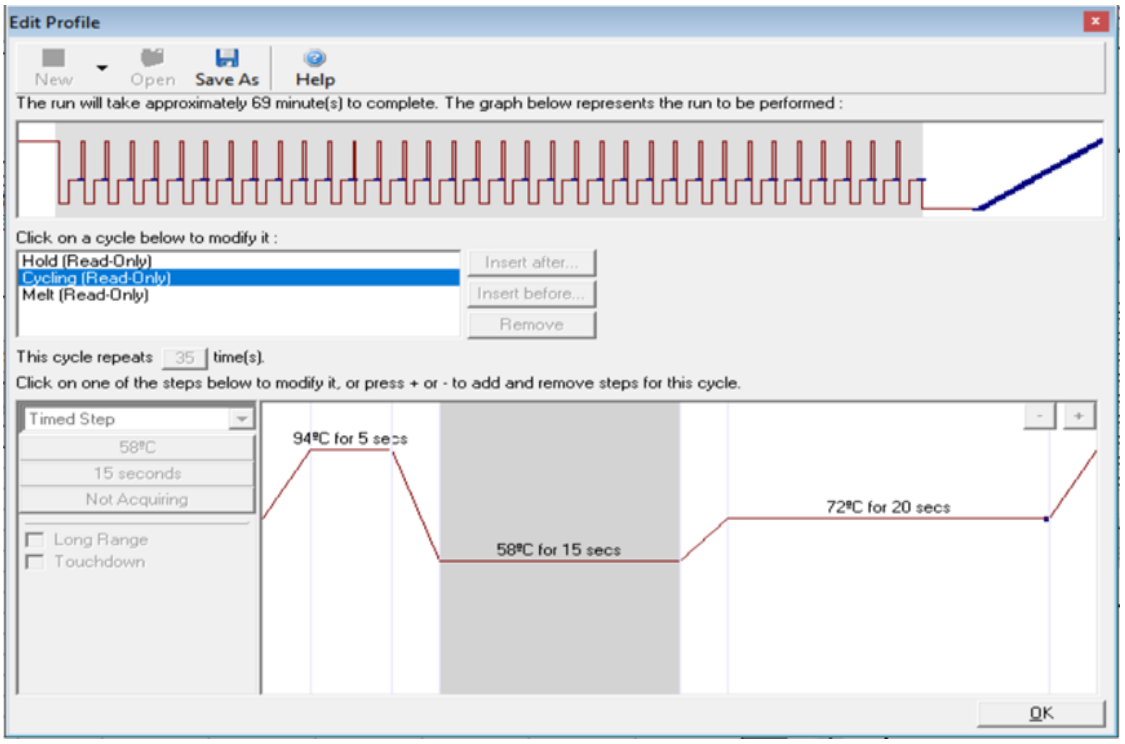

Figure 2.2: The Thermal Profile of *GAPDH, ITGAM and TNFAIP3* Gene Expression The image was taken directly from the qPCR machine.

2.2.4.5 *ITGAM* and *TNFAIP3* Genes Expression Calculation

Livak and Schmittgen (2001) initially published the relative cycle threshold (2-ΔΔCt) approach, which was used to quantify fold changes of the measured expression of the mature RNAs. It is the proportion of the test group's relative gene expression to that of the control group. A fold change of 1 implies no change, numbers between 1 and 0, respectively, indicate downregulated or decreased gene expression, and numbers more than 1 indicate upregulated or increased gene expression. By establishing suitable thresholds, the target genes' expressions were standardized in order to acquire precise Ct values from the qRT-PCR device.

The double delta Ct (threshold cycle) analysis was used to assess the expression of *ITGAM* and *TNFAIP3* genes, in which the *GAPDH* and *miRNAU6* were the housekeeping reference genes. The calculations were as the following:

i. The threshold cycle (CT) was computed for every sample using the real-time cycler program. Every sample was ran twice, and mean results were computed. the Ct values for the housekeeping genes (*GAPDH*, *miRNAU6*) and the target genes (*ITGAM* and *TNFAIP3*, *miRNA27b*) being tested in patients and controls were recorded.

ii. The difference between the CT values (ΔCt), also called the "normalized raw data" for each gene of target and the housekeeping gene was calculated by subtracting the selected normalization factor from the Ct value of each gene of interest.

$$\Delta Ct\ (control) = CT\ (gene) - CT(HKG)$$

$$\Delta Ct\ (patient) = CT\ (gene) - CT(HKG)$$

iii. Finally, the expression ratio was calculated according to the formula:

$$2^{-\Delta CT} = \text{Normalized expression ratio}$$

iv. The Double Delta Ct Value (ΔΔCt) for the genes of interest was calculated by subtracting the ΔCt value of each test group from the control group as follow:

$$\Delta\Delta Ct = \Delta Ct(patient) - \Delta Ct\ (control)$$

v. To get the expression fold change for each gene, the $2^{-\Delta\Delta Ct}$ was obtained, which represents the Relative Fold Change. Therefore, the results were expressed as a fold change in the expression level of target gene that was normalized to endogenous control (housekeeping gene) and relative to calibrator, which is the target gene in control subjects (Stephenson,2016). Finally, the fold-change value in gene expression was calculated as follow: Fold change $=2^{-\Delta\Delta Ct}$ Normalized expression ratio.

2.3 Software program

For assessment of disease progression, for measures in SLE was describe outcome (disease activity, damage from disease, and health status), based on 24 items across nine organ systems, with a possible score range of (0 to 105).and hence the SLE disease activity index (SLEDAI) was formulated in software application . The group of patients was classified according to disease activity into three categories: mild, moderate, and severe, according to the following grades :

Grade I: SMEDAI = 0 (No activity)

Grade II: SMEDAI = 1 – 5 (Mild activity)

Grade III: SMEDAI = 6 -10 (Moderate activity)

Grade IV: SMEDAI = 11 – 19 (High activity)

Grade VI: SMEDAI ≥ 20 (Very high activity)

The disease activity stages it has been to the software application RheumHub . This APP is owned by Kuwait Association of Rheumatologists (KAR) https://karkwt.org/ (Appendix D)

2.4 Statistical Analysis

The IBM SPSS (26 edition) application was used for data analysis in order to determine how various factors affected the study's parameters. The T-test and one-way ANOVA were utilized to compare means statistically. A meaningful comparison between percentages (0.05 and 0.01 probability) was made using the Chi-square test. The study's estimated odds ratio and confidence interval. GraphPad Prism 9 was utilized to create the study's figures.

Chapter Three

Results

3.1 Demographical and Clinical Characteristics of the Study Patients

The mean age of the SLE patients was 34.23±8.06 years, comprising 57 females, aged 37.35±14.03 years (range, 16 – 67 years) as well as 3 males, aged 30.00±4.00 years (26 – 34 years). The control group had the mean age of 30.22±6.24 years, comprising 57 females, aged 31.86±11.19 years (range, 16 - 62 years) as well as 3 males aged 30.00±7.55 years (range, 23 – 38 years). The age and sex of the study subjects are presented in Table (3.1). Statistical comparison between the SLE and control groups as well as between Sexes in each group, was not significant at $p < 0.05$.

