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Genetic Polymorphism of Interferon Regulatory Factor 5 Gene in Egyptian Children with Systemic Lupus Erythematosus

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ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) is considered an autoimmune disorder that influence many organs, distinguished by complement activation, synthesis of many autoantibodies, organ damage, and deposition of immune complex.

Objective: Examine the relationship between the interferon regulatory factor 5 (IRF5) gene's genetic polymorphism and lupus nephritis disease activity as well as the correlation between SLE and these conditions.

Patients and methods: Sixty-three children were included and classified into three groups as follows: 21 children with lupus nephritis (LN), 21 lupus children without nephritis, and 21 control cases**.** Blood samples from all children were tested for the IRF5 gene (rs2004640) polymorphism utilizing the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method.

Results: Cases and controls differed in high statistically significant way regarding the frequency of TT genotype (64.3% and 23.8% respectively with p value <0.001) and frequency of risk allele T (77.4% and 38.1% respectively with p <0.001). However, when it came to IRF5 genotypes and alleles, there was no statistically significant difference between those with and without nephritis.

Conclusion: Children in Egypt who have the rs2004640 T allele and TT genotype may be at increased risk of developing SLE. Egyptian children with SLE who carry the rs2004640 T allele or TT genotype do not have an increased risk of developing nephritis.

Keywords: IRF5, Lupus Erythematosus, Genotype, Polymorphism.

INTRODUCTION

Systemic lupus erythematosus (SLE) is considered an autoimmune disorder that influence many organ, distinguished by complement activation, synthesis of many autoantibodies, organ damage, and deposition of immune complex [(1,2)].

SLE manifestation severity and prevalence differ among cases. SLE is a less prominent disease among Caucasians than Asians [(3)]. The main etiological factor of SLE still unknown. The pathogenesis of SLE includes hormonal and environmental factors, and genetic susceptibility [(4)].

The genetic factors have a potential role in SLE revealed by 10 folds existence in dizygotic twins, and 20 folds in monozygotic twins [(5)].

Over the last 20 years, SLE pathogenesis involved type I interferon (IFN). IFN is a significant viral immunity mediator, and IFN levels including INF-α were associated with SLE disease presentation and severity [(6)]. IRF5 gene is expressed in dendritic and B cells and is located on chromosome 7q32. IFN regulatory factor 5 (IRF5) is a transcription factor that regulates inflammatory responses. It can alter the expression of type I IFN genes and pro-inflammatory cytokines like interleukin (IL)-6, IL-12, and tumour necrosis factor (TNF) by activating the nuclear factor-B (NF-κB) pathway in a MyD88-dependent manner [(5)].

Many studies concerning IRF5 reported that IRF5 rs2004640 single-nucleotide polymorphisms (SNP) was correlated with SLE [(6)]. Studies were out in Egypt in 2017 discovered a link between the TT genotype and the rs2004640 T allele with a higher risk of nephritis and SLE [(7-8)].

In this study, the effects of genetic polymorphisms in the interferon regulatory factor 5 gene on SLE in Egyptian children and their associations with lupus nephritis disease activity were examined.

PATIENTS AND METHODS

Between September 2018 and September 2020, the Clinical Pathology Department and the Pediatric Nephrology Unit of the Faculty of Medicine at Zagazig University Hospitals conducted this case-control research.

Ethical consent:

All research participants' parents gave their informed permission, and the Zagazig University Institutional Review Board (IRB) gave their approval to the study procedure. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Sixty-three children were involved in this study; they were classified into three groups as follows: the first group 21 lupus with nephritis (LN), the second include 21 lupus without nephritis, and the third group 21 healthy children matched for age and sex as a control group.

The cases with the following criteria were included; Children diagnosed with SLE regarding the American College of Rheumatology (ACR) criteria for

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SLE [9]. Exclusion criteria included; other associated immunological diseases, and drug induced SLE.

