TNF-α (-308) G>A and IFN-γ (+874) A>T gene Polymorphisms in Egyptian Patients with Systemic Lupus Erythematosus: Focus on Lupus Nephritis

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ABSTRACT

Background: *Lupus nephritis (LN) remains one of the most serious manifestations of systemic lupus erythematosus (SLE) associated with considerable morbidity and mortality. This study was planned to test for the association of TNF-α -308 G>A and IFN-γ +874 A>T gene polymorphisms with susceptibility of LN in Egyptian patients.*
Subjects and Methods: *A cross-sectional study was conducted on 125 patients (72 females and 53 males) suffering from SLE having LN.* ***C****ontrol group consisted of 112 age matched healthy (46 males and 66 females) individuals. For all patients, characterization of TNF-α -308 G>A and IFN-γ +874 A>T genetic polymorphisms was done using the allele-specific PCR technique.* ***Results*** *Regarding TNF-α -308 G>A, the A allele was associated with a significantly elevated odd ratio (OR) of 3.2 (95% confidence interval (CI) =2.15 -5.01, P = 0.003) in SLE cases associated with LN. The AA genotype, was statistically insignificant (95% CI = 0.61-46.5, P 0.12) increased risk. Combined genotypes (AA plus GA) versus GG was associated with a significant increase in SLE having nephritis compared to controls (OR = 2.51, 95% CI = 1.26-4.99, p=0.009). Regarding the IFN-γ +874, the T allele of this gene was not associated with increased risk of SLE having nephritis (OR 1.43, 95% CI =0.93-2.20, p 0.13). Genotype AT was associated with increased association of SLE having nephritis (OR 2.06, CI= 3.62-1.18, p = 0.01). In* ***Conclusions:*** *Polymorphisms related to TNF-α -308 G>A and IFN-γ +874 A>T genes may be considered as genetic risk factors for SLE having lupus nephritis.*
Keywords: *SLE, Cytokines, Gene polymorphism, TNF-α -308 G>A, IFN-γ +874 A>T.*

INTODUCTION

Systemic lupus erythematosus (SLE) is the prototypic immune complex disease which induces injury in multiple organs. Common targeted organs include the skin, kidneys, and joints. The disease is characterized by excessive autoantibody production, immune complex formation, and immunologically mediated tissue injury [(1,2)]. One of its most serious manifestations is lupus nephritis (LN), which leads to severe persistent proteinuria, chronic renal failure, and

end-stage renal disease, remains a cause of substantial morbidity and mortality, either secondary to kidney disease, or as a result of intense immunosuppressive drug toxicity [3–5].

The etiology of SLE is multifactorial, and both environmental and genetic factors are implicated. The contribution of these two factors may differ between individuals, but the resulting malfunctions in the immune system and the production of autoantibody plays a pivotal role in the pathogenesis of SLE [3]. Twin and family studies provide evidence that a genetic component contributes to disease susceptibility. Many genes that most likely contribute to the development of lupus have been identified [6,7].

There is strong evidence supporting the role of cytokine genes in the pathogenesis of autoimmune diseases, including SLE[8]. The tumor necrosis factor alpha (TNF-α), an important pro-inflammatory cytokine, plays an important role in the inflammatory and immune responses[9]. The single-nucleotide polymorphism (SNP) TNF-α -308 G/A is located on chromosome 6p21.3, within the class III region of major histocompatibility complex (MHC) [10,11]

Several studies analyzing the polymorphism of TNF-α -308 G/A and SLE in different populations have found contradictory results, some reported no association[12,13] others reported an association with increased risk of SLE [14,15].

Interferon-gamma (IFN-γ) secreted by activated T lymphocytes and has antiproliferative, antiviral, and immunomodulatory activities. It controls the development of T helper 1 (Th1) cells [16,17]. The human IFN-γ gene is located on chromosome 12q24.1, which spans about 5.4 kb and consists of four exons. Several polymorphisms within the IFN-γ non-coding regions, such as +874 A/T, CA repeat microsatellite and -179 T/G, have been implicated in numerous autoimmune and chronic inflammatory conditions[18,19]. There were no previous reports of polymorphisms of the IFN-γ +874 AT in cases of SLE associated with lupus nephritis.

Taking into account these findings, the aim of the present study was to establish the association between TNF-308 G/A and IFN-γ +874 A/T gene polymorphisms among Egyptian patients with SLE associated with lupus nephritis.

