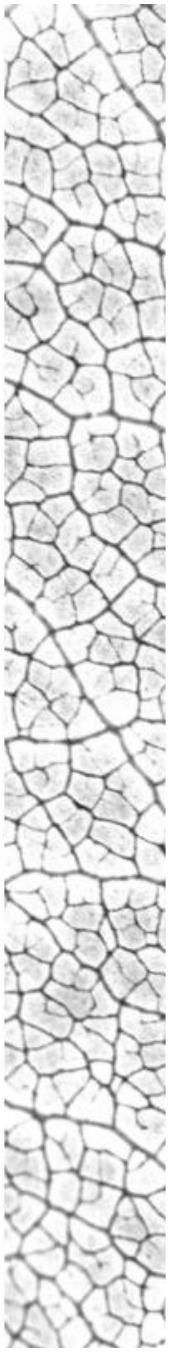

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Correlation between interleukin-10 and interleukin-12 levels with systemic lupus erythematosus and the effect of the age and gender

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Abstract

Background: Systemic Lupus Erythematosus is an autoimmune disease that affects many systems, including the skin, musculoskeletal, renal, neuropsychiatric, hematologic, cardiovascular, pulmonary, and reproductive systems. Family physicians should be familiar with the manifestations of lupus to aid in early diagnosis, monitoring patients with mild disease, recognizing warning signs that require referral to a rheumatologist, and helping to monitor disease activity and treatment in patients with moderate to severe disease.

Materials & Methods: The study included 70 samples included: 50 outpatients who attended to Al-Hilla General Teaching Hospital in Babylon Governorate who are suffering from dermatitis during the period (Feb.2019 to Aug. 2019). Their ages are ranging between (1-45) years old and from both genders. Depending on the age, the patients were divided into three groups included 1-15 years old, 16-30 years old and 31-45 years old. The negative control group included 20 healthy persons in the same age groups. A questionnaire was done to obtain the required information from the patient for the purpose of statistical analyzes.

Results: A significant difference in the level of IL-10 & IL-12 were detected in patient groups in comparison with the control group. The results also showed that O^+ blood group is the most frequent (30%) among patient groups. Furthermore, there is a genetic tendency in the frequency of onset of Systemic Lupus Erythematosus and the family history: 20%, 40%,and 30%of patients with SLE have family history of this disease in both parents, in mother only, in father only, respectively. Results also showed that the frequency of Systemic Lupus Erythematosus infection was effected by the gender (female is more vulnerable) and the age (31-45 years old has highest frequency).

Keywords: systemic lupus erythematosus**,** Interleukin-10, Interleukin-12, family history, gender, age, blood group

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that affects many systems, including the skin, musculoskeletal, renal, neuropsychiatric, hematologic, cardiovascular, pulmonary, and reproductive systems. well knowledge of the manifestations of lupus is necessary for early diagnosis and recognizing warning signs that require referral to a rheumatologist, and aid to monitor disease activity and treatment in patients with moderate to severe disease (VU LAM *et al*., 2016).

Although the diagnosis and treatment of lupus disease has dramatically developed with introduction of potent immunosuppressive therapies and better medical management for acute cases, the mortality rates are still significant among active acute cases with increasing chronic cases occurring at intervals, and there are clear evidences about atherosclerotic and cardiovascular disease that is insufficiently explained (Feng *et al*., 2006). Increased production and decreased clearance of immune complexes lead to immune complex deposition in tissue and damage to multiple organ systems. Interleukin-10 (IL-10) has the ability to induce autoantibody production by B lymphocytes, suggesting that IL-10 plays an important role in the pathogenesis of SLE, the great sources of IL-10 in patients with SLE are B-cells and monocytes. IL-10 overproduction by B lymphocytes and monocytes (Peng *et al*.,2013). Although the exact etiology of SLE is still unknown, there

