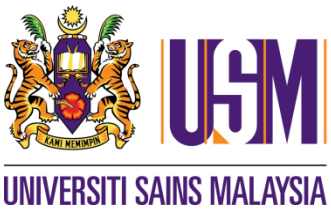
ELUCIDATION OF SERUM INTERLEUKIN-35 LEVELS AND INTERLEUKIN-35 RECEPTORS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS: ASSOCIATIONS WITH SEROLOGICAL PARAMETERS AND DISEASE ACTIVITY

DR NUR DIYANA BINTI MOHD SHUKRI

Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Pathology (Clinical Immunology)



UNIVERSITI SAINS MALAYSIA
2021

ELUCIDATION OF SERUM INTERLEUKIN-35 LEVELS AND INTERLEUKIN-35 RECEPTORS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS: ASSOCIATION WITH SEROLOGICAL PARAMETERS AND DISEASE ACTIVITY

BY

DR NUR DIYANA BINTI MOHD SHUKRI

Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Pathology (Clinical Immunology)

UNIVERSITI SAINS MALAYSIA
2021

SUPERVISOR:
ASSOC. PROF. DR CHE MARAINA CHE HUSSIN

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim

Alhamdulillah, all praises be to Allah the Almighty for giving me the opportunity and strength to finally complete this dissertation.

First and foremost, I would like to express my utmost gratitude to my supervisor, Associate Professor Dr Che Maraina binti Che Husin for giving me endless support throughout my research journey. Thank you for believing in me. My sincere thanks to my co-supervisor Associate Professor Dr Wong Kah Keng, for the guidance with the analysis and writing of my research. Every encouragement and word of wisdom had been so motivating. I would also like to express my gratitude to Encik Jamaruddin and Encik Azlan for assistance in completing the experimental work.

To my dearest friends in Immunology Laboratory, Azrini, Ellene, Suhana, Hana, Husna, Fatihah, Aina, and Ros, thank you for the knowledge, help, and support. To Farah, thank you for being my best partner and patient in teaching me laboratory and technical stuff. May our friendship lasts forever.

To my Mpath friends, Dr Siti Nadirah and Dr Wan Nur Amalina, thank you for always being my faithful listener every time I felt low and alone. I can't express how thankful I am to be blessed with the best group of girls for the past four years.

Finally, my special thanks to my beloved parents, Mohd Shukri bin Othman and Hilmiah binti Awang Noh, my husband Nik Mohd Haniff, and my dear children, Maisarah, Haris, and Zahra for understanding and unfailing support throughout this master journey. I would not have done it if it were not for them. Thank you for the endless prayers and encouragement whenever I feel impossible. Truly, no words would be enough to show how thankful I am for the greatest support. I hope I have made you proud.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF ABBREVIATIONS AND SYMBOLS	viii
ABSTRAK	xi
ABSTRACT	xiii

CHAPTER 1

1. INTRODUCTION

1.1. Systemic lupus erythematosus	1
1.1.1. Incidence and prevalence	1
1.1.2. Aetiology	2
1.1.3. Pathogenesis	4
1.1.4. Clinical symptoms and signs	6
1.1.5. Diagnosis	7
1.1.6. Disease monitoring	11
1.1.7. Management	13
1.2. Interleukin-35 (IL-35)	
1.2.1. IL-35 structure and expression	14

1.2.2. IL-35 receptor expression and signalling 14

1.2.3. The anti-inflammatory role of IL-35 15

1.2.4. Role of IL-35 in SLE 16

1.3. Justification of the study 16

1.4. References 17

CHAPTER 2

2. OBJECTIVES OF THE STUDY

2.1. General objective 24

2.2. Specific objectives 24

CHAPTER 3

3. MANUSCRIPT

3.1. Title page 25

3.2. Abstract 26

3.3. Introduction 28

3.4. Materials and methods 31

3.5. Results 37

3.6. Discussion 42

3.7. Conclusion 45

3.8. Acknowledgement 45

3.9. References 46

3.10. Tables and figures 54

3.11. Guidelines/Instructions to authors of selected journal format 58

CHAPTER 4

4. STUDY PROTOCOLS

4.1. Full study protocols 69

4.2. Patient information sheet and consent form 95

4.3. Data collection sheet 100

4.4. Ethical approval letter

101

CHAPTER 5

5. APPENDICES

5.1. Additional tables and figures 102

5.2. Raw data in GraphPad Prism 104

LIST OF TABLES

Manuscript

Table 1 : Demographic data of SLE patients and controls.

Table 2 : Disease characteristics of systemic lupus erythematosus (SLE) patients.

Table 3 : Comparison of mean $CD3^{+}CD4^{+}$ T helper (Th) cells expressing IL-35 receptor subunits levels and serum IL-35 between SLE patients and controls.

Table 4 : Correlation between serum IL-35 and IL-35 receptors (IL-12Rβ2 and gp130) levels with SLEDAI-2K scores in SLE patients.

Table 5 : Summary of IL-35 levels in SLE patients and healthy controls and its association with treatment from published studies.

LIST OF FIGURES

Figure 1 : The IL-12 family consist of heterodimeric cytokines where each member comprised of an α–subunit (p35, p19, p28) and a β–subunit (p40, Ebi3). Following binding of the cytokines to the cognate receptors, receptor-associated Janus kinases (Jak1, Jak2, Tyk2) are stimulated leading to activation of STAT family of transcription factors.

Manuscript

Figure 1 : Determination of interleukin-35 receptors; IL-12Rβ2 and gp130 expression levels by flow cytometry analysis.

Figure 2 : Comparison of mean $CD3^{+}CD4^{+}$ T helper (Th) cells expressing IL-35 receptor (IL- 12Rβ2 and gp130) levels and serum IL-35 between SLE patients and controls.

Figure 3 : Association of IL-35, IL-12Rβ2 and gp130 with serological parameters (i.e. ANA, anti-dsDNA, CRP) in SLE patients.