Each group was subjected to the following:

- Full history taking including: Age, sex, symptoms of anemia, fever, butterfly rash, discoid rash, weight loss, hair loss, arthritis, seizures, hematuria, oliguria, purpura together with the onset and duration of the clinical course.
- Clinical examination was done particularly for hypertension, fever, renal disorder, arthritis, neurologic disorder, purpura, bruising, lymphadenopathy, pleuritis and pericarditis.
- Clinical disease activity was evaluated using SLEDAI-2k [10].
- Based on the Renal Pathology Society (RPS), International Society of Nephrology (ISN) as well as pathological analysis were performed [11].
    - Routine laboratory investigations as complete blood picture, was done on automated cell counter, model XS 500i (Sysmex, Japan), C3 and serum creatinine, 24h urinary proteins were done by using cobas 6000 autoanalyzer (Roche diagnostics, Germany), and urine analysis was also performed.
    - ANA using Hep-2 cells and Anti-dsDNA using Crithedia Lucelia substrate were done by indirect immunofluorescence (Inova, diagnostics, INC. San Diego) for all SLE cases to confirm diagnosis.
    - Specific laboratory investigations: Molecular detection of IRF5 gene (rs2004640) polymorphism by PCR- REFLP.

DNA extraction: Following the manufacturer's instructions, the QIAamp DNA kit was used to extract the DNA from whole blood (QiagenHilden, Germany) and it was stored at -70°C until used. Primers used in DNA amplification were forward primer: 5′- CGG CGG GAT GAA GAC TGG A -3′and reverse primer: 5' –CCG AAA GGG TGA GGG TGG C-3'. The reaction mixture of each sample included: Master mix 10 µl, Forward primer 1.0 µl, Reverse primer 1.0 µl, Template DNA 2.0 µl, Sterile high quality nuclease free water 6 µl.

The following temperature scheme was performed for the amplification using thermal cycler (Biometra, An Analytik Jena Company, Germany). Denaturing at 95°C for five minutes at first, then 34 cycles of denaturing at 94°C for thirty seconds, annealing at 56°C for forty seconds, extending at 72°C for forty seconds, and lastly extending at 72°C for five minutes. The band 501 of a 2 percent agarose gel was used to separate the PCR products.

The amplified DNA was digested by using (Tai I) restriction enzyme Thermo Scientific FastDigest Tai I. The following components were mixed at room temperature: (10 µl of the amplified PCR product, 17 µl of nuclease free water, 2 µl of 10x FastDigest Green Buffer and 1 µl of restriction enzyme) then incubated at 37°C for 5 minutes.

The amplified fragment (501 bp) after being digested with Tai I restriction enzyme, was visualized on agarose gel 3% concentration, using ultraviolet transillumination gave rise to (Figure 1).