Table 3.1: Age and Sex of the Study Subjects

Groups	Sex	Mean (years)	Std. Dev. (years)	* SEM (years)	p-value	
SLE	**Female**	37.35	14.03	1.86	0.3 NS	1.00 NS
	Male	30.00	4.00	2.31		
Control	**Female**	31.86	11.19	1.48	0.7 NS	
	Male	30.00	7.55	4.36		

***** SEM (standard error of mean); NS (not significant)

***** Statistically significant at ($p < 0.05$) .

Regarding family history of SLE (Table 3.2), 44 (73.3%) SLE patients had a history of SLE disease, while 16 (26.7%) of them did not. However, all 60 (100%) subjects of the control had no family history of SLE disease. The difference between the study groups with respect to family history was statistically significant with $p = 0.01$ ($p < 0.05$).

Table 3.2: Family History of SLE among the Study Subjects

Family History	Groups		p-value
	Patients n *(%)*	Control n *(%)*	
No	16 *(26.7)*	60 *(100)*	0.01*
Yes	44 *(73.6)*	0 *(0)*	
Total	60	60	

***** Statistically significant ($p < 0.05$)

3.2 Categorization of the SLE Patients Based on Disease Severity

Table (3.3) shows the distribution of the patients with respect to severity of disease condition. Thirty-six (representing 60%) of the patients had mild SLE, while 19 patients (representing 31.7%) had moderate SLE and 5 patients (representing 8.3%) had severe SLE.

Table 3.3: Distribution of the SLE patients with respect to Disease Severity

SLE Disease Severity	n *(%)*
Mild	36 *(60.0)*
Moderate	19 *(31.7)*
Severe	5 *(8.3)*

3.3 Biochemical Analysis of the Study Subjects

3.3.1: Correlation between Autoantibodies (ANA , Anti dsDNA) , complement (C3, C4) , and stages of the disease activity (mild , moderate , sever) of the Study Subjects .

Table (3.4)shows the association between autoantibodies (ANA , Anti dsDNA) complement (C3 , C4) levels ,and disease activities . Negative correlation was observed between ANA , Anti dsDNA and mild, moderate and sever stages ($p = 0.8$). Relatively negative correlation was observed between C3 and disease activities stages ($p = 0.2$) while a strong positive

correlation was observed between C4 and disease activity stages (p = 0.005).

Table 3.4: Correlation between Autoantibodies (ANA ,and Anti dsDNA) , coplement (C3 ,C4) and disease activity stages of the Study Subjects

Stage of disease		ANA	Anti-DNA	C4	C3
Mild	Mean	50.8418	225.75	6.1377 c*	51.2767
	SD	88.98490	387.063	8.97385	54.35157
	SE	33.24623	136.847	2.99128	31.37989
Moderate	Mean	50.4533	83.79	78.5000 a*	
	SD	82.59209	61.898	57.27565	
	SE	27.53070	35.737	40.50000	
sever	Mean	9.6500	146.00	42.0000 b*	149.0000
	SD	10.39447	.	.	.
	SE	7.35000	.	.	.
P-VALUE		0.8	0.8	0.005**	0.2

* c , a and b: Different letters mean there is significant difference. Means followed by the same letter are not significantly different

3.3.2 Serum Analysis of Cytokines IFN-α and TLR-7

The serum levels of cytokines IFN-α and TLR-7 of the SLE patients and the control subjects were measured. For IFN-α, the SLE patients had the mean value of 344.79±137.98 U/mL which was significantly higher at $p < 0.05$, compared to that of the control which was 234.88±121.15 U/mL (p = 0.01).

For TLR-7, the SLE patients had the mean value of 786.48±244.68 U/mL which was significantly higher at $p < 0.05$, compared to that of the control which was 426.66±129.71 U/mL ($p = 0.001$) . This data is graphically presented in Figure (3.1).

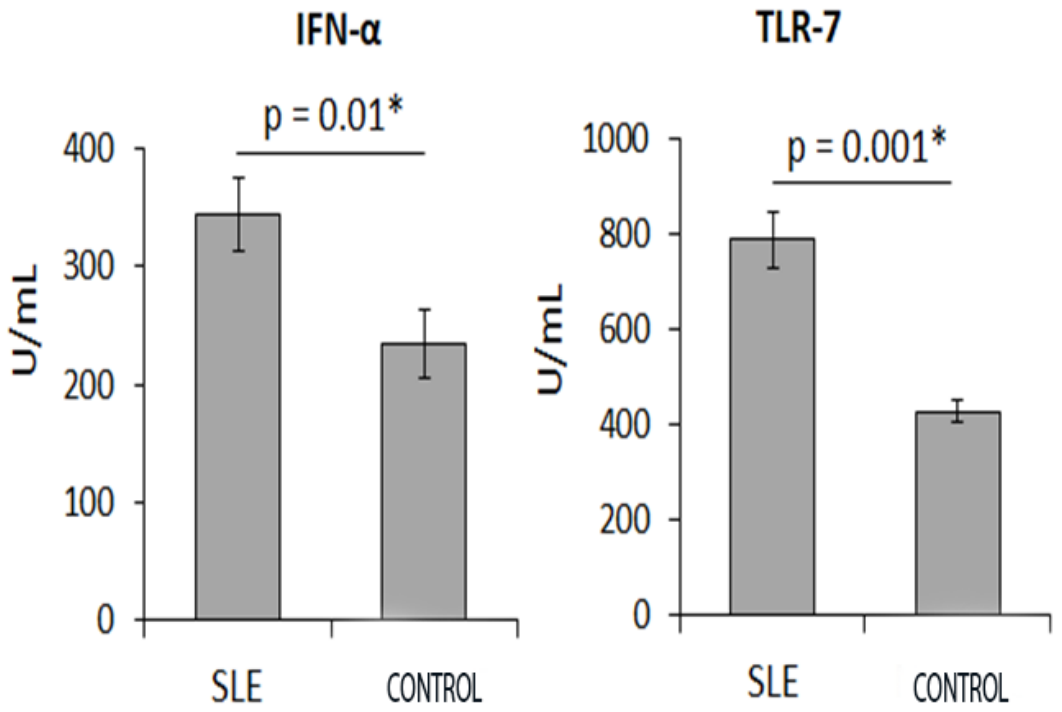

Figure 3.1: Mean Levels of Serum SLE-associated Cytokines
The mean IFN-α level was significantly higher in the sera of the SLE patients compared to the control group. Similarly, mean TLR-7 level was also significantly higher in the sera of the SLE patients compared to the control group ($p < 0.05$).
*Statistically significant at $p < 0.05$.
Error bars indicated standard error of mean (SEM).

Receiver operating characteristic (ROC) curves (Figure 3.2) data showed that while levels of TLR-7 had the specificity of 93% with the sensitivity of 50% (AUC = 0.75), IFN-α had a relatively lower specificity (i.e. 83%) with a lower sensitivity of 40% (AUC = 0.65) .