LIST OF ABBREVIATIONS AND SYMBOLS

aβ2-GPI	Anti-β2-glycoprotein-I antibodies
aCL	Anti-cardiolipin antibodies
ACR	American College of Rheumatology
ALBIA	Addressable laser bead immunoassay
APS	Antiphospholipid syndrome
ANA	Antinuclear antibodies
APC	Allophycocyanin
BILAG	British Isles Lupus Assessment Group
Breg	B regulatory cells
BLK	B lymphoid tyrosine kinase
CLIA	Chemiluminescence assay
CRP	C-reactive protein
CTLA4	Cytotoxic T lymphocyte-associated antigen 4
DC	Dendritic cells
dsDNA	Double-stranded DNA
EBI3	Epstein-Barr virus-induced gene protein 3
ECLAM	European Community Lupus Activity Measure

EDTA	Ethylenediaminetetraacetic acid
ENA	Extractable nuclear antigen
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
FEIA	Fluroenzyme immunoassay
HLA	Human Leucocyte Antigen
HRP	Horseradish peroxidase
HUSM	Hospital Universiti Sains Malaysia
IFN	Interferon
IIFA	Indirect immunofluorescence assay
IL	Interleukin
IRF	Interferon regulatory factor
JAK	Janus kinase
ml	Millilitres
NLR	Nucleotide-binding and oligomerization domain receptors
NET	Neutrophils extracellular traps
PEG	Polyethylene glycol
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
PE	Phycoerythrin

PRR	Pattern recognition receptor
RIA	Radioimmunoassay
RLS	Retinoid acid-inducible gene-I-like receptors
SELENA	Safety of Estrogens in Lupus National Assessment
SLAM	Systemic Lupus Activity Measures
SLE	Systemic lupus erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SLEDAI-2K	Systemic lupus erythematosus Disease Activity Index 2000
SLICC	Systemic Lupus International Collaborating Clinics
snRNP	Small nuclear ribonucleoproteins
STAT	Signal transducer and activator of transcription
Th	T helper
TCR	T-cell receptor
TLR	Toll-like receptor
Tyk	Tyrosine kinase
Treg	T regulatory cells
UV	Ultraviolet
µl	microlitre
α	alpha
β	beta

γ gamma

ABSTRAK

Pengenalan

Interleukin-35 (IL-35) dan reseptor IL-35 dikaitkan dengan perkembangan lupus eritematosus sistemik (SLE). Kebelakangan ini, banyak penyelidikan telah dilakukan untuk mengkaji kaitannya dengan penyakit autoimun, terutamanya SLE. Kajian ini bertujuan untuk menentukan tahap serum IL-35 dan reseptor IL-35 (IL-12Rβ2 dan gp130) pada pesakit lupus eritematosus sistemik dan kaitannya dengan parameter serologi dan aktiviti penyakit.

Metodologi

Lima puluh pesakit orang SLE didaftarkan dalam kajian ini, dan lima puluh orang sukarelawan direkrut sebagai kawalan yang sihat. Peratusan sel T pembantu $CD3^+$ $CD4^+$ dan reseptor IL-35 subunit IL-12Rβ2 dan gp130 dalam sel mononukleus darah periferi dianalisis menggunakan flow sitometri. Tahap serum IL-35 diukur dengan ujian cerakinan imunoserapan untaian enzim (ELISA). Aktiviti penyakit SLE dinilai menggunakan skor indeks aktiviti penyakit lupus erythematosus sistemik-2K (SLEDAI-2K).

Keputusan

Hasil kajian menunjukkan bahawa tahap serum IL-35 lebih tinggi pada pesakit SLE (31.53 ± 14.15 pg/ml) berbanding mereka yang berada dalam kawalan sihat (25.36 ± 7.073 pg/ml, p=0.038), manakala tidak ada perbezaan yang ketara antara tahap reseptor IL-35; IL-12Rβ2 dan gp130 pada pesakit SLE berbanding kawalan sihat. Di samping itu,

reseptor IL-35 subunit gp130 berhubung kait secara positif dengan indeks aktiviti penyakit SLE (SLEDAI) (r=0.425, *p*=0.002). Keputusan menunjukkan tiada hubung kait antara IL-35 dan reseptor IL-35 subunit IL-12Rβ2 dan gp130 dengan parameter serologi.

Kesimpulan

Kajian kami menunjukkan peningkatan yang ketara dalam tahap serum IL-35 berbanding pesakit SLE dan korelasi positif antara reseptor IL-35 subunit gp130 dan skor SLEDAI. Tahap reseptor IL-35, IL-12Rβ2 dan gp130 yang lebih tinggi mungkin memainkan peranan dalam manifestasi klinikal SLE, yang membawa kepada aktiviti penyakit yang lebih teruk. Oleh itu, mereka boleh berfungsi sebagai penanda penting untuk aktiviti dan keparahan penyakit SLE di samping memantau perkembangan penyakit.

Kata kunci: lupus eritematosus sistemik, interleukin-35, IL-12Rβ2, gp130, SLEDAI-2K.

ABSTRACT

Background

Interleukin-35 (IL-35) and IL-35 receptors are associated with the development of systemic lupus erythematosus (SLE). Many studies have been done recently to look into its association with autoimmune diseases, particularly SLE. This study aims to determine the serum IL-35 levels and the surface levels of IL-35 receptors (IL-12Rβ2 and gp130) on T helper cells in SLE patients versus healthy controls and their associations with serological parameters and disease activity.

Method

Fifty SLE patients were enrolled in the study and fifty volunteers were recruited as healthy controls. The percentage of $CD3^{+}CD4^{+}$ T helper cells and IL-35 receptors subunits IL-12Rβ2 and gp130 in peripheral blood mononuclear cells were analysed using flow cytometry. Serum IL-35 levels were measured by enzyme-linked immunosorbent assay (ELISA). SLE disease activity was evaluated using the Systemic Lupus Erythematosus Disease Activity Index-2K (SLEDAI-2K) score.

Result

The results showed that the mean serum IL-35 levels were significantly higher in SLE patients (31.53 ± 14.15 pg/ml) than those in the healthy controls (25.36 ± 7.073 pg/ml, p=0.038) whereas there was no significant difference among the mean IL-35 receptors (IL-12Rβ2 and gp130) levels in SLE patients compared to healthy control. In addition, IL-35 receptor subunit gp130 was positively correlated with the SLEDAI-2K scores (r=0.425, p=0.002). No significant association was observed between IL-35 and IL-35

receptor subunit IL-12Rβ2 and gp130 levels with the serological parameters in SLE patients.

Conclusion

Our findings demonstrated a significant increase in serum IL-35 levels in SLE patients and a positive correlation between IL-35 receptor subunit gp130 with SLEDAI-2K scores. Higher levels of gp130 on $CD3^{+}CD4^{+}$ T helper cells might play a role in the clinical manifestation of SLE, leading to more severe disease activity. Hence, they might serve as an essential biomarker and estimation for SLE disease activity and severity as well as for monitoring disease progression.

Keywords: Systemic lupus erythematosus, interleukin-35, IL-12Rβ2, gp130, SLEDAI-2K.