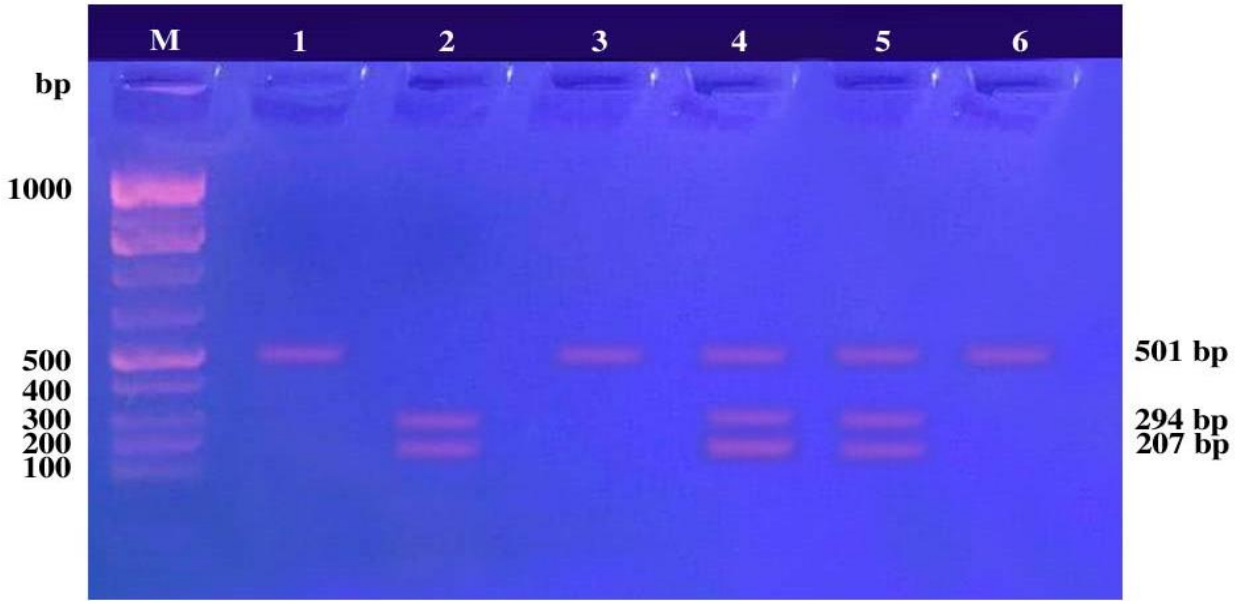

Figure (1): Genotyping of IRF5 gene (rs2004640): Lanes (1, 3, 6) = TT genotype (single band at 501), Lane 2 = GG genotype (two bands at 294 and 207), Lanes (4, 5) = GT genotype (three bands at 501, 294 and 207)

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) was used to analyse the data (IBM Corp. Version 24. Armonk, NY: IBM Corp). For numerical data, the mean and standard deviation (SD) were used. Proportion and frequency were used for of non-numerical information. The Student T test was utilised to determine the statistical significance of qualitative data between 2 groups and the ANOVA test was applied for more than 2 groups, and the Post Hoc test (LSD) was employed for pairwise comparisons. The Chi-Square test was used to examine the relationship between two qualitative variables. P value < 0.05 was considered significant.

RESULTS

Cases and control did not differ significantly regarding age (Mean ± SD 12.79±2.435 versus 12.05 ± 2.559 respectively with p value 0.269) and gender (female 88.1% versus 76.2% and male 11.9% versus 23.8% respectively with p value 0.223). The clinical characteristics and laboratory investigation of children with and without nephritis are given in table (1).

Table (1): Comparison between cases with nephritis and cases without nephritis regarding clinical and laboratory data

		Cases with Nephritis (No.= 21)	Cases without Nephritis (No.= 21)	P. value
Hb, g/Dl	Mean ± SD	10.28 ±1.85	10.005 ± 1.14	0.570
WBC, x 10^3	Mean ± SD	10.18 ± 2.43	11.93 ±2.87	0.231
Platelet, x 10^3	Mean ± SD	257.05 ±61.51	314.29 ± 77.21	0.109
CRP	Mean ± SD	35.28 ± 8.611	24.05 ± 5.34	*0.027
1st hr ESR	Mean ± SD	52.86 ± 13.22	27.05 ± 6.54	0.010**
C3	Mean ± SD	0.6400 ± 0.14	1.09 ± 0.24	0.000**
Serum creatinine	Mean ± SD	1.25 ± 0.27	0.52 ± 0.12	0.049*
24h urine protein (g)	Mean ± SD	0.48 ± 0.11	-	-
ANA		21 (100.0%)	21(100.0%)	-
Anti dsDNA		21 (100.0%)	21(100.0%)	-
Malar Rash		10 (47.6%)	6(28.6%)	0.204
Discoid Rash		1 (4.80%)	0 (0%)	0.311
Oral Ulcers		6 (28.6%)	0 (0%)	0.008**
Oedema		7 (33.3 %)	0 (0%)	0.004**
Hematuria		1 (4.80%)	0 (0%)	0.311
Arthritis		18(85.7%)	6(28.6%)	0.000**
Bleeding		0 (0%)	8(38.1%)	0.002**
Vasculitis		2(9.5%)	0 (0%)	0.147
Serositis		3(14.3%)	0 (0%)	0.072
Psychosis		0.072**???**	7 (33.3 %)	0.147
Fever		11 (52.4%)	6 (28.6)	0.116
SLEDAI score	Mean±SD	19.10 ± 6.96	8.19 ± 4.75	0.000**

*: Significant, **: Highly Significant

The rs2004640 TT genotype was significantly more common in SLE patients than in controls, and patients had a higher percentage of the T allele than controls (Table 2). Moreover, there was significant difference between control group and both LN cases and cases without nephritis as regard IRF5 genotype and allele, as frequency of TT genotype and T allele among lupus nephritis and among patients without nephritis compared to healthy. However, when it came to IRF5 genotypes and alleles, there was no statistically significant difference between those with and without nephritis (Table 3).