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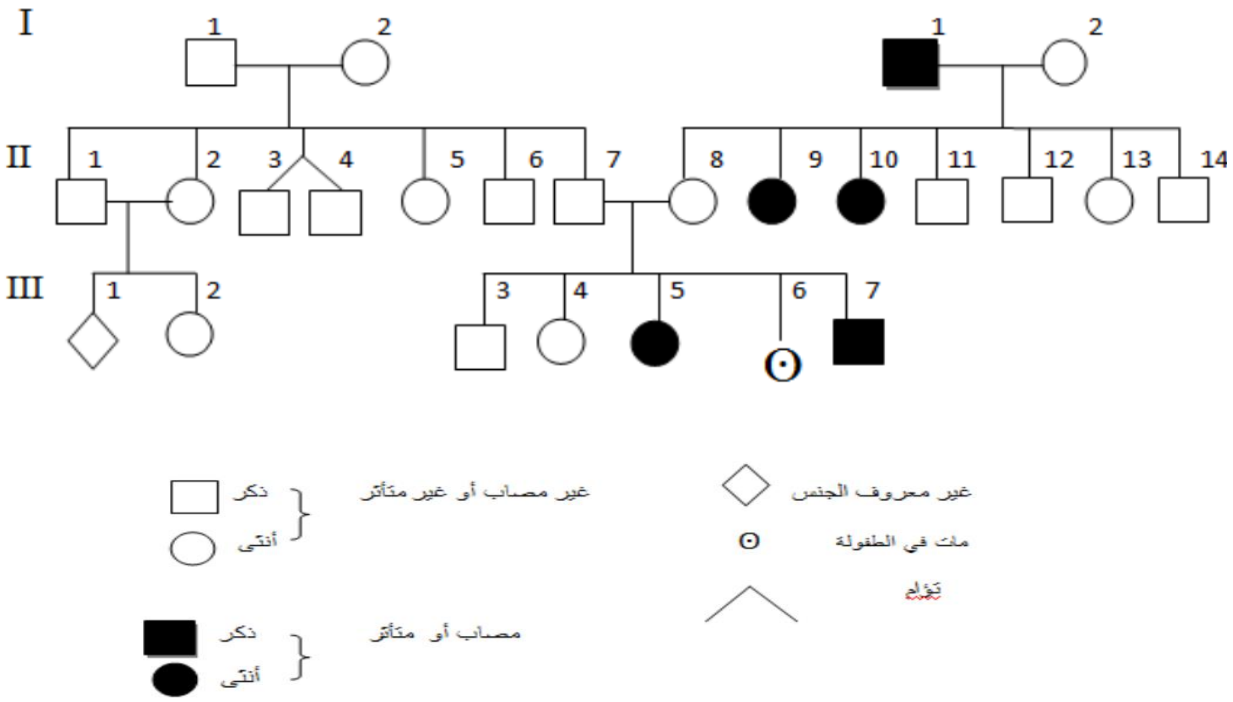

Fig. 1. A typical Pedigree tree contains agreed symbols, such as the Roman numerals on the left to define the generation, and each person in the generation with a particular number (Sutton, 2002).

is a believing that the defect of some T helper type 1(Th1) derived cytokine family which mainly activate the cellular mechanisms of the immune system, has direct contribution of this disease(Rana *et al*.,2012**;** Abbas *et al*., 2018).

It is known that, Interleukin-12 (IL-12) and Interferon gamma (IFN_γ) represent the most important cytokines in Th1 derived cytokines family. IL-12 mainly targets T cells and Natural killer cells(NK cells), it plays a key role in the differentiation of Th1 cells, which can specifically stimulate the signal transducer and activator of transcription 4 (STAT4) and transcription factor, thereby promote the differentiation of naive T cells toward the Th1 phenotype whatever in vivo or in vitro (Ye *et al.,*2017; Kadhum *et al.,* 2019; Zelalem, et al, 2018).

MATERIALS AND METHODS

Study Population

The study included 70 samples included: 50 outpatients who attended to Al-Hilla General Teaching Hospital in Babylon Governorate who are suffering from dermatitis during the period (Feb.2019 to Aug. 2019). Their ages are ranging between (1-45) years old and from both genders. Depending on the age, the patients were divided into three groups included 1-15 years old, 16-30 years old and 31-45 years old. The negative control group included 20 healthy persons in the same age groups. A questionnaire was done to obtain the required information from the patient for the purpose of statistical analyzes.

Samples Collection

Venous blood samples have been collected from control and patient groups. Using a septic technique by applying povidone iodine and 70% alcohol at the site of vein puncture, 5.0 ml of venous blood was drawn from the antecubital by the attending nurse has been transferred directly into sterile coagulation tubes for 60 min at room temperature. Then, centrifuged at 3000rpm for 15min. The serum was stored at -20C˚.

Pedigree Analysis

The pedigree tree was drawn from information obtained from children about parents and fathers if the information about the family is completed, the genetic analyst can re-create the genetic history of personal traits (Johanson *et al*, 2000).