CHAPTER 1

INTRODUCTION

1.1 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disorder with a wide range of clinical manifestations. It is characterised by dysregulated production of autoantibodies and the formation of immune complexes that resulted in tissue and organ damage. The pathogenesis of SLE is complex, which involves interaction of impaired apoptotic clearance, upregulation of innate and adaptive immune system, complement activation, immune complexes and tissue inflammation that leads to a self-sustained autoimmune process [1].

1.1.1 Incidence and prevalence

SLE is known as a global disease. Studies have reported an increased incidence of SLE, partially attributable to enhanced diagnostic capabilities, advances in technical immunologic testing and greater awareness of the disease, which leads to patient identification in the early stages of the disease [2].

SLE has a predilection for women of reproductive age group, with female to male ratio up to 13:1, while only 2:1 in children and elderly [1]. The reported values of incidence and prevalence vary considerably worldwide. The overall incidence ranging from 0.3 per 100,000 per year in Ukraine to 31.5 per 100,000 per year among Afro-Caribbean people living in the UK, while the overall prevalence ranging from 3.2 per 100,000 in India to 517.5 per 100,000 among Afro-Caribbean people living in the UK

[2]. The worldwide incidence and prevalence differences are influenced by ethnic and geographical areas, study design and environmental exposures.

The prevalence of SLE in Asians is within 30 to 50 per 100,000 population. In Malaysia, a prevalence of 43 per 100,000 population has been reported. The highest prevalence of SLE was Chinese (57/100,000), followed by Malays (33/100,000) and Indians (14/100,000) [3].

1.1.2 Aetiology

The aetiology of SLE is complex and multifactorial. The clinical manifestation requires the interaction of genetic, environmental and hormonal factors.

1.1.2 (a) **Genetic factors**

The genetic susceptibility is supported by the 11-50% monozygotic twin concordance and increased risk among family members. Certain genes have been associated with a predisposition to develop lupus, for example, human leucocyte antigen (HLA) genes, interferon regulating factor 5 (IRFS) and signal transducer and activator of transcription 4 (STAT4) [1].

1.1.2 (b) **Environmental factors**

Many environmental triggers have been implicated in SLE development, namely ultraviolet (UV) light, drugs, smoking, infections with Epstein-Barr virus, silica and mercury [1]. Several of these factors are potent activators of the type I interferon (IFN) system by various mechanisms.

Ultraviolet light is the most recognized physical factor that is known to induce SLE. The crucial mechanism involved is the induction of type I and III IFN as well

as chemokines. UV light (290-320 nm) exposure causes the redistribution of nuclear antigens on the cell surface and stimulates apoptosis and secondary necrosis of keratinocytes. This, in turn, leads to the recognition of normally hidden autoantigens, such as nucleoprotein by autoantibodies that later form immune complexes that trigger type I IFN production by the plasmacytoid dendritic cells (pDC) in the skin [4]. Apart from that, UV light can trigger the release of reactive oxygen species that cause DNA strand breaks and pyrimidine dimer formation in DNA, hence promoting the formation of the immune complex by increasing the availability of nucleic acids [4].

Chemical agents or drugs that have been implicated with the development of SLE are aromatic amines (procainamide, practolol, and sulfapyridine) or aromatic hydrazines (hydralazine, isoniazid), echinacea and antibiotics like trimethoprim/ sulfamethoxazole [1, 5]. These conditions are collectively called drug-induced lupus and tend to subside after the withdrawal of the offending drugs. The pathogenesis behind this is likely attributed to the effects of the medication on the T cell function.

Additionally, psychological stress has been associated with a 50% increased risk of developing lupus [1].

1.1.2 (c) **Hormonal factors**

Hormonal factors can influence the patient's susceptibility to SLE. Most autoimmune disease, particularly SLE, is predominantly seen in women of reproductive age group and exacerbated during puberty, pregnancy, and postpartum periods [6]. This suggests the role of sex hormones in the pathogenesis of SLE. Studies have shown that exposure to oestrogen is associated with a higher risk of SLE. Simultaneously, progesterone and testosterone appear to be protective due to their counteracting oestrogen effects [7]. Different levels of oestrogen stimulate different types of helper

T (Th) cells. Low doses increase Th1 cell responses, while high oestrogen levels enhance Th2 responses. In a genetically predisposed individual, oestrogen stimulates the production of type 1 IFN, increases the survival of autoreactive B cells and production of pathogenic immunoglobulins and enhances the differentiation of $CD4^+$ Th cells [8]. Furthermore, prolactin hormone has been shown to contribute to the development of SLE. Hyperprolactinemia causes impaired B cell receptor-mediated clonal deletion and reduces B cell apoptosis, which enhances the survival of self-reactive B cells and breaks down B cell self-tolerance, thus resulting in the development of autoimmunity [7, 9].

1.1.3 Pathogenesis

SLE involves a multifactorial process and requires the interaction of genetic predisposition, environmental triggers and hormonal factors. A variety of immunological defects contribute to SLE. The primary pathogenesis of SLE involved the breakdown of self-tolerance and sustained autoantibody production [10]. The dysregulation of the immune system, which includes the innate and adaptive immune system towards self-antigen, stimulates the production of autoantibodies and production of immune complexes deposited in various tissues, leading to the activation of complement, accumulation of neutrophils, monocytes and self-reactive lymphocytes [11].

The major cell types involved in SLE are dendritic cells (DC), neutrophils, T lymphocytes and B lymphocytes. Dendritic cells promote the loss of T cell and B cell self-tolerance in SLE by inappropriate antigen presentation. The increased number of pDC subset in SLE patients, which is the primary cell type responsible for type I IFN, indirectly caused an increase in the secretion of IFN, particularly IFN-α, leading to

activation of lymphocytes, DC, natural killer cells and breakdown of the autoimmune tolerance [10, 11]. Besides that, DC also interacts with the pattern recognition receptors (PRRs) such as the toll-like receptors (TLRs), the nucleotide-binding and oligomerization domain receptors (NLRs) and the retinoid acid-inducible gene-I-like receptors (RLRs). The TLR9 acting via the interferon regulatory factor (IRF) can stimulate pDCs to produce a large amount of type I IFN, whereas TLR7 ligand activates the pDCs leading to increased expression of IL-1β and IL-23 and promote differentiation of Th17 [11]. TLR can also stimulate B cells, resulting in autoantibodies that later form immune complexes containing ribonucleic acid and nucleosome.

In addition, inappropriate activation of neutrophils, which is an important component of the innate immune system, causes the release of many cytokines, chemokines, protease and reactive oxygen species leading to disorders of immune regulation and tissue damage in SLE [12]. Studies also revealed that neutrophils are involved in autoimmune disease with a new structure, neutrophils extracellular traps (NETs), which consists of fibrous networks assembled from nuclear and granular components. NETs formation and imbalance in degradation can cause stimulation of type I IFN synthesis and endothelial damage as well as the involvement of a large amount of complements resulting in activation of the classical pathway of complement [11].