MATERIALS & METHODS

SLE patients and healthy controls selection:

This is cross-sectional study which was conducted on 125 patients (72 females and 53 males) suffering from SLE. Each patient met more than four of the revised criteria for the diagnosis of SLE proposed by the American College of Rheumatology (ACR) criteria at the time of evaluation[20]. SLE patients were selected from the Rheumatology Departments, Mansoura University, Mansoura, Egypt, for two and half years (2010-2013). Their mean age was 23.4±4.4 years. Demographic data, clinical manifestations and immunological parameters, including antinuclear antibodies (ANA), double-strands (ds) - DNA, and C3 and C4

were collected retrospectively (**table 1**). SLE patients were classified as having nephritis if they fulfilled ACR criteria for renal involvement (persistent proteinuria > 500 mg/ 24 hours (or > 3+) or cellular casts) [(21)]. Control group consisted of 112 age matched healthy (46 males and 66 females) individuals, selected from laboratory personnel, undergraduates, nursing staff, and blood donors of the same area with patients, their mean age was 28.3±6.2years. Exclusion criteria were the presence of autoimmune diseases as SLE or rheumatoid arthritis in a first-degree relative. An approval was obtained from The Mansoura Local Ethical Committees, as well as an informed consent that was obtained from all participants in the study before venipuncture.

DNA extraction, purification and amplification:

About 5 mL of peripheral venous blood was collected under aseptic conditions into sodium-citrated tubes and were delivered to centrifuge tubes containing K_2DTA (stored as EDTA anti-coagulated blood sample at -30ºC for DNA extraction)[(22)]. Genomic deoxyribonucleic acid (DNA) was extracted and purified according to the kit provided by Gentra Systems, USA. The average DNA concentration (0.161±0.002µg/µl) was determined from absorbance at 260 nm (Jenway, Genova Model, UK). All samples had a 260/280 nm absorbance ratio between 1.4 and 1.63. The integrity of the DNA was checked by electrophoresis on 1% agarose gel stained with ethidium bromide

Genotyping of TNF-α -308 gene:

TNF-α -308 gene polymorphism study was carried out by allele-specific polymerase chain reaction (ASPCR) method described by **Tronchon et al. (2008)** [(23)] as follow : **A** TNF-R primer (5′-TCTCGGTTTCTTCTCCATCG) was used with either 308-**G** (5′-ATAGGTTTTGAGGGGCATGG)or308-**A** (5′-ATAGGTTTTGAGGGGCATGA) to amplify a 184 bp fragment of the TNFα gene, which includes the polymorphic site at the nucleotide position −308. The primer pair TNF-F (5′-GAGTCTCCGGGTCAGAATGA)/TNF-R was used to amplify a 531 bp TNF gene fragment that was used as an internal control in the allele-specific polymerase chain reaction (ASPCR). Primer TNF-F was also used as a competitor for the TNF-R/A and TNF-R/G primer pairs to improve the specificity of the ASPCR assay **(Zhu and Clark, 1996).**[(24)]

Gene specific primers were purchased from Biolegio. BV, PO Box 91, 5600 AB Nijmegen, Netherlands. PCR was carried out in 50 microliters final reaction volume using Ready Mix (RED.Taq-PCR Reaction Mix), purchased from Sigma Aldrich, Saint Louis, USA. The following mixture was prepared for each sample: 25µl RED-Taq PCR reaction Mix(1X),1µl (20 pmole) of forward primer for each allele, 1µl (20 pmole) of reverse primer, 2µl (200ng) of genomic DNA and 20 µl of double distilled deionized water. This mix was put in a thin wall PCR microcentrifuge tube and gently centrifuged to collect all components

to the bottom of the tube. Amplification was performed in a Thermal Cycler (TECHEN TC-312, Model FTC3102D, Barloworld Scientific Ltd. Stone, Stafford Shire St., 150 SA, UK) using the following program: After initial heating at 95°C for 10 minutes, 30 PCR cycles were performed and consisted of heat denaturation (95°C for 45 seconds), annealing (for 50 seconds at 60°C for the TNF primer pair) and extension (72°C for 45 seconds). A final extension (72°C for 9 minutes) was performed. The resulting PCR product was 184 bp in length for each allele and 531 bp TNF gene fragment for the internal control.

The products was subjected to agarose gel electrophoresis using 2 % agarose stained with ethidium bromide and visualized via Light UV Transilluminator (Model TUV-20, OWI Scientific, Inc.800 242-5560, France) and photographed (fig1).