Statistical Analysis

Statistical analysis was performed with the SPSS, Version 23 (statistical package for social sciences) and also Chi-square. Data analysis was done using T-Test for tables with means. P-value of ≤ 0.05 was considered as a level of significance. All statistical analysis were performed according to statistician directions.

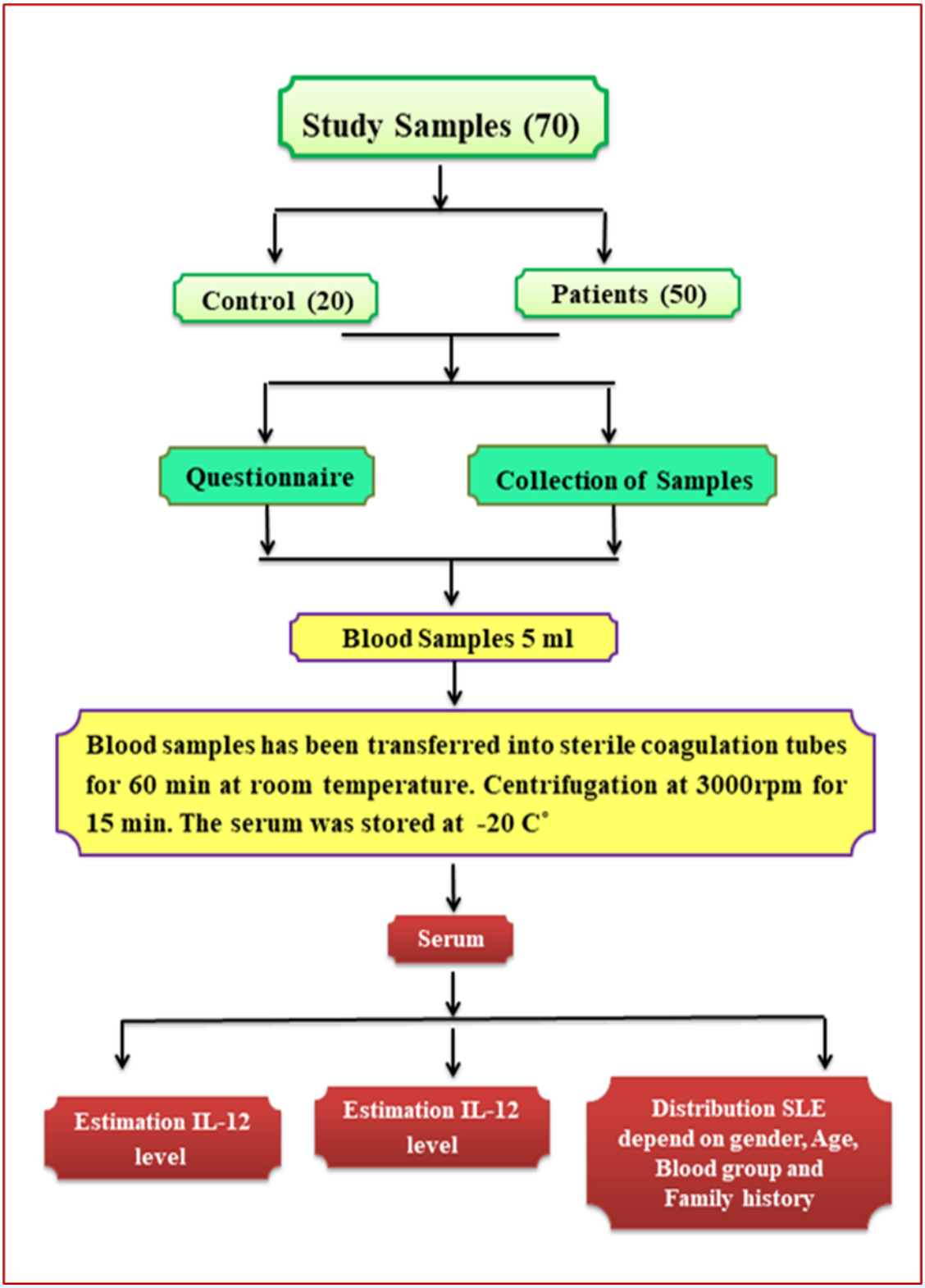

Fig. 2. Design of Study

Table 1. Serum level of IL-10 in Systemic Lupus Erythematosus patients and controls

Subject	Number of samples	IL-10 Mean ± S.D(pg /ml)
Systemic Lupus Erythematosus patients	50	244.40 ± 52.55**
Control	20	128.25 ± 36.39

**Significant differences compared to the control group at a potential level (p<0.01).

Table 2. Serum level of IL-12 in Systemic Lupus Erythematosus patients and controls

Subject	Number of samples	IL-12 Mean ± S.D(pg /ml)
Systemic Lupus Erythematosus Patients	50	87.24 ± 25.72 **
Control	20	176.50 ± 52.39

**Significant differences compared to the control group at a potential level (p<0.01).

RESULTS & DISCUSSION

Serum level of IL-10 in Systemic Lupus Erythematosus Patients and controls

The results of the present study showed a significant increase in IL-10 level in patients as compared with the control group regardless of the age and the gender.