T cells play an essential role in the pathogenesis of SLE due to their association with MHC proteins. Apart from causing abnormal cytokine secretion and cell signal transduction, it can also lead to inappropriate recruitment and activation of B cells and DCs [13]. T-cell signalling pathways that are affected include T-cell receptor (TCR)-CD3 signalling pathway, CD44-Rock-ERM signalling pathway and PI3K-Akt-mTOR signalling pathway [11]. Patients with SLE also display an imbalance in the T helper cell subset population. The imbalance of Th1 and Th2 cells are considered to be critical in the

development of SLE as the different subtypes secrete different cytokines. Th1 cells secrete TNF-α, IL-2 and IFN-γ, which are involved in the activation of macrophages and cytotoxic T cells. In contrast, Th2 cells secrete IL-4, IL-6 and IL-10, which stimulate the activation of B lymphocytes and induce the production of IgG1 [11]. In active SLE, there is a decreased function of Th1 but hyperfunction of Th2, resulting in excessive activation of B cells, production of autoantibodies and tissue injury [11]. Besides, there is also an imbalance of Th17 and regulatory T cells (Treg). Th17 cells produce IL-17A, IL-17F and IL-22, which mediate inflammatory responses and the occurrence of autoimmune disease. IL-17 and B-cell stimulating factor (BLys) stimulates humoral immunity to produce autoantibodies by upregulating the differentiation and survival of B cells. The T reg cells are involved in self-tolerance and can modulate the effector T cells function, maintains immunological homeostasis and prevents autoimmunity [11]. Hence, impaired Treg function leads to the development of the autoimmune disease. Furthermore, abnormal central and peripheral tolerance of B cells produces many self-reactive B cells resulting in the production of autoantibodies and the manifestation of SLE [11].

1.1.4 Clinical symptoms and signs

SLE presents no single characteristic clinical pattern. The pattern of clinical manifestation is heterogeneous among SLE patients and can involve constitutional symptoms with a mixture of skin, musculoskeletal, renal disease, haematological and central nervous system. The symptoms vary, depending on the parts of the body involved and can be mild, moderate or severe. Constitutional symptoms include fever, weight loss, malaise and lethargy.

Skin involvement is common in SLE, with almost 90% of patients develop cutaneous lupus. This includes lupus-specific manifestations such as acute cutaneous lupus, subacute cutaneous lupus, chronic cutaneous lupus and non-lupus-specific manifestation, including alopecia vasculitis, livedo reticularis and Raynaud's phenomenon. The important feature in cutaneous lupus is photosensitive distribution following exposure to ultraviolet light, which is raised, delayed and lasting more than three weeks [1].

Musculoskeletal involvement, including arthralgia and true synovitis is common and occurs in almost 90% of patients, presenting as symmetrical polyarthritis involving the metacarpophalangeal, proximal interphalangeal and knee joints [1]. Additionally, renal involvement is found in 50% of patients with typical manifestations of lupus nephritis, including proteinuria, increased urine protein-to-creatinine ratio, decreased complement levels, the elevation of anti-dsDNA antibodies and anti-C1q antibodies. Specific neuropsychiatric manifestations associated with SLE are seizures, psychosis, mononeuritis multiplex, peripheral and cranial neuropathy and acute confusional state. However, other differential diagnoses such as infection, metabolic causes and malignancy need to be excluded prior to diagnosis [1].

1.1.5 Diagnosis

1.1.5 (a) ACR criteria

The diagnosis of SLE is made based on the diagnostic criteria of the 1982 revised American College of Rheumatology (ACR) SLE classification criteria (revised in 1997). It consists of 9 clinical manifestations and two immunological laboratory tests. The clinical manifestations are malar rash, discoid lupus, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurological disorder and haematological

disorder. Meanwhile, the serological laboratory test includes positive ANA and any of the following; anti-dsDNA, anti-Sm, antiphospholipid antibodies or a false positive serological test for syphilis, which is known to be positive for more than six months [14]. Those that satisfy a minimum of 4 out of 11 criteria, either simultaneously or at any time are highly suggestive of SLE.

The latest classification criteria for SLE is the 2019 European League against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for SLE published in 2019. This new classification criteria have excellent sensitivity and specificity of 96.1% and 93.4%, respectively, compared to 82.8% sensitivity and 93.4% specificity of the ACR 1997 and 96.7% sensitivity and 83.7% specificity of the Systemic Lupus International Collaborating Clinics (SLICC) 2012 criteria [15].

1.1.5 (b) Laboratory testing (included in the ACR criteria for SLE classification)

Antinuclear antibody

Antinuclear antibody (ANA) is the hallmark of SLE and is considered an excellent screening test for most autoimmune diseases, including SLE. It shows extreme sensitivity for SLE with >98% but has a low specificity [14]. About 20-23% of normal healthy individuals test positive for ANA, especially older people [16]. The detection and semi-quantification of ANA by the indirect immune-fluorescent assay (IIFA) method using human epithelial (Hep2) cells as the substrate has been considered the gold standard method and widely used [17]. IIFA involves initial screening, serial serum dilution and determination of the distinct ANA staining pattern on Hep-2 slides. The results are reported in terms of titre and the pattern of cellular staining present. A positive ANA result indicates the detection of antibodies

to a wide variety of nuclear molecules and also antibodies to antigens located in the cytoplasm or expressed by mitotic cells [17]. On the other hand, the staining pattern can provide some insight into the molecule targeted by ANA, such as anti-centromere antibodies. Subsequent immunological tests need to perform to determine the specific autoantibodies, for example, anti-dsDNA and anti-extractable nuclear antigen (ENA). Other methods used in the detection of ANA include ELISA, fluroenzyme immunoassay (FEIA), chemiluminescence assay (CLIA) and laser addressable bead multiplex assays (ALBIA), which utilize purified or cloned antigens.