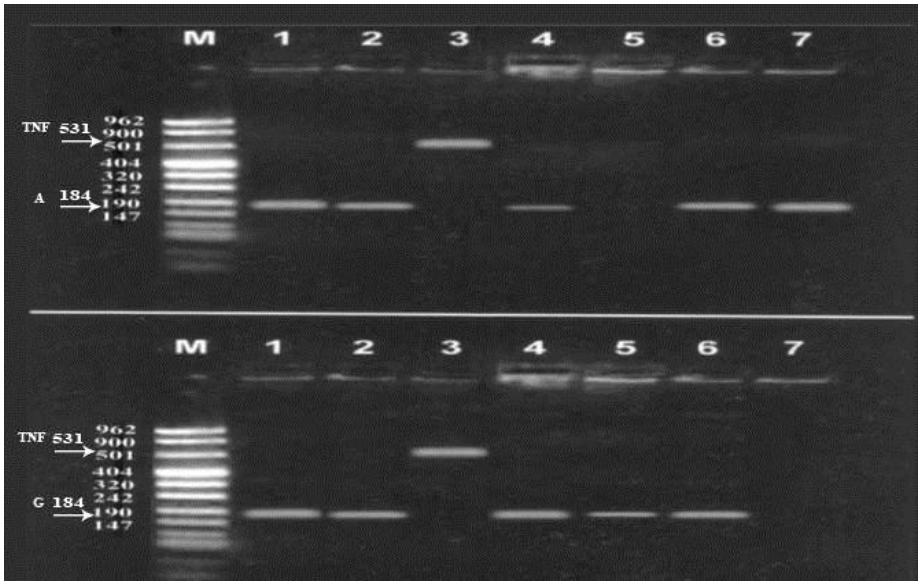

Fig (1): Agarose gel electrophoresis of TNFα gene 308 gene polymorphism showed **lane(M):** DNA marker ,**lane (1,2,4and 6**): **AG** TNF gene polymorphism which is one band at 184 bp in both gels **(lane 5)** : **GG** showing one band at 184 bp in gel (b, lower), **lane (7) AA** TNF polymorphism showing one band at 184 bp in gel (a, upper). **Lane (3)** there is one band at 531 indicating TNF- α gene control gene.

IFN-γ +874 A/T (rs2430561) polymorphism: It was determined by allele-specific polymerase chain reaction (ASPCR) method described **[amplification refractory mutational system (ARMS) PCR**] followed by gel electrophoretic analysis as described by **Pravica et al.**,[19]. The T and A polymorphism sequences were identified using a specific single oligonucleotide, synthesized to cover a 24 bp region for each allele. The following primers were used for amplification: 5'-TCA ACA AAG CTG ATA CTC CA-3' (consensus primer), 5'-TTC TTA CAA CAC AAA ATC AAA TCA-3' (**A** allele specific), 5'-TTC TTA CAA CAC AAA ATC AAA TCT-3' (**T** allele specific). (Fermentas Life Science, Ontario, Canada) Amplification yielded a **263-**bp PCR product. The PCR conditions consisted of an initial denaturation step at 95 ºC for 2 minutes, 10 cycles of incubation at 95 ºC for 15 seconds, 62 ºC for 50 seconds and 72 ºC for 40 seconds, followed by 20 cycles of incubation at 95 ºC for 20 seconds, 56ºC for 50 seconds and 72ºC for 50 seconds, with a final extension at 72ºC for 5 minutes. The amplified products were visualized by electrophoresis using 2% agarose gels containing ethidium bromide, and bands were visualized by Light UV Transilluminator (Model TUV-20, OWI Scientific, Inc.800242-5560, France) and photographed (Fig2).