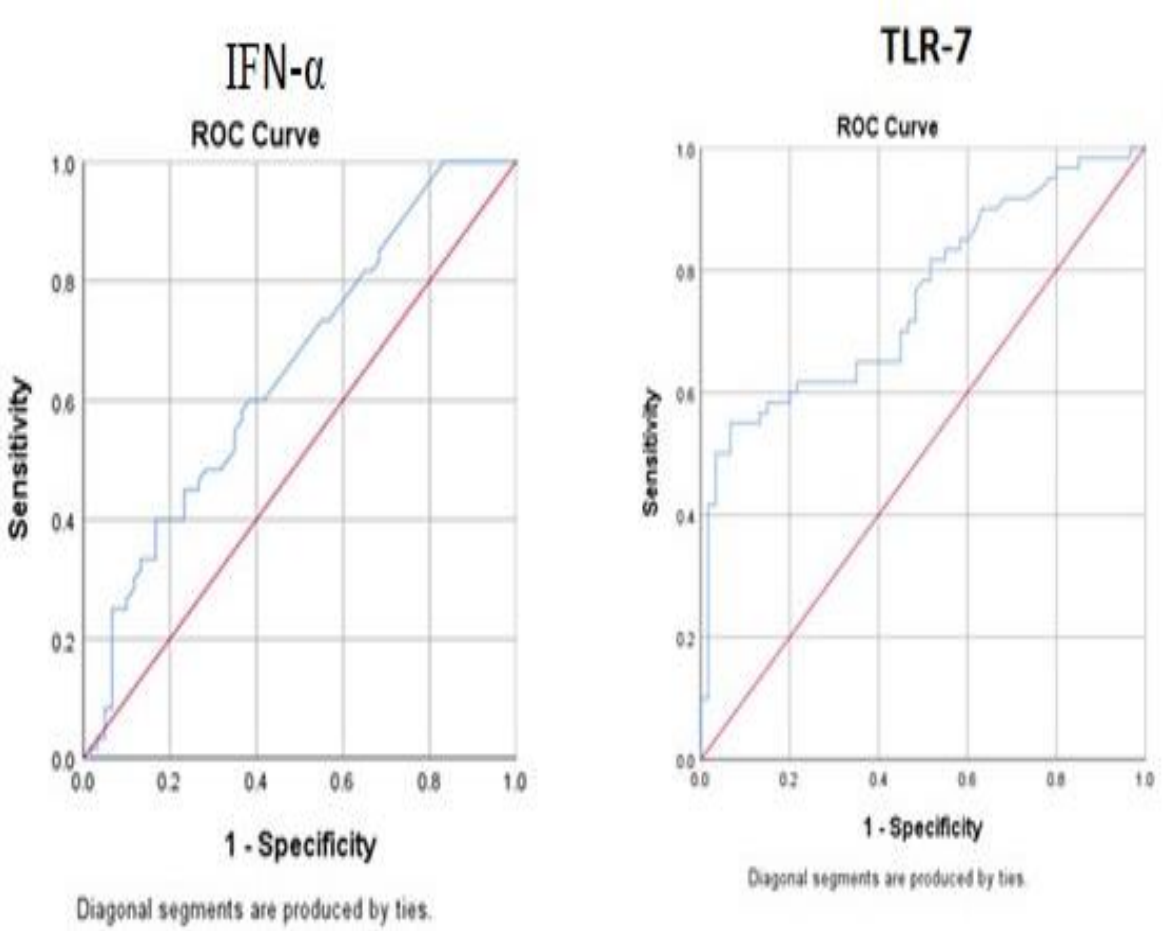

Figure 3.2: ROC curve for the Measured IFN-α and TLR-7
The results indicate that TLR-7 levels had a specificity of 93% and a sensitivity of 50% (AUC = 0.75); in contrast, IFN-α levels had a considerably lower specificity of 83 % and a sensitivity of 40% (AUC = 0.65) .

3.4 Analysis of *ITGAM* and *TNFAIP*3 Genes Expression Levels in the Study Subjects

Gene expression analysis showed significantly (p = 0.0001) increased expression of *ITGAM* in SLE patients by 15.48 folds compared to the non-SLE group. Similarly, the expression level of *TNFAIP3* was significantly (p = 0.0001) increased by 43.08 folds in the SLE patients relative to the non-SLE group (Table 3.5).

Table 3.5: Gene Expression Fold of *ITGAM* and *TNFAIP3* Genes between SLE patients and non-SLE group

	ITGAM		***TNFAIP3***	
	SLE	**Control**	**SLE**	**Control**
Mean Ct (Gene of Interest)	20.82	24.74	20.13	25.53
Mean Ct (*GAPDH*)	20.32	20.29	20.32	20.29
ΔCt (Means Ct)	0.496	4.446	-0.186	5.243
$2^{-\Delta Ct}$	0.709	0.046	1.137	0.026
Gene Expression Fold	15.48	1	43.08	1
p-value	0.0001*		0.0001*	

*Statistically significant ($p < 0.05$) .

The ROC curves (Figure 3.3) data showed that *ITGAM* had the specificity of 100% with the sensitivity of 98% (AUC = 1.00). While *TNFAIP3* had the specificity of 98% with the sensitivity of 93% (AUC = 0.97) (Table 3.6)

Table 3.6: ROC Curve Data for the Measured *ITGAM* and *TNFAIP3*

Parameters	***ITGAM***	***TNFAIP3***
***AUC**	01.00	0.97
Interpretation	Excellent	Excellent
p-value	0.004	0.001
Best Cut-off	3.507	12.735
Sensitivity (%)	98	93
Specificity (%)	100	98

*AUC (Area under curve)

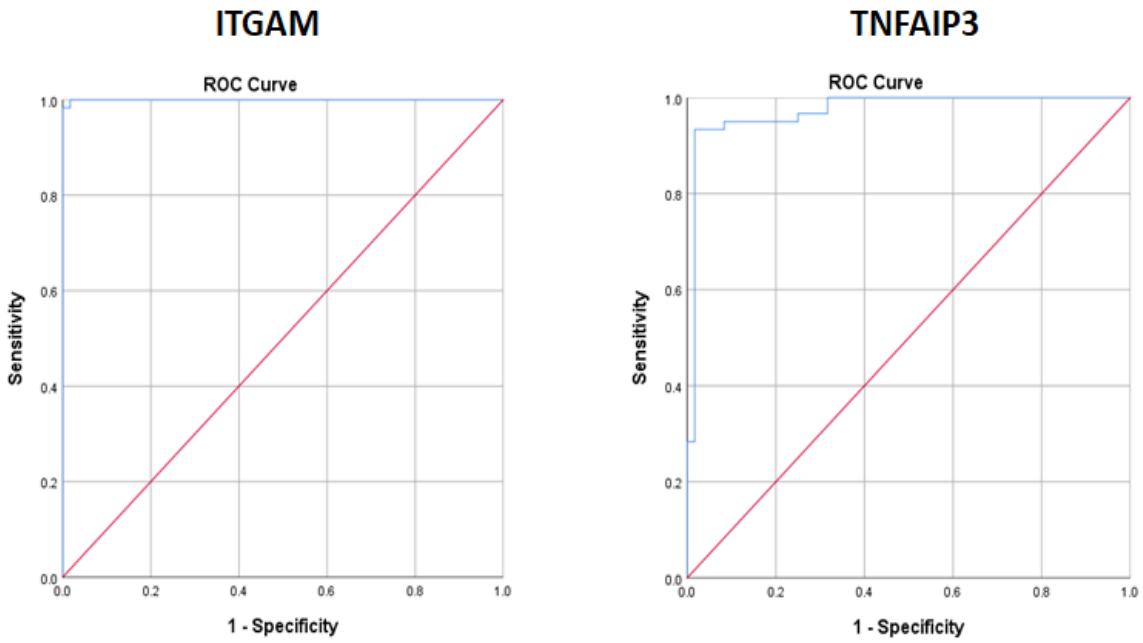

Figure 3.3: ROC curve for the Measured *ITGAM* and *TNFAIP3.*

ITGAM had a 100% specificity and a 98% sensitivity, according to the ROC curves (AUC = 1.00). Conversely, *TNFAIP3* (AUC = 0.97) had a 98% specificity and 93% sensitivity.