Table (2): Systemic lupus erythematosus group genotypes and alleles of the IRF5 gene (rs2004640) in comparison to controls

	Cases (No.= 42)	Control (No.=21)	P	OR	95% CI	
					LL	UL
IRF5 gene						
GG	4 (9.5%)	10 (47.6%)	Reference			
GT	11 (26.2%)	6 (28.6%)	0.073	0.218	0.047	1.005
TT	27 (64.3%)	5 (23.8%)	<0.001*	0.074	0.017	0.333
Alleles						
G	19 (22.6%)	26 (61.9%)	Reference			
T	65 (77.4%)	16 (38.1%)	<0.001*	0.170	0.076	0.384

Table (3): IRF5 gene (rs2004640) genotypes and alleles distribution in cases with nephritis, cases without nephritis and controls

	Cases with nephritis (No.=21)	Cases without nephritis (No.=21)	Control (No.=21)	P
IRF5 gene				
GG	2 (9.5%)	2 (9.5%)	10 (47.6%)	0.003*
p1=1.0, p2=0.006*, p3=0.006*				
GT	4 (19.0%)	7 (33.3%)	6 (28.6%)	0.569
p1=0.294, p2=0.469, p3=0.739				
TT	15 (71.4%)	12 (57.1%)	5 (23.8%)	0.007*
p1=0.334, p2=0.002*, p3=0.028*				
Alleles				
G	8 (19.1%)	11 (26.2%)	26 (61.9%)	<0.001**
p1=0.434, p2<0.001*, p3=<0.001*				
T	34 (80.9%)	31 (73.8%)	16 (38.1%)	<0.001**
p1=0.434, p2<0.001*, p3=<0.001*				

*: Significant, **: Highly Significant, p: for comparing between the studied groups. p1: for comparing between cases with nephritis and cases without nephritis. p2: for comparing between cases with nephritis and control. p3: for comparing between cases without nephritis and control.

Related to clinical data of the cases, malar rash and psychosis were significantly different between GT and TT, however, in instances with LN, there was no statistically significant difference between the IRF5 gene and Renal Biopsy Pathological Grade. Similarly, there was no difference between the IRF5 gene and SLEDAI score in subjects with or without nephritis (Table 4).

Table (4): Clinical data among patients and IRF5 gene (rs2004640) genotypes association

		IRF5 gene			P. value	
		GG	GT	TT		
Malar Rash		1 (25.0%)	1 (9.1%)	14 (51.9%)	0.041*	P1=0.422 P2=0.640 P3=0.037
Discoid Rash		0 (.0%)	0 (.0%)	1 (3.7)	0.752	
Oral Ulcers		0 (.0%)	1 (9.1%)	5 (18.5%)	0.521	
Oedema		0 (.0%)	1 (9.1%)	6 (22.2%)	0.396	
Hematuria		0 (.0%)	0 (.0%)	1 (3.7)	0.752	
Fever		1(25%)	3(27.3 %)	13(48.1%)	0.396	
Arthritis		3(75.0%)	3(27.3%)	18(66.7%)	0.063	
Bleeding		1(25%)	0 (.0%)	7 (25.9%)	0.173	
Vasculitis		0 (.0%)	0 (.0%)	2 (7.45)	0.558	
Serositis		0 (.0%)	1 (9.1%	2 (7.45)	0.830	
Psychosis		0 (.0%)	7(63.9%	3(11.1%)	0.001**	P1= 0.109 P2=0.483 P3=0.003
Renal Biopsy Pathological Grade	**Class II**	1(25%)	0 (.0%)	7 (25.9%)	0.630	
	Class III	1(25%)	2(18.2%)	4(14.8%)		
	Class IV	0 (.0%)	2(18.2)	4(14.8%)		
SLEDAI score among LN cases	**Mean±SD**	21.50±2.21	18.50±6.14	18.93±7.74	0.453	
SLEDAI score among Cases without nephritis	**Mean±SD**	9.00±4.24	6.71±2.14	8.92±5.90	0.626	

*: Significant, **: Highly Significant, P1: between GG and GT P2: between GG and TT P3: between GT and TT

DISCUSSION

Systemic lupus erythematosus (SLE) is a multisystem organ, chronic autoimmune disease that produces a wide range of autoantibodies, activates complement, deposits immune complexes, and damages target organs[(2)]. Regarding sex distribution in our study, among all SLE groups, our study included 88.1 % females and 11.9 % males with no statistically significant differences were found between both SLE and healthy control groups as regard age and sex distribution. This sex distribution agreed with the study of **El-Hefnawy *et al.* [(4)] and Hammad *et al.* [(8)]** who found that females versus males were (93.6% vs 6.7 %) and (3:1) respectively, and reported that between SLE patients and controls, there were no gender differences that were notable.