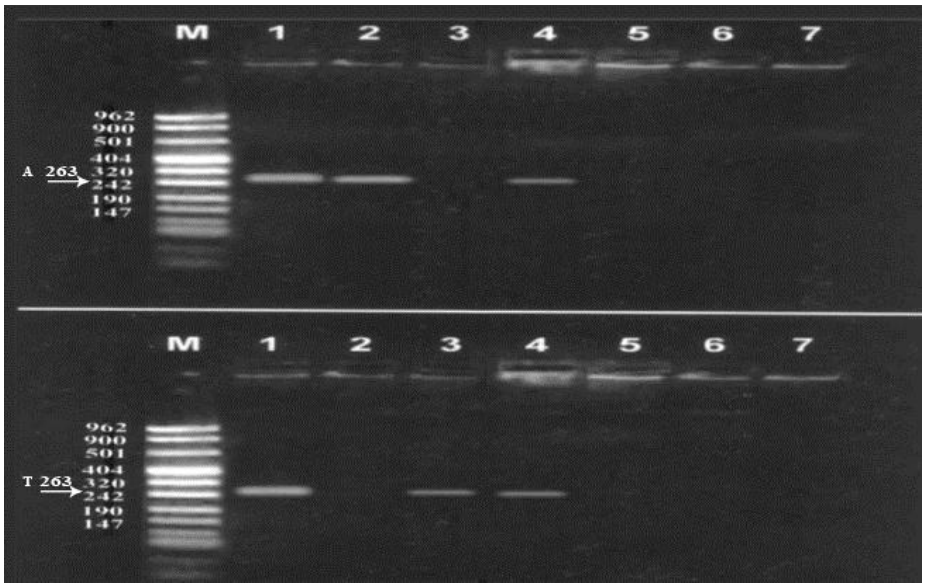

Fig (2): Agarose gel electrophoresis of IFN-γ **lane (m):** DNA marker, **Lane (1,4)** showing **AT genotype** , **Lane 2 : AA** genotype revealed A band in 263 bp gel(a, upper), **Lane3: TT** genotype with T band at 363 in gel (b, lower).

STATISTICAL ANALYSIS:

Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 17.0). The frequencies of studied genotypic and allelic polymorphisms among cases were compared to that of controls using Fisher's exact test and odds ratio (OR) with the 95% confidence interval (95% CI). Quantitative traits were compared using the Student t-test while nominal traits were compared using the Chi square test. Conformity with the Hardy Weinberg law of genetic equilibrium was assured by a non-significant Chi square test comparing the observed Vs the expected genotypes among studied cases and controls. A minimum level of statistical significance was considered at a *p* level of <0.05.

RESULTS

The present study was conducted on 125 patients (72 females and 53 males) suffering from SLE having nephritis (persistent proteinuria > 500 mg/ 24 hours (or > 3+) or cellular casts), their mean age was 23.4± 4.4 years. Demographic data, clinical manifestations, duration of symptoms, were shown in table 1. Control group consisted of 112 age matched healthy (46 males and 66 females) individuals from the same area with patients, their mean age was 28.3 ± 6.2 years.

The frequencies of the genotypes of -308 G/A of TNF-α in the SLE nephritis and control groups are shown in Table 2. The frequencies of TNF-α genotypes were in agreement with Hardy-Weinberg equilibrium in both SLE nephritis and control groups (p>0.05). Using G allele as reference, the A allele was associated with a significantly elevated OR of 3.2 (95% CI =2.15 -5.01, P = 0.003) in SLE cases associated with lupus nephritis (**Table 2**). Using the GG genotype as the reference genotype, the AA genotype, although, it was associated with a significantly elevated OR 5.33, it was statistically insignificant (95% CI = 0.61-46.5, P 0.12). However, addition of genotypes (AA plus GA) versus GG was associated with a significant increase in SLE having nephritis compared to controls (OR = 2.51, 95% CI = 1.26-4.99, p=0.009) **table 2**.

The frequencies of the genotypes of IFN-γ +874 A/T in the SLE nephritis and control groups were shown in Table 3 and were in agreement with Hardy-Weinberg equilibrium in both SLE nephritis and control groups (*p*>0.05). Using A allele as reference, the T allele was associated with insignificant increased risk of SLE having nephritis (OR 1.43, 95% CI =0.93-2.20, = 0.13). Also, using the AA genotype as a reference, TT genotype was associated with insignificant increased risk of SLE having nephritis (OR 1.15, 95% CI =0.38-3.45, p= 1.0). On the other hand, genotype AT was associated with increased association of SLE having nephritis, OR 2.06, CI= 1.18-3.62, p= 0.01. The genotype of AT versus combined genotypes (AA+TT) was associated with increased OR 2.04, CI, 1.17-3.54, p= 0.01) in patients with SLE having lupus nephritis.

Table 1: Clinical characteristics of SLE patients with lupus nephritis (125 patients

Gender -Females (n, %) -Males (n, %)	 72/125, (57.6%) 53/125, (42.4%)
Age at diagnosis (years)	23.4±4.4
Duration of the disease (months)	30.6±2.6
ACR criteria (%)	
Malar rash	67/125, (53.6%)
Discoid rash	39/125, (31.2%)
Photosensitivity	61/125, (48.8%)
Oral ulcers	29/125, (23.3%)
Arthritis	37/125, (29.6%)
Serositis	25/125, (20%)
CNS involvement	39/125, (31.2%)
Hematological abnormalities	61/125, (48.8%)

Results were expressed as number and frequency

Table 2: Allelic and genotype frequencies of the -308 G/A of TNF-α in the SLE nephritis and control groups.