Anti-dsDNA

Anti-double stranded DNA (Anti-dsDNA) is one of the immunological criteria in the 1997 ACR criteria for SLE diagnosis. It is a characteristic of SLE and rarely occurs in other autoimmune disorders. A study by Durcan *et al*. demonstrates a sensitivity of 90.5% of anti-dsDNA for disease activity by standard ELISA with a specificity of 35.1% [18]. Anti-dsDNA titre is considered one of the standard serological tests for disease activity evaluation [14]. The titre fluctuates over time and can disappear with treatment; hence it can be used as a biomarker for disease activity [19, 20]. The changes in the autoantibody level or titre, rather than the absolute autoantibody level, correlate to changes in the disease activity. There are different methods used for the detection of anti-ds DNA. Some of the early techniques include double immunodiffusion (Ouchterlony), radioactive assays such as Farr radioimmunoassay (RIA), the Polyethylene glycol (PEG) precipitation assay and the filter binding assay. Farr assay, which is first described in 1969, still considered as the gold standard due to its high specificity for SLE. The specificity for SLE in Farr assay is due to the use of high salt (ammonium sulphate) concentrations during the precipitation step, which

detects high avidity antibodies [21]. The indirect immunofluorescence (IIF) test using the hemoflagellate *Crithidia luciliae* as a substrate (*Crithidia Luciliae* Immunofluorescence Test-CLIFT) developed in 1975 is still widely used for the detection of anti-dsDNA because it has high specificity and positive predictive value for SLE. The *Crithidia* cells contains the kinetoplast; a modified mitochondrion that contains highly compacted native dsDNA. However, the IIF gives a semi-quantitative result, making it less suitable for monitoring the disease activity and predicting flares of SLE. Moreover, the diagnostic sensitivity of the CLIFT assay for SLE is low, usually 20–35% depending on assay conditions, serum dilution and the kit test used [21].

Anti-Smith (anti-Sm)

Anti-Smith antibodies are highly specific for SLE and represent one of the immunological diagnostic criteria for the disease. However, the sensitivity is low and shows a marked variation in different ethnic populations; about 20% in Caucasian patients and 30-40% in African, African-American and Asian patients [22]. Anti-Sm antibodies are directed against Sm antigens that are a set of seven proteins (B, D1, D2, D3, E, F, G), which form a ring for small nuclear ribonucleoproteins (snRNP). The Sm core proteins are assembled in the cytoplasm where they attach to the snRNP before being transported to the nucleus [23]. Hence, anti-Sm antibodies coexist with anti-RNP in many patients with SLE.

A recent study also suggests that the anti-Sm antibody level is associated with disease activity in patients with new-onset SLE and that monitoring of anti-Sm antibody levels could help to assess the disease activity [24]. In addition, studies also reported the possibility of anti-Sm antibodies as neural toxicity in the pathogenesis of neuropsychiatric manifestation of SLE [25].

Antiphospholipid antibodies, lupus anticoagulant and false-positive syphilis serological test

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by recurrent arterial or venous thrombosis, pregnancy-related morbidity and increased levels of antiphospholipid antibodies, which are lupus anticoagulant (LA), anti-cardiolipin antibodies (aCL) and anti-β2 -glycoprotein-I antibodies (aβ2-GPI).

Antiphospholipid antibodies are found in 30-40% of SLE patients [23, 26]. They are not specific for SLE and can be present in other autoimmune diseases such as rheumatoid arthritis and Sjögren's syndrome, infections, drug-induced disorders and healthy people. Approximately half of SLE patients with antiphospholipid antibodies will develop antiphospholipid syndrome. The laboratory test to determine antiphospholipid antibodies presence includes the detection of anti-cardiolipin by ELISA, β2GP1 antibodies by ELISA and functional lupus anticoagulant assay that demonstrates the ability of antiphospholipid antibodies to prolong phospholipid-depending clotting reactions [23].

Apart from that, the false-positive result of syphilis screening test is a characteristic finding in patients with SLE. This is because the non-specific serological test for syphilis, such as VDRL and RPR test, uses normal mammalian tissues such as beef heart extract as subtract. Therefore, the non-specific antibodies produced following syphilis infection can be directed against the cardiolipin in the substrate mixture, giving rise to false-positive results.

1.1.6 Disease monitoring

The assessment or monitoring of disease activity is essential in managing SLE patients as it forms the basis of most treatment decisions. Since the 1950s, there have been more

than 60 attempts at developing disease activity indices in SLE [27]. Multiple disease monitoring indices have been developed; however, few are reliable or consistent. Examples of validated activity indices commonly used include BILAG (British Isles Lupus Assessment Group), SLAM (Systemic Lupus Activity Measures), ECLAM (European Community Lupus Activity Measure) and SLEDAI (Systemic Lupus Erythematosus Disease Activity Index). Among the various methods, the most widely used worldwide in clinical practice SLE disease activity measure is the SLE Disease Activity Index (SLEDAI).

SLEDAI is a global index that was developed in 1985 to determine disease activity in patients with lupus. It was derived by agreement among experts in the field of lupus research and modelled based on experienced clinician global judgment. It consists of 24 weighted clinical and laboratory variables of nine organ systems. Each descriptors scores range from 1 to 8, with a total possible score for all 24 descriptors is 105. Other versions of SLEDAI are SELENA-SLEDAI, SLEDAI-2000 (SLEDAI-2K) and SLEDAI-2K (30-day).

SELENA-SLEDAI is a modified version of SLEDAI, which is specifically used in the Safety of Estrogens in Lupus National Assessment (SELENA) study. In this version, the scoring was modified to report for persistent active disease in some descriptors, for example, rash, mucosal ulcers and alopecia, which were not scored before. In the year 2002, the SLEDAI-2000 (SLEDAI-2K) was introduced as a measure of global disease activity and also a strong predictor of mortality in SLE [28]. SLEDAI-2K, which was a validated and revised version of the original SLEDAI was introduced to allow the documentation of persistent disease activity in the descriptors and verified to be sensitive in describing changes in disease activity from one visit to another [28].

1.1.7 Management

The goals of treatment in SLE are to maintain the lowest degree of activity or in remission, avoiding triggers of flares, prevent end-organ damage from active lupus and to reduce comorbidities or complications due to lupus and its treatment. Various drugs are used in the treatment of SLE, depending on the grading or severity of the disease, from mild, moderate to severe. They can be broadly divided into immunomodulators (hydroxychloroquine), corticosteroids, immunosuppressive drugs (cyclophosphamide, azathioprine, methotrexate, mycophenolate, and calcineurin inhibitors) and biological agents. The use of hydroxychloroquine is recommended for all patients as it has been shown to increase the survival in lupus, reduce lupus flare and prevent organ damage [1]. The mechanism of action involves many pathways; for example, the drug increases the lysosomal pH in the antigen-presenting cells, leading to disruption in antigen presentation, inhibition in various cytokine secretions and decreased toll-like receptor (TLR) signalling [29]. Corticosteroid use and route of administration are tailored according to the severity of the disease where in case of life or organ threatening manifestation, pulses of intravenous methylprednisolone are given to rapidly control the autoimmune response. However, long-term use of corticosteroid therapy can lead to irreversible organ damage, hence it must be used with caution and the daily dose must be minimised with the aim to off.

