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. Control 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.

INTRODUCTION

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. 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±6.2 years. 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-citrate tubes and were delivered to centrifuge tubes containing K$_2$DTA (stored as EDTA anti-coagulated blood sample at -30°C 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'-TCTCGGTTCCTTCTCCATCG) was used with either 308-G (5'-ATAGGTTTGAGGGGCATGG) or 308-A (5'-ATAGGTTTTGAAGGGCATGA) 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'-GAGTCTCCGGGTCAGATGA)/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](image)

**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)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Females (n, %)</th>
<th>Males (n, %)</th>
<th>Age at diagnosis (years)</th>
<th>Duration of the disease (months)</th>
<th>ACR criteria (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>72/125, (57.6%)</td>
<td>53/125, (42.4%)</td>
<td>23.4±4.4</td>
<td>30.6±2.6</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Count (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malar rash</td>
<td>67/125, (53.6%)</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>39/125, (31.2%)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>61/125, (48.8%)</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>29/125, (23.3%)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>37/125, (29.6%)</td>
</tr>
<tr>
<td>Serositis</td>
<td>25/125, (20%)</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>39/125, (31.2%)</td>
</tr>
<tr>
<td>Hematological abnormalities</td>
<td>61/125, (48.8%)</td>
</tr>
</tbody>
</table>

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.

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

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

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

Acknowledgement:
I would like to thank Dr, Dina Shahein, Assistant professor of Rheumatology, Department of Internal medicine, Mansoura University, Egypt, for her kind help in patient assessment.
REFERENCES


التعذد الجيني لجين محفز التنفرز الورمي -الفا والانترفيرون -جاما في التهاب الكلي الناتج عن مرض الذبابة الحمراء في المرضى المصريين

اموكامل سليم(1) -احمد ستين(2) -رفاء الخولي(3) - يلد فتحي(4) عفاف محمد(4)

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الإصابة بمرض الذبابة الحمراء قد يكون له خلفية جينية، وقد سجلت دراسات عديدة ارتباطاً وثيقاً بين محفز جين التنفرز الورمي -الفا والانترفيرون -جاما. هذا الوضع الذي قد يؤدي إلى الوفاة يعد هذا البحث إلى دراسة تردد التهابات الفيروسية لكل من محفز جين التنفرز الورمي -الفا والانترفيرون -جاما في المرضى الذين يعانون من التهاب في الكلي نتيجة مرض الذبابة الحمراء ودراسة ما إذا كان كل منهما من العوامل الحيوية المسببة لهذا الوضع.

تم إجراء هذه الدراسة على 125 مريضاً يعانون من التهاب الكلي نتيجة مرض الذبابة الحمراء و112 شخص غير مصاب بالمرض كمجموعة مضافة ملائمة بالنسبة للعمر والمرض. تم استخلاص الحمامات النووية من الدم الوريدي من جميع الحالات والضوابط، وأجري تحليل الحمامات النووية ARMS PCR بطريقة المراقبة المتسلسلة كما تم دراسة التعدد الجيني لكل الحالات بطريقة الاستنتاج: استخلصنا من هذا البحث أن التعدد الجيني لانزيم محفز التنفرز الورمي -الفا والانترفيرون -جاما في المرضى المصابين بالتهاب الكلي نتيجة مرض الذبابة الحمراء يلعب دوراً واضحاً في منشأ هذا الوضع.