3.5 Correlation between Cytokine (IFN-α), (TLR-7 receptor) Expression and Gene (*ITGAM* and *TNFAIP3*) Expression of the Study Subjects

Table (3.7) shows Pearson's correlation matrix indication the association between cytokine expression levels and gene expression levels of the study subjects. Positive moderate correlation was observed between IFN-α and TLR-7 (r = 0.345, p = 0.000). Relatively weak positive correlation was observed between TLR-7 and *TNFAIP3* (r = 0.218, p = 0.017) while a strong positive correlation was observed between *ITGAM* and *TNFAIP3* (r = 0.824, p = 0.000).

Table 3.7: Pearson's Correlation Matrix for Cytokine (IFN-α and TLR-7) Expression and Gene (*ITGAM* and *TNFAIP3*) Expression of the Study Subjects

		INF-α	TLR-7	*ITGAM*	*TNFAIP3*
INF- α	Pearson Correlation	1	.345**	.064	.104
	Sig. (2-tailed)		.000	.485	.258
TLR-7	Pearson Correlation		1	.151	.218*
	Sig. (2-tailed)			.099	.017
ITGAM	Pearson Correlation			1	.824**
	Sig. (2-tailed)				.000
TNFAIP3	Pearson Correlation				1
	Sig. (2-tailed)				
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

3.6 Correlation between Cytokine (IFN-α), (TLR-7 receptor) levels Gene (*ITGAM* and *TNFAIP3*) Expression and disease activity stages , of the Study Subjects.

Table (3.9) shows correlation between cytokine expression levels , gene expression levels , and disease activity stages (mild , moderate , sever) of the study subjects. Negative correlation was observed between IFN-α , TLR-7, *ITGAM* , *TNFAIP3* and stages of disease activity which was p values respectively (p = 0.4 , p = 0.06 , p = 0.3 , p = 0.2).

Table 3.8: Correlation between Cytokine (IFN-α and TLR-7) , Gene (*ITGAM* and *TNFAIP3*) Expression and disease activity stages of the Study Subjects.

Stage of disease		IFN-α	TLR-7	*ITGAM*	*TNFAIP3*
Mild	Mean	270.7364	718.2119	24.058532	48.200616
	Std. Deviation	124.884	409.2644	17.87039	27.87573
	Std. Error of Mean	37.480	68.21074	2.978399	4.645956
Moderate	Mean	246.66	659.8138	26.41130	51.23550
	Std. Deviation	109.17	411.914	19.474857	27.87484
	Std. Error of Mean	47.9885	94.4997	4.467838	6.39492
sever	Mean	135.720	1141.52	13.83683	73.617263
	Std. Deviation	34.8559	359.492	6.0525271	45.04804
	Std. Error of Mean	15.5880	160.769	2.7067724	20.14609
Total	Mean	251.861	734.9954	23.951770	51.279720
	Std. Deviation	151.663	419.389	17.83996	29.72660
	Std. Error of Mean	27.325	54.1429	2.30312	3.83768
p-value		0.4	0.06	0.3	0.2

Chapter Four

Discussion

4.1 Discussion

The complicated inflammatory disease known as systemic lupus erythematosus (SLE) eventually affects every organ in the human body and has the potential to be fatal. It is characterized by increased apoptosis along with impaired clearance of apoptotic cells, resulting in the emergence of high levels of autoantibodies. Autoantibody synthesis also leads to tissue harm through the formation and deposition of autoantibody-autoantigen immune complexes. Race, region, and sex all affect the severity, risk, and clinical manifestation of SLE, with women and some non-European-derived populations having a higher prevalence (Bertsias et al., 2010). This study evaluated the demographical and clinical features of SLE patients in comparison to control. Also, the serum levels of SLE-associated cytokines (IFN-a) and TLR-7 receptor as well as expression levels of *ITGAM* and *TNFAIP3* genes were evaluated and comparisons were made between SLE patients and the non-SLE subjects.

4.2 Demographical and clinical characteristics of the study patients

More importantly, this study revealed a fundamentally significant difference ($p < 0.01$) between the SLE and non-SLE group with respect to family history of SLE. While 73.6% of the SLE patients had family history of SLE, none of the non-SLE participants had a family history of SLE. This is consistent with the majority of the scientific literature. For instance, it is consistent with the study of (Abrass, et al 1980), which shows that heritable circulating immune complexes (CIC) were measured using fluid phase (FClq) and solid phase (SClq) binding assays, and that the presence of SLE manifestations ($p < 0.001$), such as active renal disease ($p < 0.005$) and arthritis ($p < 0.001$), as well as changes in the degree of disease activity that prompted medical intervention. Eighty-two percent of the time ($p <0.005$),

a change in the SClq findings accurately indicated a change in disease activity.

4.3 Correlation between Autoantibodies (ANA , Anti dsDNA) , complement (C3, C4) , and stages of the disease activity (mild , moderate , sever) of the Study Subjects

Complex variables that affect immune modulation play a major role in the pathogenesis and etiology of SLE (Pan, et al 2020). Multiple autoantibodies, such as ANA in serum, are the primary manifestation of immunological abnormalities associated with SLE (Salaman and Isenberg 2017). Moreover, immune complex allergies, which can trigger allergic reactions through both conventional and unconventional pathways while including cytokine indicators, are the primary cause of tissue damage in SLE (Liu et al 2010). The current results , determining the association between SLE disease activity and laboratory parameters (ANA , Anti dsDNA , C3, C4) did not show a significant correlation , that were no association between them, and this is not consistent with a study that indicates that, patients with Lupus Nephritis (NL) had high SLEDAI scores Elevated Anti-dsDNA titer, low complement levels and were significantly associated with high SLEDAI scores (Shamim,*et al* 2020). This may explain the effect of some medications that patients take on disease activity. (Kim,*et al* 2014)There were several correlations of levels of ANA, anti-RNP, anti-dsDNA, and anti-ssDNA IgG with systemic disease activity measures in all patients with discoid lupus (DLE) with or without ANA positivity or SLE. High levels of ANA, anti-RNP, anti-dsDNA, or anti-ssDNA IgG in patients with DLE could enhances association of medication use, which can reduce their levels. Use of quinacrine hydrochloride decreased anti-dsDNA antibody levels in patients with SLE. Prospective studies tracking autoantibody levels and

disease activity in patients with DLE receiving oral lupus medications may validate this hypothesis .In this study, C3 and C4 are associated with disease activity of a SLE disease, and this is supported by the study (Weinstein, *et al* 2021). That indicate C4 better correlates with lupus disease activity.

4.4 IFN- α and TLR-7 levels of the studied groups

Additionally, this study reveals higher IFN-α in SLE patients when compared to the normal values, which may be a strong indicator of an effective immune-protective response in the patients leading to elevated levels of this cytokine. One of the type I interferons (IFN) family's members, IFN-α is primarily engaged in innate immunity. In response to a viral infection, immune (lymphocytes, NK cells, B-cells, T-cells, macrophages) and non-immune (fibroblasts, endothelial cells, osteoblasts, and others) cells release IFN-α 1. IFN-α's primary roles include alerting the body to viral infection by identifying aberrant double stranded DNA and preventing virus proliferation by affecting translation in infected cells. IFN-α production that isn't normal can cause tissue inflammation or immunological malfunction, including autoimmune disorders like rheumatoid arthritis and SLE (Veronique, *et al*. 2023). According to Brohawn et al (2019), patients with SLE can be divided into two groups based on their IFN-α levels: high and low. Concurrently, there was a strong correlation between the complexity of SLE and low-level INF-α. Conversely, low levels of IFN-α were associated with a considerable enrichment of IFN-γ-specific ($Q = 0.005$), eosinophil ($Q < 0.001$), and T-cell or B-cell ($Q < 0.001$) signatures, which may further support the previously demonstrated low levels of C4 and C3.