The new advances in SLE include the use of targeted therapy towards B cells that specifically block pathways involved in SLE, such as rituximab, belimumab, anifrolumab, ustekinumab, baricitinib and atacicept.

1.2 Interleukin-35

1.2.1 IL-35 structure and expression

IL-35 is a novel anti-inflammatory cytokine belonging to the IL-12 family. It is unique in structure as it is composed of two subunits; IL-12A (p35) and Epstein-Barr virus-induced 3 (EBI3). IL-35 is similar to other IL-12 families of cytokines, which function as heteromers; where each member comprises of heterodimeric subunits (an α-subunit and a β-subunit). The α-subunit has 4-α-helical bundles, a typical cytokine structure, and the β-subunit is homologous to the soluble cytokine receptor [30]. IL-35 is produced primarily by resting and activated T reg cells by converting naïve T cells into IL-35-dependent-induced T regs (iTr35) cells, which are strongly suppressive. Studies have reported that IL-35 can directly suppress in vitro effector T cell proliferation [31].

1.2.2 IL-35 receptor expression and signalling

The IL-35 receptor is composed of dimers, which are IL-12Rβ2 homodimers (IL-12Rβ2/IL-12Rβ2), gp130 homodimers (gp130/gp130) or IL-12Rβ2/gp130 heterodimers [30]. IL-35 signalling is mediated either through the heterodimer of receptor chains IL-12Rβ2/gp130 or the homodimer of each chain, where binding of the cytokine on the receptor induces signalling by the JAK-STAT pathway to introduce a specific intracellular response. IL-12Rβ2 or gp130 homodimers can only activate STAT4 or STAT1 respectively. Only the IL-12Rβ2/gp130 heterodimer can activate STAT1 and STAT4 activated signalling pathways to mediate the T reg cell function [30].

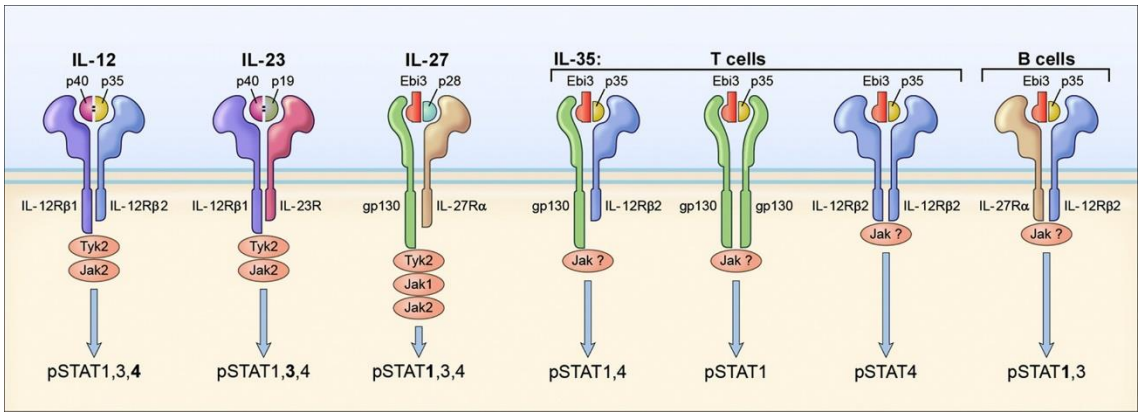


Figure 1: The IL-12 family consist of heterodimeric cytokines, where each member comprised of an α-subunit (p35, p19, p28) and a β-subunit (p40, EBI3). Following binding of the cytokines to the cognate receptors, receptor-associated Janus kinases (Jak1, Jak2, Tyk2) are stimulated leading to activation of STAT family of transcription factors. Picture adapted from Egwuagu CE, et al. Interleukin 35: Critical regulator of immunity and lymphocyte-mediated diseases. Cytokine Growth Factor Rev (2015). 26(5); 587-593.

1.2.3 The anti-inflammatory role of IL-35

IL-35 is the newest member of the IL-12 family, which comprise of IL-12, IL-23, IL-27, and IL-35. In contrast to the other family members that are pro-inflammatory, IL-35 is an anti-inflammatory or immunosuppressive cytokine and strongly inhibits immune function. It plays a critical role as a regulator of immunity in autoimmune and infectious diseases [32]. Studies have shown that the predominant mechanism of suppression associated with IL-35 is its ability to suppress T-cell proliferation, effector functions and suppress Th17 cells differentiation, hence those $CD4^+$ T reg lacking of IL-35 production will have a significant reduced ability to suppress T cell proliferation [33]. Besides that, studies also revealed that reduced IL-35 levels had been associated with an increase in incidence, development and exacerbation of inflammatory diseases while the induction of IL-35 expression has been shown to alleviate various disease symptoms [33]. These findings support the anti-inflammatory role of IL-35.

1.2.4 Role of IL-35 in SLE

IL-35 is an important anti-inflammatory cytokine and may play a significant role in autoimmune diseases such as SLE. It can inhibit inflammation in many autoimmunity models, suppress the T effector cell activity, inhibit Th17 differentiation and reduce the progression of inflammatory disease and autoimmune disease. Studies have shown that IL-35 production was significantly reduced in patients with active SLE, indicating that the IL-35 levels may play a vital role in regulating SLE. Therefore, the reduced level of IL-35 might lead to an imbalance in Th17 cells, which is crucial in regulating the T reg cells leading to the development of SLE [34].

1.3 Justification of the study

Cytokines have been shown to play an important role in the pathogenesis of SLE. However, the abnormal expression and secretion of IL-35 and its receptor present in SLE patients remain unknown. The recently identified cytokine, Interleukin-35, has been described for its anti-inflammatory and immune-modulating actions and its contribution to disease pathogenesis [35]. Therefore, to better understand its interrelationship and immunopathologic roles in SLE, this study aims to determine the levels of IL-35 and its receptors in SLE patients and healthy controls. Moreover, this study is to investigate the association of IL-35 and its receptors with serological parameters such as C-reactive protein (CRP), antinuclear antibodies (ANA) and anti-double stranded DNA (anti-dsDNA). Furthermore, the study is also to evaluate the association of IL-35 and its receptors with clinical manifestation (SLE Disease Activity Index; SLEDAI score).