	SLE nephritis (n= 125)	Control (n=112)	OR	95 %CI	P
Alleles					
G	212 (84.8%)	209 (93.3%)	2.50	1.33-4.68	0.003**
A	38 (15.2%)	15 (6.7%)			
Genotypes					
GG	92 (73.6%)	98 (87.5%)			
GA	28 (22.4%)	13 (11.6%)			
AA	5 (4.0%)	1 (0.9%)			
AA vs. GG			5.33	0.61-46.5	0.12
AA+GA vs. GG			2.51	1.26-4.99	0.009**
AA vs. (GG+GA)			4.63	0.53-40.21	0.22

Table 3: Allelic and genotype frequencies of the genotypes of IFN-γ +874 A>T polymorphisms in the SLE nephritis and control groups.

	SLE nephritis (n= 125)	Control group (n=112)	OR	95 %CI	P
Alleles					
A	184 (73.6%)	179 (79.9 %)	1.43	0.93-2.20	0.13
T	66 (26.4%)	45 (20.1%)			
Genotypes					
AA	66 (52.8%)	76 (67.9%)			
AT	52 (41.6%)	29 (25.9%)			
TT	7 (5.6%)	7 (6.2%)			
TT vs. AA			1.15	0.38-3.45	1.0
AT vs. AA			2.06	1.18-3.62	0.01*
AT vs. AA+TT			2.04	1.17-3.54	0.01*

DISCUSSION

Systemic lupus erythematosus is a multifactorial disease caused by interaction of genetic risk factors and environmental events that lead to disease initiation and progression, which can affect many organs. Glomerulonephritis, which leads to severe persistent proteinuria, chronic renal failure, and end-stage renal disease, remains one of the most severe complications of SLE and is associated with significant morbidity and mortality [3,5]. The levels of various cytokines have been found elevated in SLE patients; so they have been considered essential elements in the etiology of the disease. TNF-α is a pro-inflammatory cytokine involved in the severity of different immune-regulated diseases including autoimmune, infectious, and malignant diseases [23]. IFN-γ is a signature cytokine of the Th1 subset of helper T cells. Several studies have indicated that alterable expression of the IFN-γ level has significant effects to the susceptibility of various autoimmune diseases, particularly in SLE [14, 15].

TNF-α -308 G/A in SLE having Lupus nephritis:

The current study analyzes the association of the TNF-α -308 G/A SNP with SLE nephritis in 125 Egyptian patients with SLE having LN. The presence of a single copy of the A allele of TNF-α -308 G/A SNP was associated with a significantly elevated OR of 3.2 (95% CI =2.15 - 5.01, P = 0.003) in SLE cases associated with lupus nephritis, the A allele behaves as a dominant variant, the present results were in agreement with those reported by others [10,11]. The transition of guanine (G) to adenine (A) at position -308 directly affects gene regulation and has been associated with altered transcriptional activity of TNF-α in various disorders. The adenine (A) allele has been reported to be a stronger transcriptional activator in vitro than the common guanine (G) allele, However, in the case of homozygous genotype (AA) for the TNF-α -308 G/A SNP, there was no significant difference, which may be due to a low frequency of this genotype in the study population (5 cases, 4%). On the other hand, addition of genotypes (AA plus GA) in 33 cases versus GG was associated with a significant increase in SLE having nephritis compared to controls (OR = 2.51, 95% CI = 1.26-4.99, p=0.009).

The TNF gene polymorphism at position -308 have been demonstrated to influence TNF expression and results in differential binding of nuclear factors, leading to six to seven fold increase in the inducible level of TNF gene transcription[25].

The present results were in agreement with the previously reports in Colombian[26], Mexican [14], North American [27], Spanish [15], Asian [28] and Caucasian [29] populations. However the results were not in line with that reported in other different ethnic groups as Japanese[13], Italian[30], Chinese [31], Thai [32] and Portuguese[11] populations.