A substantial amount of evidence suggests that Toll-like receptors (TLRs), particularly TLR7, are crucial for the pathophysiology of SLE. They have a critical role in the development and maintenance of certain autoimmune

disorders, most notably SLE (Hurst and von Landenberg 2008). Autoantigen expression is elevated in patients with systemic lupus erythematosus (SLE) due to disruptions in apoptosis and deficiencies in the removal of apoptotic bodies. Furthermore, evidence from the literature points to a possible link between TLR anomalies and the onset of autoimmune processes (Klonowska-Szymczyk et al 2017). The study's findings included a review of the general literature and demonstrated an unusually high level of TLR7.

According to the results of the current study, TLR-7 level can be a very useful tool for management and prognoses SLE; its specificity is higher at 93%, although its sensitivity was only about 50%.AUC=0.75 indicates a decent value .This agrees with the study (Jassim, *et al* 2023). that indicated serum level of TLR-7, was evaluated in SLE patients compared to healthy people and they may be suggested as a prognostic tool in SLE patients. This was not consistent with the study that was also conducted in Iraq (Jassim,*et al* 2023). Which indicated that TLR-7 was less associated with SLE , The reason may be due to genetic variation as was explained in this same study the TLR-7 (rs3853839) GG genotype is correlation with SLE pathogenesis in Iraqi SLE patients while the TLR-7 rs3853839 CG genotype is common in SLE-free control. .INF-α is a useful prognoses and management tool for SLE, but its low specificity (83%) means it cannot be used in isolation. Its sensitivity was just 40%. (AUC of 0.65 indicates a good value.)

4.5 Quantitative Expression of *ITGAM* and *TNFAIP3* Genes

One of the most notable findings of this study is the substantial difference in the fold of gene expression in the tumor necrosis factor (TNF) Alpha Induced Protein 3 (*TNFAIP3*) between the patient and control groups (p = 0.0001). The protein A20, which is a negative regulator of NF-κb, is

encoded by *TNFAIP3*. Inflammation and autoimmunity have been related to its deficit in immune cells (Ciccacci et al 2019). According to preliminary expression investigations, patients with SLE have lower levels of *TNFAIP3* than healthy controls (Ciccacci et al, Qian, et al 2017); However, the idea that *TNFAIP3* is a causal gene linked to both SLE and rheumatoid arthritis is supported by genetic and functional studies. Thus, *TNFAIP3* might have a significant role in the development of certain autoimmune disorders. The results showed that the *TNFAIP3* nonsynonymous coding SNP rs2230926 had the highest odds ratio (OR). Additionally, the minor allele of rs2230926 was linked to an increased risk of SLE (Musone, 2008)

The study's results also demonstrate a significant difference in the fold of gene expression in the *ITGAM* groups of patients and controls ($p = 0.0001 < 0.01$), which is consistent with the body of general literature. For instance, it is consistent with the study conducted by (Rhodes, et al 2008), which examined the rs1143679 variant of *ITGAM*, encoding the R77H variant of CD11b (part of complement receptor 3; CR3). The study found that there was a 31% decrease in the phagocytosis of iC3b (CR3 ligands) by 77H cells opsonized sheep erythrocytes (sRBCiC3b) ($p=0.003$), as well as decreased adhesion to a variety of ligands, most notably a 24% reduction in adhesion to iC3b ($p=0.014$). A 42% decrease in phagocytosis by CD11b (77H) expressing cells was seen in transfected COS7 cells, which lack phagocytic receptors but are functionally capable of phagocytosis when receptors are supplied ($p=0.004$). When CR3 was pre-engaged using sRBCiC3b, there was a notable inhibition observed in the release of pro-inflammatory cytokines induced by Toll-like receptor 7/8 from WT monocytes. These findings also raise the possibility of a relationship between the Toll-like receptor and the rs1143679 (R77H) lupus associated variant of *ITGAM*. It is noteworthy to mention that the findings of (Rhodes, et al 2012) differ slightly from those of our study, indicating that the correlation between Toll

and *ITGAM* was not significant (p = 0.099). However, since this is not an interval conclusion, it is possible that the rs1143679 variant was not present in our study. This is a crucial point for additional academic research. The results of the study showed that SLE can be accurately diagnosed using the genes *ITGAM* and *TNFAIP3*. In contrast to *TNFAIP3*, which displayed a sensitivity of 93% and a specificity of 98%, *ITGAM* demonstrated a sensitivity of 98% and a specificity of 100%. *ITGAM* (AUC value of 1.00 indicates great value) and *TNFAIP3* (AUC value of 0.97 indicates excellent value) both showed good performance in differentiating SLE cases from non-SLE cases. These results for the gene *TNFAIP3* (Kim,*et al* 2020). were consistent with a study that observed a synergistic interaction between this gene *TNFAIP3*and SLE disease when compared with controls .As show in study (Leffers,et al 2022), which proved the existence of a significant association between many genes, including the *ITGAM* gene with SLE disease, and this agrees with current result about *ITGAM* gene and its role in the SLE disease.

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4.6 Correlation matrix between immune-related genes (*ITGAM* and *TNFAIP3*) and Cytokine Markers (IFN- α and TLR-7)

The results of this study also indicate a significant correlation between, IFN-α TLR7, and SLE. These findings are consistent with a study by (Lyn-Cook *et al*. 2014) that found that SLE patients had significantly higher levels of TLR7 level ($p < 0.01$) and INF-α level ($p < 0.0001$) when compared to controls. It was also agreed with the study (Paradowska *et al* 2021). The relationship between interferon (IFN- α) and SLE may be due to the fact that immune responses occur through IFN- α and the activation of interferon genes that are important in the pathogenesis of SLE . As for the IFN- α

relationship with TLR7 , the reason may be attributed to the fact that the receptor TLR7is considered one of the main stimulators of interferon type 1 in SLE according to a study (Tanaka , *et al* 2024).

Moreover, there is a strong link between current results , there was a significant correlation between ITGAM and TNFAIP3 that effects of SLE and the reason for this may be attributed to the interaction of genes and one effect on the other in its functions that drives it in the SLE disease. Other study also indicated that many genes are linked to each other and are susceptible to causing the SLE disease (Fagerholm, et al 2013).

4.7 Correlation between Cytokine (IFN-α and TLR-7) , Gene (*ITGAM* and *TNFAIP3*) Expression and disease activity stages of the Study Subjects .

The results in table (3.10) show no association between genes (*ITGAM* and *TNFAIP3*) , cytokine (IFN-α) and (TLR-7) receptor therefore found a negative relationship between this factors and disease activity .The interferon α, in the study of (Maharani, *et al*. 2020)showed that it is responsible in the early stages of the disease and that there is no correlation with disease activity.This may be due to the discrepancy of the use of immunosuppressive treatments that cause an imbalance in the regulation of immune responses While this was not consistent with other studies, such as this study, this may be due to the discrepancy . the same applies to the TLR-7 as the study indicated (Azab , et al 2022).