1.4 References

1. Fava A, Petri M. Systemic lupus erythematosus: Diagnosis and clinical management. Journal of Autoimmunity. 2019;96:1-13.
2. Carter EE, Barr SG, Clarke AE. The global burden of SLE: prevalence, health disparities and socioeconomic impact. Nature Reviews Rheumatology. 2016;12(10):605-20.
3. Chai HC, Phipps ME, Chua KH. Genetic risk factors of systemic lupus erythematosus in the Malaysian population: a minireview. Clinical and Developmental Immunology. 2011;2012.
4. Eloranta M-L, Rönnblom L. Cause and consequences of the activated type I interferon system in SLE. Journal of molecular medicine. 2016;94(10):1103-10.
5. Vaglio A, Grayson PC, Fenaroli P, Gianfreda D, Boccaletti V, Ghiggeri GM, et al. Drug-induced lupus: Traditional and new concepts. Autoimmunity reviews. 2018;17(9):912-8.
6. Tedeschi SK, Bermas B, Costenbader KH. Sexual disparities in the incidence and course of SLE and RA. Clinical Immunology. 2013;149(2):211-8.
7. Pan Q, Chen X, Liao S, Chen X, Zhao C, Xu Y, et al. Updated advances of linking psychosocial factors and sex hormones with systemic lupus erythematosus susceptibility and development. PeerJ. 2019;7:e7179. https://doi.org/10.7717/peerj.7179
8. Tabor DE, Gould KA. Estrogen receptor alpha promotes lupus in (NZB× NZW) F1 mice in a B cell intrinsic manner. Clinical Immunology. 2017;174:41-52.
9. Elera-Fitzcarrald C, Ugarte-Gil M, Gamboa-Cardenas R, Zevallos F, Medina M, Cucho-Venegas J, et al. Prolactin levels are associated with a pro-inflammatory

body mass distribution among women with systemic lupus erythematosus. Lupus. 2017;26(8):808-14.

10. Tsokos GC, Lo MS, Reis PC, Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. Nature Reviews Rheumatology. 2016;12(12):716-30.

11. Pan L, Lu M-P, Wang J-H, Xu M, Yang S-R. Immunological pathogenesis and treatment of systemic lupus erythematosus. World Journal of Pediatrics. 2019;16:19-30. https://doi.org/10.1007/s12519-019-00229-3

12. Smith CK, Kaplan MJ. The role of neutrophils in the pathogenesis of systemic lupus erythematosus. Current opinion in rheumatology. 2015;27(5):448-53.

13. Comte D, Karampetsou MP, Tsokos GC. T cells as a therapeutic target in SLE. Lupus. 2015;24(4-5):351-63.

14. Mok C. Systemic lupus erythematosus: what should family physicians know in 2018. Hong Kong Medical Journal. 2018;24:501-11.

15. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Arthritis & Rheumatology. 2019;71(9):1400-12.

16. Pisetsky DS. Antinuclear antibody testing-misunderstood or misbegotten? Nature Reviews Rheumatology. 2017;13(8):495-502.

17. Pisetsky DS, Bossuyt X, Meroni PL. ANA as an entry criterion for the classification of SLE. Autoimmunity reviews. 2019;18(12):102400.

18. Durcan L, Thomason JL, Kuo D, Wener MH. AB0467 Antibodies To Double Stranded DNA: Combined Standard Elisa and High-Salt Elisa Assays for The

Detection of SLE Disease Activity. Annals of the Rheumatic Diseases. 2016;75(Suppl 2):1065-6.

19. Pisetsky DS. Anti-DNA antibodies-quintessential biomarkers of SLE. Nature Reviews Rheumatology. 2016;12(2):102-10.

20. de la Varga Martínez R, Rodríguez-Bayona B, Añez GA, Medina Varo F, Pérez Venegas JJ, Brieva JA, et al. Clinical relevance of circulating anti-ENA and anti-dsDNA secreting cells from SLE patients and their dependence on STAT-3 activation. European journal of immunology. 2017;47(7):1211-9.

21. Mummert E, Fritzler MJ, Sjöwall C, Bentow C, Mahler M. The clinical utility of anti-double-stranded DNA antibodies and the challenges of their determination. Journal of immunological methods. 2018;459:11-9.

22. Arroyo-Ávila M, Santiago-Casas Y, McGwin G, Cantor RS, Petri M, Ramsey-Goldman R, et al. Clinical associations of anti-Smith antibodies in PROFILE: a multi-ethnic lupus cohort. Clinical rheumatology. 2015;34(7):1217-23.

23. Dema B, Charles N. Autoantibodies in SLE: specificities, isotypes and receptors. Antibodies. 2016;5(1):2.

24. Ahn SS, Jung SM, Yoo J, Lee S-W, Song JJ, Park Y-B. Anti-Smith antibody is associated with disease activity in patients with new-onset systemic lupus erythematosus. Rheumatology international. 2019;39(11):1937-44.

25. Hirohata S, Sakuma Y, Yanagida T, Yoshio T. Association of cerebrospinal fluid anti-Sm antibodies with acute confusional state in systemic lupus erythematosus. Arthritis research & therapy. 2014;16(5):450.

26. Ünlü O, Zuily S, Erkan D. The clinical significance of antiphospholipid antibodies in systemic lupus erythematosus. European journal of rheumatology. 2016;3(2):75.

27. Fernando MMA, Isenberg DA. How to monitor SLE in routine clinical practice. Annals of the Rheumatic Diseases. 2005;64(4):524-7.

28. Mikdashi J, Nived O. Measuring disease activity in adults with systemic lupus erythematosus: the challenges of administrative burden and responsiveness to patient concerns in clinical research. Arthritis research & therapy. 2015;17(1):183.

29. Durcan L, Petri M. Immunomodulators in SLE: clinical evidence and immunologic actions. Journal of autoimmunity. 2016;74:73-84.

30. Huang A, Cheng L, He M, Nie J, Wang J, Jiang K. Interleukin-35 on B cell and T cell induction and regulation. Journal of Inflammation. 2017;14(1):16.

31. Cai Z, Wong C, Kam N, Dong J, Jiao D, Chu M, et al. Aberrant expression of regulatory cytokine IL-35 in patients with systemic lupus erythematosus. Lupus. 2015;24(12):1257-66.

32. Egwuagu CE, Yu C-R, Sun L, Wang R. Interleukin 35: Critical regulator of immunity and lymphocyte-mediated diseases. Cytokine & Growth Factor Reviews. 2015;26(5):587-93.

33. Olson B, Sullivan J, Burlingham W. Interleukin 35: A Key Mediator of Suppression and the Propagation of Infectious Tolerance. Frontiers in Immunology. 2013;4(315).1-12.

34. Ouyang H, Shi Y-b, Wang Z, Feng S, Kong S-m, Lu Y, et al. Decreased Interleukin 35 and CD4+EBI3+T cells in Patients With Active Systemic Lupus Erythematosus. The American Journal of the Medical Sciences. 2014;348(2):156-61.