As detailed in **Table 1**, The mean serum levels of IL-10 for patients were 244.40 pg/ml, while it was 128.25 pg/ml for control. The difference was statistically significant at a potential level (p<0.01). This finding is in agreement with Rönnelid *et al.*, 2003 who showed that the production of IL-10 has a higher significant increase in the cell culture which has been incubated with SLE sera as compared with those cell culture which has been incubated with control sera.. The possible explanation is, the enhanced production of IL-10 in patients with SLE leads to B-cell hyperactivity, autoantibody production, immune complex production, PBMC stimulation, and also production of IL-10, Stimulation of this cycle leads to increased deposition of immune complexes in tissues and SLE-related pathology. Immune complexes that are produced in SLE acting through Fcγ receptor II stimulate IL-10 production from peripheral blood mononuclear cell (PBMC), thus perpetuating the pathological cycle

Serum level of IL-12 in Systemic Lupus Erythematosus Patients and controls

As detailed in **Table 1**, the mean serum levels of IL-12 for patients were 87.24 pg/ml, while it was 176.50 pg/ml for control. The difference was statistically significant at a potential level (p<0.01). Tucci *et al.*,2008 suggested that, Th1 cytokines (IL-12 and IFN-γ) may be involved in pathogenesis of SLE. IL-12 is produced by macrophage and dendritic cells (DC) and represents a pro-inflammatory cytokine which induces the differentiation of Th1 cells, and links innate immunity and adaptive immunity. IFN-γ, principally produced by T cells, CD4+ as well as CD8+, and natural killer cells, links innate and acquired response of macrophages. A study by Chun *et al*., 2007 made a comparison and reported that IL-6, IL-10, IL-12, and IFN-γ levels in SLE patients are higher than normal controls, but IL-2 level in SLE patients is lower than normal controls. On the other hand, IL-6 level in the serum was significantly high in active SLE patients and has positive correlation with many factors such as: SLE activity index(SLEDAI), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). While it has negative correlation with C3, C4, and lymphocyte counts. No significant differences in the levels of cytokines were observed between SLE patients with nephritis and those without nephritis; These data suggest that IL-6 and IL-10 may be a useful biomarker for disease activity in SLE. These findings are in agreement with some previous reports showed that inflammatory cytokines (IL-12 and IFN-γ levels) in SLE patients were higher than the healthy controls. on the other hand, some Previous experimental studies had detected that IFN-γ can increase in rate of disease of SLE, while anti IFN-γ antibody and soluble recombinant IFN-γR (sIFNR) can delay onset of the disease. Thus, overexpression of IL-12 and IFN-γ causes further inflammation and tissue injury and contributes to the immunopathogenesis of SLE (Harigai *et al.,*2008; Ye *et al.*, 2017).

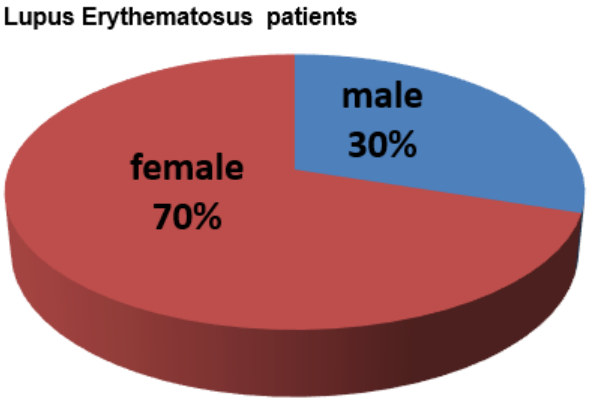

Fig. 3. Distribution Systemic

Frequency of infection in Systemic Lupus Erythematosus and gender

The data in the present study showed an increase in frequency of SLE in female compared with male, **Fig. 3**. This finding is in disagreement with another studies who found that no clear impact of the gender as a risk factor of SLE infection, although male group members with a family history with SLE have 10 fold risk probability for developing SLE as compared with 8-fold risk probability in female group members (Kuo *et al*.,2015). A study by Abdou *et al*. (2008) made a comparison for the effects of blocking the action of Estrogen Receptor alpha (ERα) in order to detecting signaling pathways that could participate in improving of disease activity in women with SLE. On the other hand, a study by Ghedira *et al*. (2002)