4.8 Possible idea of a novel SLE markers

It is difficult to find the perfect biomarker for SLE because it needs to meet certain requirements: (1) it must reflect the underlying pathophysiology or treatment target; (2) it must have validity, reliability, high predictive values, sensitivity, and specificity; (3) it must be able to monitor SLE activity or flare-ups; (4) it must be able to be reliably measured in tissues, cells, or fluids; (5) it must be stable, reproducible, easily detected, and testing should be readily available in most laboratories at a reasonable cost (Yu, et al 2021) While the AUC for TNF-α in this study was 0.97, the study by (Idborg H et al, 2018 found that the AUC for TNF-α was 0.86 (0.83–0.89). This suggests that TNF-α is an important and potentially effective approach for the diagnosis of SLE.The study's ability to highlight the significance of *ITGAM* is a huge plus. As a result, ROC curves showed that *ITGAM* had a 98% sensitivity and 100% specificity for diagnosing SLE ($p = 0.004 < 0.001$), making it a promising method for monitoring the course of SLE in Iraqi patients.

Chapter Five

Conclusions
and
Recommendations

5.1 Conclusion

From the findings of this study, the following conclusions can be made:

1. Socio-demographical factors such as Sex, and family history affect susceptibility to SLE. Females, married individuals as well as those with family history of SLE are more likely to develop the disease.
2. SLE patients can be characterized by elevations in titre values of ANA and anti-dsDNA autoantibodies as well as low levels of complement proteins C3 and C4.
3. The cytokines IFN-α and TLR-7 are high serum level in SLE patients and the expression levels of these cytokines may be useful in monitoring disease progression in SLE patients.
4. The upregulation of genes *ITGAM* and *TNFAIP3* is observable in SLE patients and may indicate susceptibility to SLE even in non-SLE individuals.
5. Evaluation of the, cytokines (IFN-α and TLR-7) as well as expression levels of *ITGAM* and *TNFAIP3* genes are useful markers for prognosis and management of SLE.

5.2 Recommendations

1. Multi-center, nationwide studies, involving larger sample size as well as follow up analysis of SLE patients should be conducted.
2. The disease prognosis prowess of the genetic markers identified in this study (i.e. *ITGAM* and *TNFAIP3*) should be evaluated in a larger study using high throughput molecular applications in order to elucidate the possible molecular mechanism and cell signaling pathways associating these markers with SLE.

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Appendix

Appendix A : SLE QUESTIONNAIRE I am a researcher , Fatima Abdul Hussein a master student at the university of Karbala /collage of medicine , I perform a progression research on patients with systemic lupus erythematosus (SLE) Do you agree to participate to complete my research with information & blood extraction ?

Yes ☐
No ☐

PERSONAL HEALTH HISTORY

Name : ……

Phone number : ……..

Age : …….

Address : ……

Status

single ☐
married ☐

Sex

female ☐
Male ☐

Other Autoimmune Dis ?

Yes , if yes what are ? ☐
No ☐

Family have history for SLE?

Yes ☐ 1st ☐ 2ND ☐
No ☐

Disease activity ?

Appendix

Mild ☐
Moderate ☐
sever ☐

LABRATORY INVESTIGATION ?

ANA ☐
Anti dsDNA ☐
C3 ☐
C4 ☐
GUE ☐
CBC ☐

blood sample

Yes ☐
No ☐

Patient classify according to sle severity scoring (SLEDAI) ?