Regarding SLEDAI score, mean value of SLEDAI score among cases with nephritis was significantly higher than among cases without nephritis. This agree with **Halim *et al.* [(12)],** who found the SLEDAI score was elevated in SLE cases with LN in comparison with cases without nephritis. Also **Mora *et al.***, as they reported a significant association between SLEDAI and LN [(13)]. **Azab *et al.* [(14)],** revealed that SLEDAI mean score of SLE cases was (5.6 ± 1.9).

Regarding IRF5 gene SNP there was a significant variance between cases and control with the percent of TT genotype was (64.3 %) in SLE patients versus (23.8%) in control group and the percent of GG genotype was (47.6 %) in control group versus (9.5) in SLE patients. In addition, our study suggested that T allele is a risk allele for SLE as 77.4 % of SLE patients were carriers of T allele versus 38.1 % among the control group. This agrees with **Hammad *et al.* [(8)],** who found that the TT genotype was prominent in SLE cases (37%) compared to healthy cases (20%) and the T allele was also elevated in patients (58%) than healthy cases (41%). Also, the current study was matched with **El-Hefnawy *et al.* [(4)],** who stated that there was a significant difference as regards genotype frequency of IRF5 rs2004640 between the SLE and controls, with high frequency of TT genotype among SLE patients and GG genotype among the control group. Allelic distribution showed that T allele was the abundant in SLE cases.

Matching with our result, **Ruhizadeh *et al.* [(15)] and Zervou *et al.* [(16)],** found a correlation between control and genotype of cases, as GT genotype and TT genotype were correlated with higher risk of SLE and the T allele frequency was significantly higher in SLE cases than in healthy cases considering the T allele as a susceptibility allele.

Moreover, in the current study, there was significant variance between control and both LN cases and cases without nephritis as regard IRF5 genotype and allele, as frequency of TT genotype and T allele among lupus nephritis (71.4% and 80.9% respectively) and among patients without nephritis were (57.1 and 73.8% respectively) compared to healthy control (23.8% and 38.1% respectively). Regarding IRF5 and allele the relation between patients with and without nephritis did not differ significantly. Matching with our result, **El-Hefnawy *et al.* [(4)],** found that genotype of IRF5 rs2004640 and its allelic distribution had no significant variation between lupus patients with and without nephritis.

The current findings agreed with **Vuong *et al.* [(17)],** as they revealed no significance of SLE correlated SNPs for lupus nephritis presence. In contrary **Qin *et al.* [(6)], and Hammad *et al.* [(8)],** reported that the rs2004640 T allele elevates nephritis development risk.

This discrepancy in results may be due to differences in sample sizes, research design, genetic base of each population, clinical heterogeneity, different age groups and difference in ethnicity [(4)]. Additionally, because population stratification alters outcomes or hides the genuine genetic impact of polymorphism in research, it is challenging to determine the true impact of gen in case-control groups [(18-19)].

Regarding association between IRF5 gene and clinical manifestation, the present study showed significant association between IRF5 gene and psychosis. Despite the fact that studies examining the relationship between various IRF5genotypes and neuropsychiatric lupus are extremely rare, there were no genotype differences between lupus patients with central nervous system, affection for IRF5 rs2004640, and psychosis was more common in patients with the GG genotype than the AA and AG genotypes. The IRF5 gene and arthritis may be related, although this study was unable to show that. Research by **Qin *et al.* [(6)] and Hammad *et al.* [(8)],** which showed no obvious variations in genotype distribution in people with arthritis, corroborated this.

CONCLUSION

The presence of the TT genotype and T allele increased the chance of developing SLE, and the study revealed no correlation between the IRF5 polymorphism and the disease activity of lupus nephritis. This study showed a relationship between genetic polymorphism of the IRF5 gene and SLE.

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