35. Bello RO, Chin VK, Isnadi AR, Faruq M, Abd Majid R, Atmadini Abdullah M, et al. The role, involvement and function (s) of Interleukin-35 and Interleukin-37

in disease pathogenesis. International journal of molecular sciences. 2018;19(4):1149.

36. Hamilton JA, Hsu HC, Mountz JD. Autoreactive B cells in SLE, villains or innocent bystanders? Immunological reviews. 2019;292(1):120-38.

37. Vignali DA, Kuchroo VK. IL-12 family cytokines: immunological playmakers. Nature immunology. 2012;13(8):722.

38. Larosa M, Zen M, Gatto M, Jesus D, Zanatta E, Iaccarino L, et al. IL-12 and IL-23/Th17 axis in systemic lupus erythematosus. Experimental Biology and Medicine. 2019;244(1):42-51.

39. Van Vollenhoven RF, Hahn BH, Tsokos GC, Wagner CL, Lipsky P, Touma Z, et al. Efficacy and safety of ustekinumab, an IL-12 and IL-23 inhibitor, in patients with active systemic lupus erythematosus: results of a multicentre, double-blind, phase 2, randomised, controlled study. The Lancet. 2018;392(10155):1330-9.

40. Muhammad Yusoff F, Wong KK, Mohd Redzwan N. Th1, Th2, and Th17 cytokines in systemic lupus erythematosus. Autoimmunity. 2020;53(1):8-20.

41. Vijayan D, Redzwan NM, Avery DT, Wirasinha RC, Brink R, Walters G, et al. IL-27 directly enhances germinal centre B cell activity and potentiates lupus in sanroque mice. The Journal of Immunology. 2016;197(8):3008-17.

42. Lee MH, Gallo PM, Hooper KM, Corradetti C, Ganea D, Caricchio R, et al. The cytokine network type I IFN-IL-27-IL-10 is augmented in murine and human lupus. Journal of Leukocyte Biology. 2019;106(4):967-75.

43. Shen P, Roch T, Lampropoulou V, O'Connor RA, Stervbo U, Hilgenberg E, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. Nature. 2014;507(7492):366-70.

44. Wang R-X, Yu C-R, Dambuza IM, Mahdi RM, Dolinska MB, Sergeev YV, et al. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. Nature medicine. 2014;20(6):633-41.

45. Choi J, Leung PS, Bowlus C, Gershwin ME. IL-35 and autoimmunity: a comprehensive perspective. Clinical reviews in allergy & immunology. 2015;49(3):327-32.

46. Catalan-Dibene J, McIntyre LL, Zlotnik A. Interleukin 30 to interleukin 40. Journal of Interferon & Cytokine Research. 2018;38(10):423-39.

47. Wirtz S, Billmeier U, Mchedlidze T, Blumberg RS, Neurath MF. Interleukin-35 mediates mucosal immune responses that protect against T-cell-dependent colitis. Gastroenterology. 2011;141(5):1875-86.

48. He D, Liu M, Liu B. Interleukin-35 as a new biomarker of renal involvement in lupus nephritis patients. The Tohoku journal of experimental medicine. 2018;244(4):263-70.

49. Ye Z, Jiang Y, Sun D, Zhong W, Zhao L, Jiang Z. The Plasma Interleukin (IL)-35 Level and Frequency of Circulating IL-35+ Regulatory B Cells are Decreased in a Cohort of Chinese Patients with New-onset Systemic Lupus Erythematosus. Scientific reports. 2019;9(1):1-12.

50. Yin L, Ge Y, Yang H, Peng Q, Lu X, Zhang Y, et al. The clinical utility of serum IL-35 in patients with polymyositis and dermatomyositis. Clinical rheumatology. 2016;35(11):2715-21.

51. Bassiouny M, Sonbol A, Eissa H, El-Shanawany A, Labeeb A. CD4+ Foxp3+ T cells, interleukin-35 (IL-35) and IL-10 in systemic lupus erythematosus patients: relation to disease activity. The Egyptian Rheumatologist. 2019;41(3):209-14.

52. Assad S, Khan HH, Ghazanfar H, Khan ZH, Mansoor S, Rahman MA, et al. Role of sex hormone levels and psychological stress in the pathogenesis of autoimmune diseases. Cureus. 2017;9(6).

53. Apostolidis SA, Lieberman LA, Kis-Toth K, Crispin JC, Tsokos GC. The dysregulation of cytokine networks in systemic lupus erythematosus. Journal of Interferon & Cytokine Research. 2011;31(10):769-79.

54. Pan L, Lu M-P, Wang J-H, Xu M, Yang S-R. Immunological pathogenesis and treatment of systemic lupus erythematosus. World Journal of Pediatrics. 2020;16(1):19-30.

55. Ohl K, Tenbrock K. Inflammatory cytokines in systemic lupus erythematosus. Journal of Biomedicine and Biotechnology. 2011;2011.1-15.

56. Ning-Wei Z. Interleukin (IL)-35 is raising our expectations. Rev Med Chil. 2010;138(6):758-66.

57. Li X, Mai J, Virtue A, Yin Y, Gong R, Sha X, et al. IL-35 is a novel responsive anti-inflammatory cytokine-a new system of categorizing anti-inflammatory cytokines. PloS one. 2012;7(3):e33628.

58. Pylayeva-Gupta Y. Molecular pathways: interleukin-35 in autoimmunity and cancer. Clinical Cancer Research. 2016;22(20):4973-8.

59. Qiu F, Song L, Yang N, Li X. Glucocorticoid downregulates expression of IL-12 family cytokines in systemic lupus erythematosus patients. Lupus. 2013;22(10):1011-6.

60. Mao H, Gao W, Ma C, Sun J, Liu J, Shao Q, et al. Human placental trophoblasts express the immunosuppressive cytokine IL-35. Human Immunology. 2013;74(7):872-7.

CHAPTER 2

OBJECTIVES OF THE STUDY

2.1 General objective

To determine the IL-35 and its receptors in SLE patients and their association with serological parameters and SLEDAI score.

2.2 Specific objectives

1. To determine the correlation of IL-35 levels between SLE patients and healthy controls.
2. To determine the levels of IL-35 receptors within $CD3^{+}CD4^{+}$ T helper cells between SLE patients and healthy controls.
3. To determine the association of IL-35 and its receptors (IL-12Rβ2 and gp130) levels with SLEDAI-2K scores in SLE patients.
4. To determine the association of IL-35 and its receptors (IL-12Rβ2 and gp130) levels with serological parameters (i.e. ANA, anti-dsDNA autoantibodies and CRP) in SLE patients.