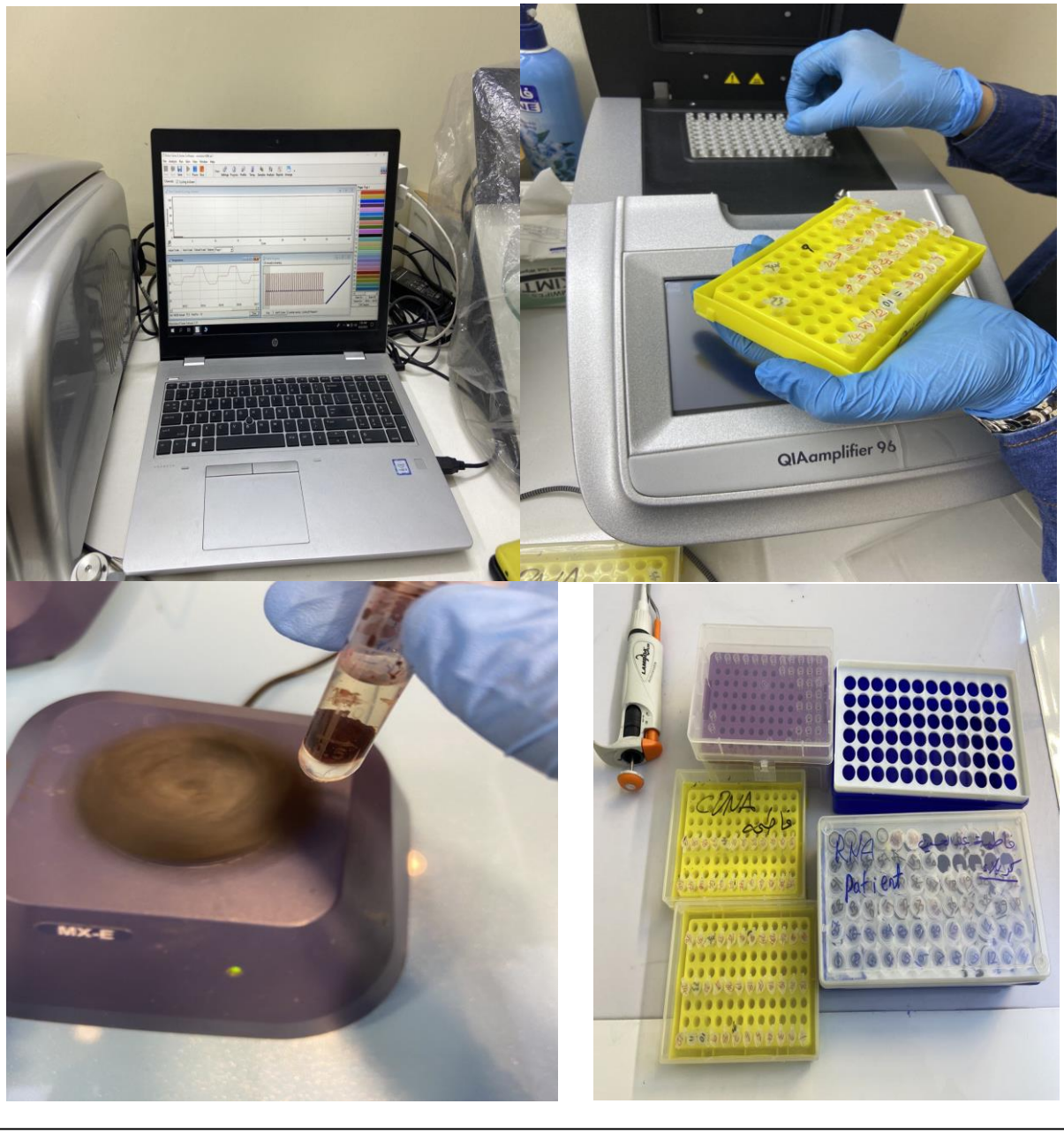
Yes ☐
No ☐

Appendix

Appendix B

Gene extraction Kits

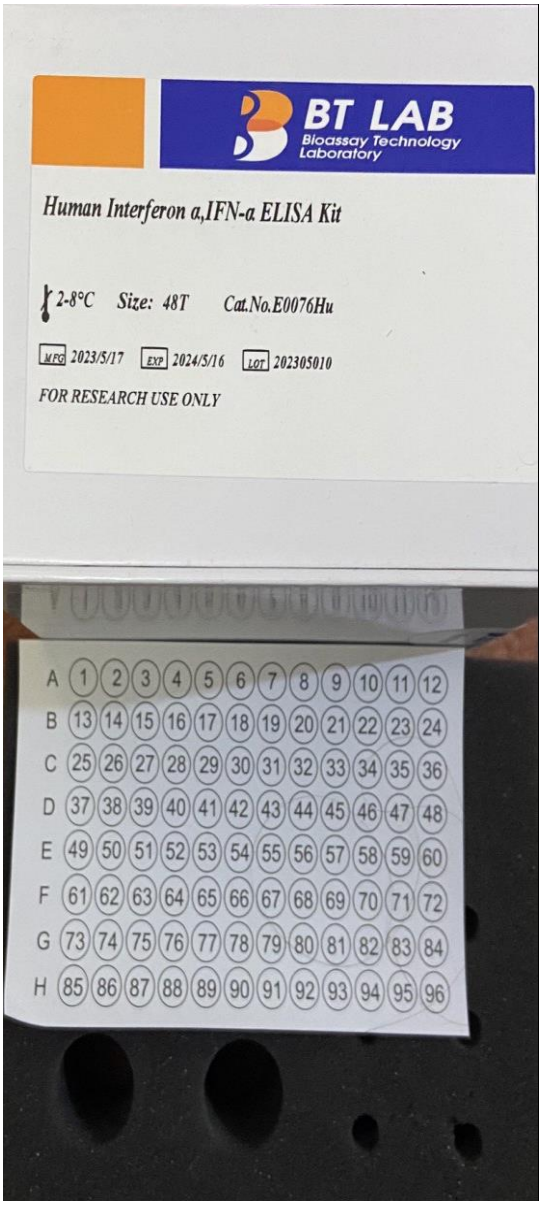
Appendix

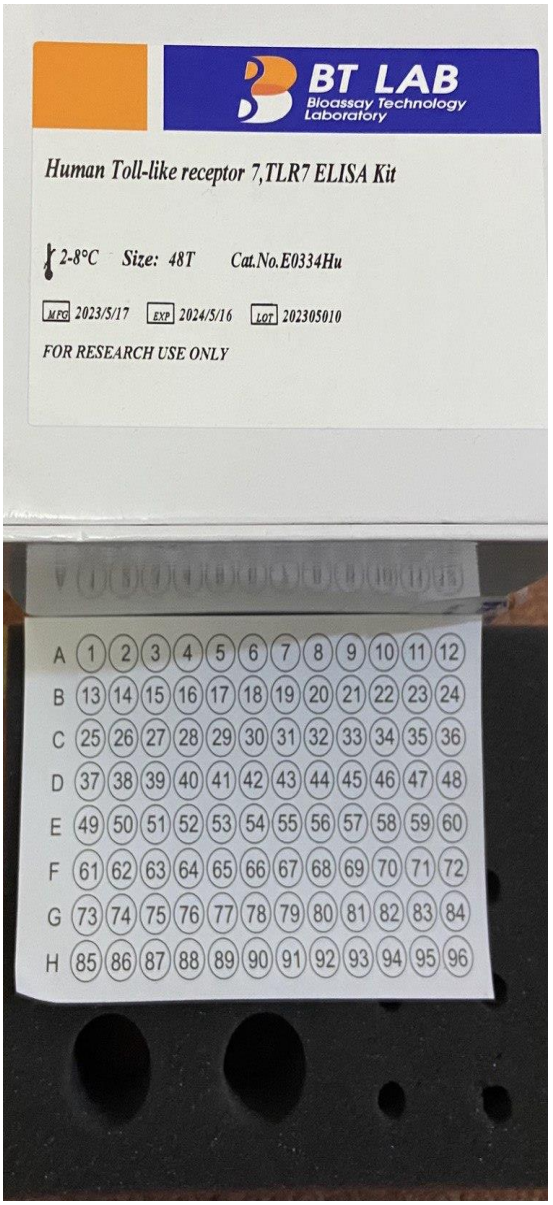


Gene expression devices

Appendix

Appendix C

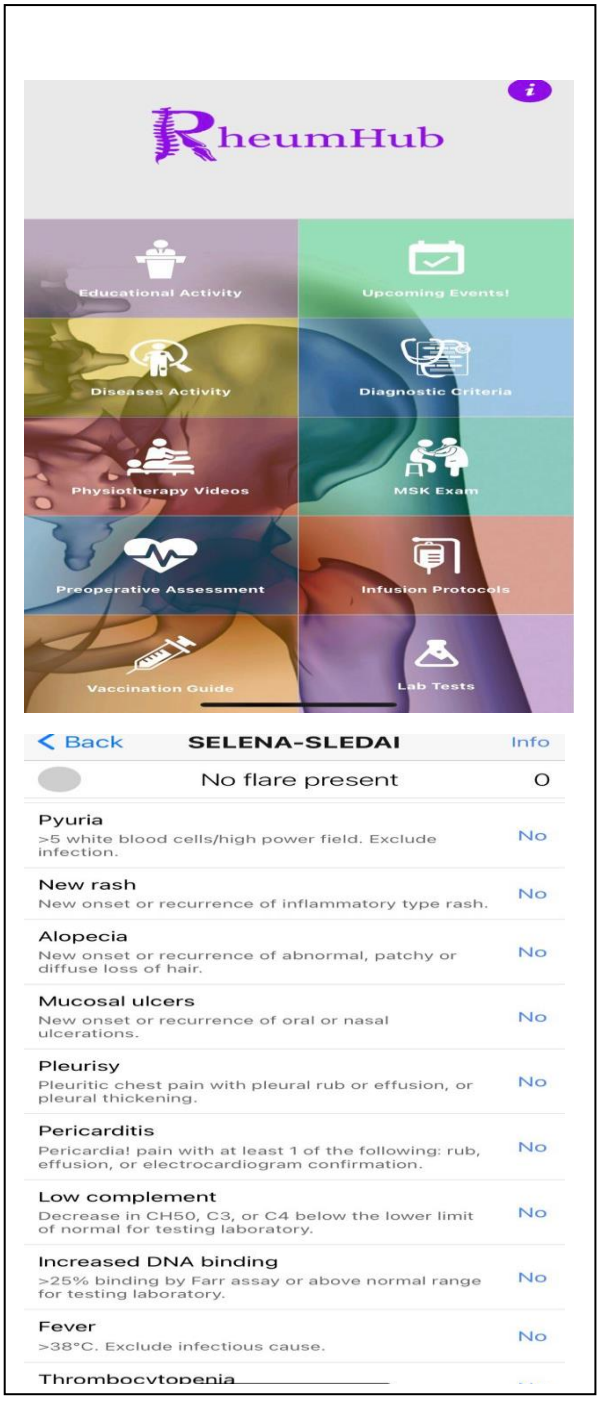


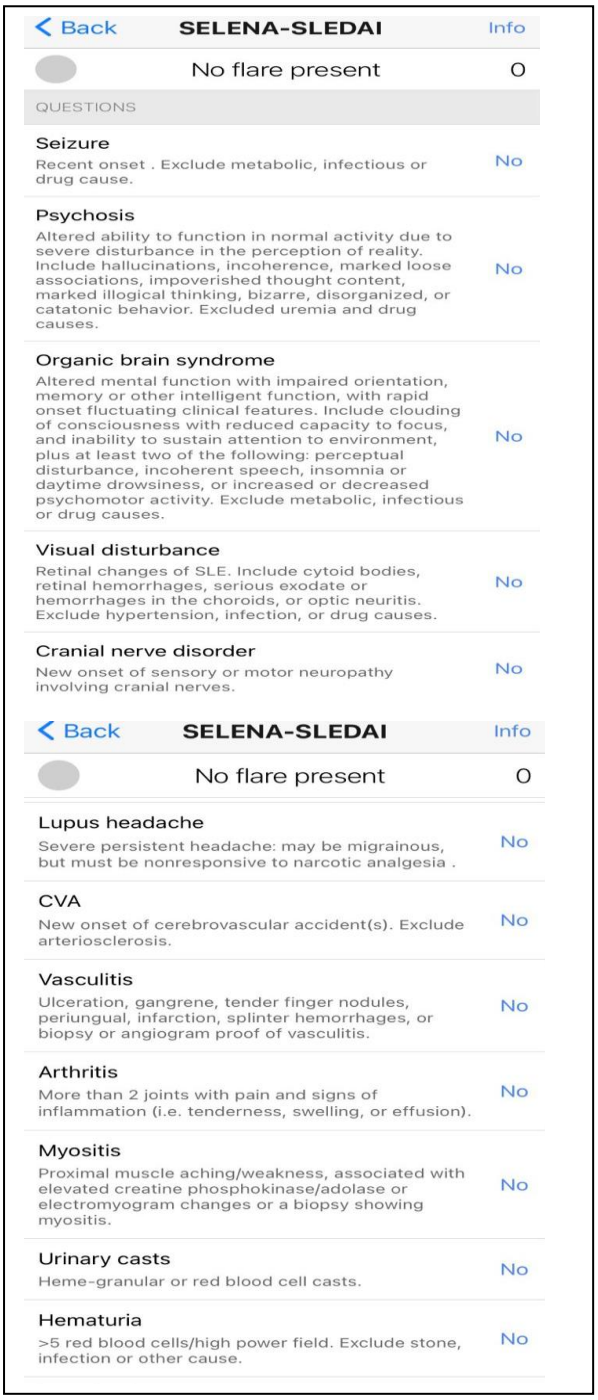


ELISA Kits

Appendix

Appendix D





The software application RheumHub for SLEDAI SCOR

الخلاصة

داء مرض الذئبة الحمراء الجهازية (SLE) هو مرض مناعي ذاتي مزمن يصـــيب العديد من أجهزة الجسم السمة المهمة لهذا المرض متمثلة في زيادة انتاج الاجسام المضـادة الذاتية المتولدة نتيجة أختراق نظام تحمل الجهاز المناعي للمســـتضـــد الذاتي . بعد ذلك يتم تلف أعضـاء الجسم من خلال ترسـب المعقدات المناعية في ألانسـجة مؤديا ذلك الى تنشـيط نظام المكمل , تراكم خلايا العدلة والوحيدة وتنشـيط الخلايا اللمفاوية .على الرغم من الصـعوبات المرتبطة بإجمالي الحالات المصـــابة بمرض الذئبة الحمامية المقدر بـــــ 134 حالة لكل 100,000 شـــخص في جميع أنحاء العالم . أظهرت الأبحاث أن الكشـف المبكر عن مرض (SLE) يقلل من مخاطر ظهور وتوهج الأعراض، وبالتالي يخفف من الأعباء المالية ويقلل من اسـتخدام خدمات الرعاية الصـحية المطلوبة لكل حالة. لهذه الأسـباب، عمل العلماء على إيجاد مؤشـرات وراثية وإفرازية محتملة لمرض الذئبة الحمراء على مر السـنين في محاولة لمســاعدة المرضـــى الذين لا يعانون من أعراض أو اعراض دون ســريرية على اكتشـــاف المرض في وقت مبكر. ولذلك فإن تحديد العلامات الحرجة والمهمة للتشـــخيص المبكر غير الجراحي لمرض الذئبة الحمراء لدى الأشـخاص الذين قد يكونون معرضـين للخطر أمر بالغ الأهمية.

ألبيانات لمســتويات المصـــل للأجســـام المضـــادة الذاتية (وanti-dsDNA) ، وبروتينات االمكمل (C3) و(C4) ، تم أخذها من تقارير المرضـــى لتقييم لنشـــاط المرض , الســمات الديمغرافية والســريرية لمرضـــى الذئبة الحمراء الجهازية تم تقيمها، كذلك اسـتخدمت نتائج سـايتوكين (IFN- α) و(TLR-7) ، فضـلاً عن مسـتويات التعبير للجينات (ITGAM , TNFAIP3) و عمل تقييم لها ومقارنات لمستوياتها بين مرضـى الذئبة الحمراء الجهازية والأشخاص غير المصابين بالذئبة الحمراء الجهازية (ألاصحاء).