IFN-γ +874 A/T with SLE nephritis:

The present study analyzes the association of the IFN-γ +874 A/T SNP with SLE nephritis in Egyptian population. Up to the best of our knowledge, this is the first report on

IFN-γ +874 A/T SNP with SLE having LN. The results of this study demonstrated that, T allele was not associated with increased risk of SLE having nephritis (OR 1.43, 95% CI =0.93-2.20, p =0.13). Also, using the AA genotype as a reference, TT genotype was associated with insignificant increased risk of SLE having nephritis (OR 1.15, 95% CI =0.38-3.45, p =1.0). These results may be due to low frequency of TT genotypes in the study population versus the control group. On the other hand, the genotype AT was associated with increased association of SLE having nephritis (p= 0.01). A single nucleotide polymorphism (SNP) located in the first intron of the human IFN-γ gene at the 50 end, adjacent to a CA repeat region (+874 A/T polymorphism, rs2430561), can influence the secretion of IFN-γ[19]. Analysis of the biological role of this SNP suggested that +874 A allele carriers are low IFN-γ producers [33]. However, the T allele of this SNP is associated with the high IFN-γ production [19]. Interestingly, several SNPS in IFN are associated with SLE susceptibility, and the risk allele of an associated SNP (rs2430561) located in an NF-kB binding site has elevated IFN expression versus the non risk allele, supporting that elevated IFN expression is associated with increased SLE susceptibility[34].

In addition, the positive association was found between the amino acid polymorphism (Val 14 Met) within the IFN-γ receptor 1 gene and SLE[19,33]. These findings support the suggestion that the enhanced production of IFN-γ by mononuclear cells may trigger inflammatory responses [35].

Both IFNγ and TNFα are pleiotrophic cytokines that play often critical roles in inflammatory reactions, both cytokines independently exert a number of biological activities in a cell type-specific fashion, they have been shown in many circumstances to function cooperatively or antagonistically in controlling expression of a variety of cytokines and cell surface molecules[36]. The signal transduction of IFN-γ/TNF-α synergism may be relevant to the pathophysiology of many autoimmune diseases as SLE having lupus nephritis. The limitations of the present study were small number of cases, cases were collected from single institution, and a hospital based not a community based study. We advise a large multicenter study to confirm our findings.

In conclusion, from this study, gene polymorphisms related to TNF-α308 G/A and IFN-γ +874 A/T may play an important role in the susceptibility and pathogenesis of SLE having LN among Egyptian patients.

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REFERENCES

1. **Ardoin SP, Pisetsky DS(2008):** Developments in the scientific understanding of lupus. Arthritis Res. Ther .,10(5):218–25.
2. **Sánchez E, Sabio JM, Callejas JL, de Ramón E, Garcia-Portales R, García-Hernández FJ, Jiménez-Alonso J, González-Escribano MF, Martín J, Koeleman BP.(2006):** Association study of genetic variants of pro-inflammatory chemokine and cytokine genes in systemic lupus erythematosus. BMC Med. Genet., 7:48- 55.
3. **Sestak AL, Nath SK, Sawalha AH, Harley JB.(2007):** Current status of lupus genetics.Arthritis Res. Ther. ,9(3):210–9.
4. **Hiraki LT, Feldman CH, Liu J, Alarcón GS, Fischer MA, Winkelmayer WC, Costenbader KH.(2012):** Prevalence, incidence, and demographics of systemic lupus erythematosus and lupus nephritis from 2000 to 2004 among children in the US Medicaid beneficiary population. Arthritis Rheum. ,64(8):2669-76.
5. **Pons-Estel GJ, Serrano R, Plasín MA, Espinosa G, Cervera R.(2011):** Epidemiology and management of refractory lupus nephritis. Autoimmun.Rev., 10 (11):655-63.
6. **Wakeland EK, Liu K, Graham RR, Behrens TW. (2001)**: Delineating the genetic basis of systemic lupus erythematosus. Immunity. ,15(3):397-408.
7. **Walport MJ, Black CM, Batchelor JR.(1982):** The immunogenetics of SLE. Clin Rheum Dis. ,8(1):3-21.
8. **Lin YJ, Chen RH, Wan L, Sheu JC, Huang CM, Lin CW, Chen SY, Lai CH, Lan YC, Hsueh KC, Tsai CH, Lin TH, Huang YM, Chao K, Chen DY, Tsai FJ. (2009):** Association of TNF-alpha gene polymorphisms with systemic lupus erythematosus in Taiwanese patients. Lupus 18(11):974–9.
9. **Aggarwal BB, Gupta SC, Kim JH.(2012**): Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood 119(3):651-65.
10. **Zou YF, Feng XL, Tao JH, Su H, Pan FM, Liao FF, Fan Y, Ye DQ (2010)**: Meta-analysis of TNF-α promoter -308A/G polymorphism and SLE susceptibility in Asian populations. Rheumatol. Int., 31(8):1055–64
11. **Santos MJ, Carmona-Fernandes D, Caetano-Lopes J, Perpétuo IP, Vidal B, Capela S, Canas da Silva J, Fonseca JE. (2011):**TNF promoter −308 G>A and LTA 252 A> G polymorphisms in Portuguese patients with systemic lupus erythematosus. Rheumatol. Int., 32(8): 2239 – 44.
12. **Zúñiga J, Vargas-Alarcón G, Hernández-Pacheco G, Portal-Celhay C, Yamamoto-Furusho JK, Granados J.(2001**): Tumor necrosis factor-alpha promoter polymorphisms in Mexican patients with systemic lupus erythematosus (SLE). **Genes Immun ., 2(7):363–6.**