Table 3. The relationship between gender and IL-10 concentration in Systemic Lupus Erythematosus patients and control group

Age Groups (Years)	Systemic Lupus Erythematosus Patients Mean ± S.D(Pg /Ml)		Control Mean ± S.D(Pg /Ml)	
	Male	Female	Male	Female
1-15	**237.33 ± 64.18	**250.43 ± 72.50	120.00 ± 60.68	125.16 ± 58.24
16-30	**255.00 ± 30.75	*230 .55 ± 75.3	162 .85 ± 34.0	140.65 ± 61.3
31-45	**244.4 ± 53.1	*190.56 ± 44.2	97.5 ±13.32	90.38 ± 12.9

*Significant differences compared to the control group at a potential level (p<0.05).
**Significant differences compared to the control group at a potential level (p<0.01).

Table 4. The relationship between gender and IL-12 concentration in Systemic lupus erythematosus patients and control group

Age Groups (Years)	Systemic Lupus Erythematosus Patients Mean ± S.D(IU/Ml)		Control Mean ± S.D(IU/Ml)	
	Male	Female	Male	Female
1-15	**78.00 ±17.64	*79.07±1.50	195.71 ± 17.18	153.16± 67.24
16-30	*102.60 ± 29.99	*100.1±45.3	204.28 ± 59.12	190.65±16.3
31-45	*86.64 ± 26.11	*62.56 ± 4.2	121.66 ± 32.16	100.38 ± 12.9

*Significant differences compared to the control group at a potential level (p<0.05).
**Significant differences compared to the control group at a potential level (p<0.01).

Table 5. The correlation between frequency of SLE infection and the age of Patients

Age/Years	Systemic Lupus Erythematosus Patients Number of SLE infection %
1 - 15	15 (30%)
16 – 30	10 (20%)
31 – 45	25 (50%)
Total	50 (100%)

identified that female group is less risk in patients with older onset SLE than in younger onset. Soto *et al*. (2004) Confirmed that SLE in childbearing women, accounting 80-90% of cases, while, male accounting 4-30% of cases in different series. Borteli *et al*. (2006) found the same result in SLE patients with disease onset after 50 years of age. Nevertheless, in other studies, the sex ratio was between 1.1 and 2.8 for patients older than 65 years (Achour *et al*., 2012).

The Relationship between IL-10 and the Gender

Compared with control group, there is a high significant increase in IL-10 level in both male and female patients. **Table 3**.

The Relationship between IL-12 and the Gender

The results of the present study showed a significant decrease in the level of IL-12 in patients as compared to the control group regardless of the age and the gender. **Table 4**.

The correlation between frequency of SLE infection and the age of Patients

In the present study, the findings showed that the patients in age group (31-45) years old have frequency of SLE infection higher than other age groups, **Table 5**. a Tunisian study by Achour *et al.* (2012) for the period 1994-2009 included analysis of the clinical and laboratory datain four hospitals for 342 SLE patients found that eighteen patients among them are aged over 65 years (5.3%).

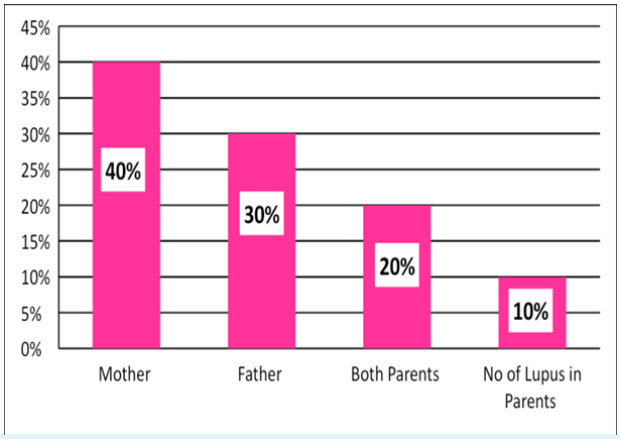

Fig. 4. Frequency of SLE in families of Systemic Lupus Erythematosus patients

Frequency of Systemic Lupus Erythematosus in family Patients

The results of the present study indicated that 20% of patients with SLE have family history of this disease in both parents. While 40% have family history of this disease in mother only. However, 30% have family history of this disease in father only, **Fig. 4**. The rest 10% have no family history with SLE. **Fig. 4** shows that in family 1, the patient (30 years old) is suffering from SLE and has a family history in grandmother who was infected too. This finding is in a comparable with a Danish study by Somers et al.(2013) to examine the risk of SLE in offspring of SLE-infected parents, the relative risk of SLE in female offspring has 17-fold increase than risk of SLE in male offspring.