أشارت النتائج التي تم الحصول عليها إلى أن العوامل الاجتماعية والديموغرافية مثل الجنس والتاريخ العائلي تؤثر على القابلية للإصـابة بمرض الذئبة الحمراء , حيث تم تقيم مسـتويات المصـــل للعوامل الالتهابية الأتية : حيث لوحظ التعبير بشـكل مفرط عن (IFN-α و TLR-7في مرضـى SLE مقارنة بالمجموعة الضابطة الاصحاء)حيث ان (IFN-α

، كان متوسـطه (344.79 ±137.98 U / Ml) لمرضـى SLE) مقابل (U / Ml 121.15 ± 234.88 للأصحاء SLE) مع قيمة (P = 0.01) وTLR-7 كان (786. 48 ± 244.68 U / Ml لمرضـى SLE مقابل 129.71 U / mL ± 426.66 لغير المرضـى ب SLE) مع قيمـة (P =0.001). كـان التعبير عن الجينـات TNFAIP3 و ITGAM أعلى في مرضـى الذئبة الحمراء مقارنة بالمجموعة الضـابطة بمقدار(15.48 و43.08) مرة على التوالي .

تشير الدراسة الحالية إلى أن تقييم الأجسام المضادة الذاتية (ANA) و (anti-dsDNA) والبروتينات التكميلية (C3 وC4), والسيتوكينات (IFNα- TLR7) بالإضـافة إلى مسـتويات التعبير عن جينات (ITGAM و TNFAIP3) مفيدة كعلامات إدارة العلاج ومراقبة مرض الذئبة الحمراء الجهازية.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب
فرع الاحياء المجهرية الطبية

تقييم دور الجينات المرتبطة بالمناعة (سلسلة Integrin alpha M (ITGAM))، وعامل نخر الورم (TNF) البروتين المستحث ألفا 3 (TNFAIP3)) وعلامات السيتوكين (Interferon-alpha (IFN α ، ومستقبل (Toll-like-7 (TLR7)) في أمراضية داء الذئبة الحمراء الجهازية لدى المرضى العراقيين

رسالة مقدمة الى مجلس كلية الطب جامعة كربلاء كجزء من متطلبات نيل
شهادة الماجستير
في الأحياء المجهرية

من قبل
فاطمة عبدالحسين كاظم
كلية العلوم _قسم علوم بايولوجي

أشراف

الاستاذ المساعد الدكتور
ستار جبار راهي
كلية الطب/ جامعة كربلاء

الأستاذ الدكتور
رياض ضيهود الزبيدي
كلية الطب/ جامعة كربلاء

2024 ميلادي

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