13. **Takeuchi F, Nakano K, Nabeta H, Hong GH, Kawasugi K, Mori M, Okudaira H, Kuwata S, Tanimoto K. (2005):** Genetic contribution of the tumor necrosis factor (TNF) B + 252*2/2 genotype, but not the TNFa,b microsatellite alleles, to systemic lupus erythematosus in Japanese patients**.** Int. J. Immunogenet., 32(3):173-8.
14. **Jiménez-Morales S, Velázquez-Cruz R, Ramírez-Bello J, Bonilla-González E, Romero-Hidalgo S, Escamilla-Guerrero G, Cuevas F, Espinosa-Rosales F, Martínez-Aguilar NE, Gómez-Vera J, Baca V, Orozco L. (2009**): Tumor necrosis factor-alpha is a common genetic risk factor for asthma, juvenile rheumatoid arthritis, and systemic lupus erythematosus in a Mexican pediatric population**.** Hum. Immunol., 70(4): 251–6.
15. **Suárez A, López P, Mozo L, Gutiérrez C.(2005**): Differential effect of IL10 and TNF{alpha} genotypes on determining susceptibility to discoid and systemic lupus erythematosus. Ann.Rheum. Dis., 64(11): 1605-10.
16. **Silva JS, Morrissey PJ, Grabstein KH, Mohler KM, Anderson D, Reed SG.(1992):** Interleukin 10 and interferon gamma regulation of experimental Trypanosoma cruzi infection. J. Exp. Med., 175(1):169-174.
17. **Torrico F, Heremans H, Rivera MT, Van Marck E, Billiau A, Carlier Y.(1991):**Endogenous IFN-gamma is required for resistance to acute Trypanosoma cruzi infection in mice. J. Immunol., 146(10):3626-32.
18. **Chong WP, Ip WK, Tso GH, Ng MW, Wong WH, Law HK, Yung RW, Chow EY, Au KL, Chan EY, Lim W, Peiris JS, Lau YL.(2006)**: The interferon gamma gene polymorphism +874 A/T is associated with severe acute respiratory syndrome. BMC Infect. Dis., 6:82- 85.
19. **Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. (2000):** A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum. Immunol., 61(9):863-6.
20. **Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. (1982):** The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum., 25(11):1271–7.
21. [21**]Hochberg MC (1997)**: Updating the American College of Rheumatology revised criteria for the classification of SLE. Arthritis Rheum., 40**(9)**: 1725-8.
22. **Schur BC, Bjereke J,Nuwayhid N and Wong SH (2001):** Genotyping of cytochrome p450 2D6*3 and *4 mutations using conventional PCR. Clin. Chim. Acta 308(1-2):25-31.
23. **Tronchon V, Vialard F, El Sirkasi M, Dechaud H, Rollet J, Albert M, Bailly M, Roy P, Mauduit C, Fenichel P, Selva J,**

Benahmed M.(2008): Tumor necrosis factor-alpha -308 polymorphism in infertile men with altered sperm production or motility. Hum. Reprod., 23(12):2858-66.