The frequency of blood groups and Rh factor in SLE patient groups and control group

As detailed in **Table 6**, The frequency of blood groups and Rh factor is different between patient groups and control group. Among the patient groups, O^+ blood group has the highest frequency (30%) followed by 20 % and 18 % for A^+ and B^+ blood groups, respectively.

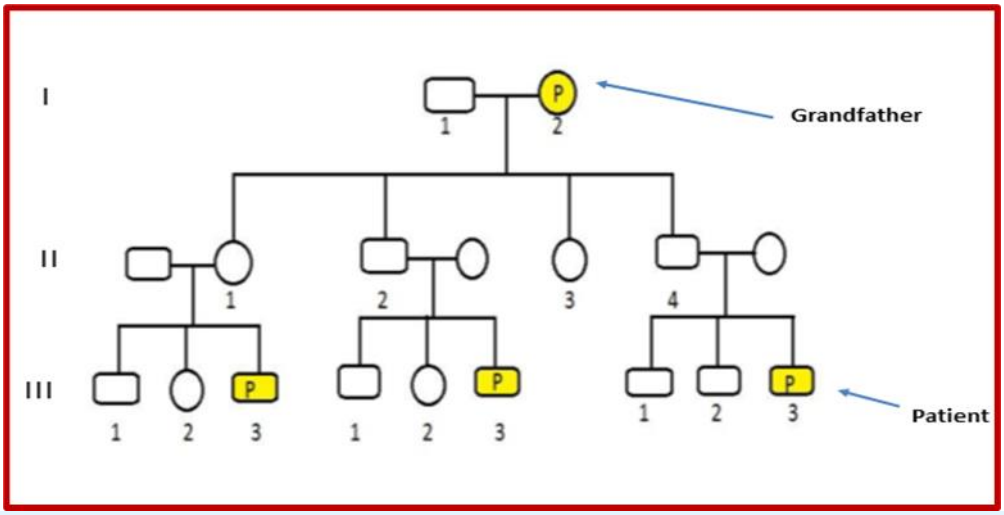

Fig. 5. Pedigree Analysis for family 1

Table 6. The frequency of blood groups and Rh factor in the patient groups and control group

Frequency %	Blood Groups & Rh factor							
	A^+	A^-	B^+	B^-	AB^+	AB^-	O^+	O^-
In The Patient groups	20% (N=10)	6% (N=3)	18% (N=9)	4% (N=2)	12% (N=6)	10% (N=5)	30% (N=15)	0%
In The Control Group	35% (N=7)	5% (N=1)	5% (N=1)	0%	20% (N=4)	0%	10% (N=2)	25% (N=5)

While, O^- blood group has no frequency among these patients. On the other hand, among control group persons, A^+ blood group has the highest frequency followed by 25% for O^- blood group. Cases of transient change of blood group from A to AB in SLE patients were reported and explained as a result of agglutination to anti-B antibodies although, Blood group changes have been reported in leukemia and stem cell autoimmune disease such as SLE. (Bornhäuser *et al.,* 1997; Nakamura *et al*.,2006).

In a Danish study by Ulff-Møller *et al*. (2017), the impact of having a family history of SLE on the risk of developing an Alzheimer's disease (AD) was assessed. the findings reported that the risk of developing AD was significantly 51% and 28% elevated in individuals with a first-degree or second/third-degree relative with SLE, respectively. The risk was increased for SLE, but also for Rheumatoid arthritis (RA), Inflammatory bowel disease (IBD), type 1 diabetes mellitus.

CONCLUSIONS

1- There was a significant increase in IL-10 for patients with Systemic Lupus Erythematosus compared with the control group.

2- There was a significant decrease in IL-12 for patients with Systemic Lupus Erythematosus compared with the control group.

3- Patients with family history are at high risk to develop Systemic Lupus Erythematosus.

4-The age group (31 – 45) found to have high incidence of Systemic Lupus Erythematosus as compared with other age groups.

5- O^+ blood group may be at high risk to have Systemic Lupus Erythematosus

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