24. **Zhu KY& Clark JM(1996)**: Addition of a competitive primer can dramatically improve the specificity of PCR amplification of specific alleles. Biotechniques 21(4):586. 90.
25. **Wilson AG, Symons JA, Mcdowell TL, McDevitt HO, and Duff GW (1997):** Effect of polymorphism in the tumor necrosis factor-alpha (TNF-α) promoter on transcriptional activation. Proc. Natl. Acad. Sci.USA 94(7):3195-9.
26. **Guarnizo-Zuccardi P, Lopez Y, Giraldo M, Garcia N, Rodriguez L, Ramirez L, Uribe O, Garcia L, Vasquez G (2007):** Cytokine gene polymorphisms in Colombian patients with SLE. Tissue Antigens 70**(5)**: 376–82.
27. **Parks CG, Pandey JP, Dooley MA, Treadwell EL, St Clair EW, Gilkeson GS, Feghali-Bostwick CA, Cooper GS. (2004):** Genetic polymorphisms in tumor necrosis factor (TNF)-alpha and TNF-beta in a population-based study of systemic lupus erythematosus: associations and interaction with the interleukin-1alpha-889 C/T polymorphism. Hum. Immunol., 65(6):622–31.
28. **Lin YJ, Chen RH, Wan L, Sheu JC, Huang CM, Lin CW, Chen SY, Lai CH, Lan YC, Hsueh KC, Tsai CH, Lin TH, Huang YM, Chao K, Chen DY, Tsai FJ. (2009):** Association of TNF-alpha gene polymorphisms with systemic lupus erythematosus in Taiwanese patients. Lupus 18(11):974–9.
29. **van der Linden MW, van der Slik AR, Zanelli E, Giphart MJ, Pieterman E, Schreuder GM, Westendorp RG, Huizinga TW. (2001):** Six microsatellite markers on the short arm of chromosome 6 in relation to HLA-DR3 and TNF-308A in systemic lupus erythematosus. Genes Immun., 2(7):373–80.
30. **D'Alfonso S, Colombo G, Della Bella S, Scorza R, Momigliano-Richiardi P.(1996):** Association between polymorphisms in the TNF region and systemic lupus erythematosus in the Italian population. Tissue Antigens 47(6):551-5.
31. **Fong KY, Howe HS, Tin SK, Boey ML, Feng PH.(1996);** Polymorphism of the regulatory region of tumour necrosis factor alpha gene in patients with systemic lupus erythematosus. Ann. Acad. Med. Singapore 25(1):90-3.
32. **Hirankarn N, Avihingsanon Y, Wongpiyabovorn J.(2007):** Genetic susceptibility to SLE is associated with TNF-alpha gene polymorphism -863, but not -308 and -238, in Thai population. Int. J. Immunogenet., 34(6):425–30.
33. **López-Maderuelo D, Arnalich F, Serantes R, González A, Codoceo R, Madero R, Vázquez JJ, Montiel C.(2003):** IFN-γ and IL-10 gene polymorphisms in pulmonary tuberculosis. Am. J.

Respir. Crit. Care Med., 167(7):970-5.

34. **Kim K, Cho SK, Sestak A, Namjou B, Kang C, Bae SC. (2009):** Interferone –gamma gene polymorphism increased susceptibility to systemic lupus erythematosus. Ann Rheum Dis. 69(6):1247-50.
35. **Baccala R, Kono DH, (2005):** The ofilopoulos AN. Interferons as pathogenic effectors in autoimmunity. Immunol. Rev., 204:9-26.
36. **Ohmori Y, Schreiber RD, Hamilton TA (1997):** Synergy between interferon gamma, and tumor necrosis factor-alpha in transcriptional activation is mediated by cooperation between signal transfer and activator of transcription 1 and nuclear factor Kappa B. J. Biol. Chem., 272(23):14899-907.

التعدد الجيني لجين محفز التنقرز الورمي -الفا والانترفيرون -جاما في التهاب الكلي الناتج عن مرض الذئبة الحمراء في المرضي المصريين

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الاصابة بمرض الذئبة الحمراء قد يكون له خلفية جينية، وقد سجلت دراسات عديده ارتباطا وثيقا بين محفزجين التنقرزالورمي -الفا والانترفيرون-جاما وهذا المرض الذي قد يؤدي الي الوفاة
يهدف هذا البحث إلى دراسة تردد النمط الوراثي لكل من محفز جين التنقرز الورمي -الفا والانترفيرون -جاما في المرضي الذين يعانون من التهاب في الكلي نتيجة مرض الذئبة الحمراء ودراسة ما اذا كان كل منهما من العوامل الخطرة المسببة لهذا المرض.
تم اجراء هذه الدراسة على ١٢٥ مريضا يعانون من التهاب الكلي نتيجة مرض الذئبة الحمراء و١١٢ شخص غير مصاب بالمرض كمجموعة ضابطة متطابقة بالنسبة للعمرمع المرضي.
تم استخلاص االحامض النووى من الدم الوريدى من جميع الحالات والضوابط .وأجرى تحليل الحامض النووى بطريقة البلمرة المتسلسلة كما تم دراسة التعدد الجينى لكل الحالات بطريقة ARMS PCR
الإستنتاج: استخلص من هذا البحث أن التعدد الجينى لانزيم محفز التنقرز الورمي- الفا والانترفيرون- جاما في المرضى المصابين بالتهاب الكلي نتيجة مرض الذئبة الحمراء يلعب دورا واضحا فى منشا هذا المرض.

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