DNase I Activity and Gene Polymorphism: Role in SLE Susceptibility and Auto-Antibody Production

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Abstract

Background: Previous studies have suggested that interrupted clearance of nuclear DNA-protein complexes after cell death might initiate and propagate systemic lupus erythematosus (SLE). Deoxyribonuclease I (DNase I) may be responsible for the removal of DNA from nuclear antigens at sites of high cell turnover, thus preventing the onset of SLE.

Objectives: To investigate the association of serum DNase I activity and single nucleotide polymorphism (SNP) +2373A>G (Gln244Arg) of DNase I gene with susceptibility to systemic lupus erythematosus (SLE) and the production of auto-antibodies to double-stranded DNA.

Subjects and Methods: A total of 42 SLE patients, all fulfilled the revised criteria of the American College of Rheumatology for the diagnosis of SLE, were enrolled in the study and 17 healthy individuals with matching age and sex as a control group. 27 out of the 42 SLE patients had lupus nephritis proved by renal biopsy. DNase I gene +2373A>G SNP was studied by polymerase chain reaction followed by restriction fragment length polymorphism analysis. Serum DNase I activity (measured as percent of activity reduction; %AR) and anti-double-stranded DNA (anti ds-DNA) level were determined by solid phase enzyme immunoassay (ELISA).

Results: There was a significant decrease in DNase I enzyme activity (increase %AR) in the sera of SLE patients compared to the healthy individuals (p=0.000). Anti ds-DNA antibody level was significantly higher in SLE patients compared to control group (p=0.000). There was a significant positive correlation between DNase I enzyme (%AR) and the level of anti ds-DNA antibody (r=0.596, p=0.000). Comparing the results of lupus patients with and without nephritis revealed an increase in both DNase enzyme %AR and the level of Anti ds-DNA antibody in the nephritis group but the difference is not statistically significant. There was no association of the +2373A>G SNP genotypes or alleles with SLE susceptibility. However, SLE patients with GG genotype showed significant increase in both DNase I %AR (p=0.007) and anti ds-DNA antibody level (p=0.022) than those with AG & AA genotypes.

Conclusion: The observed association of +2373A>G SNP of DNase I gene with DNase I activity and production of anti ds-DNA antibodies but not with SLE susceptibility calls into question how this SNP could contribute to SLE pathogenesis.

A wider scale study with special emphasis on other auto-antibodies and genetic polymorphisms is recommended.


Introduction

SYSTEMIC lupus erythematosus (SLE) is a complex autoimmune disease characterized by a broad spectrum of auto-antibodies directed to different intracellular antigens particularly against double-stranded DNA that are critically involved in tissue damage [1], so a clue to understanding the pathogenesis of SLE may come from unraveling the mechanisms involved in the generation of anti ds-DNA and other auto-antibodies.

As many complex human diseases, SLE involves multiple, interacting genetic and environmental determinants and identifying genes and enzymes for complex traits is challenging and has had limited success so far [2]. Researchers have long suspected a strong genetic predisposition to the development of systemic lupus erythematosus. Most genes identified as having a role in SLE however, have been associated with the immunologic response (e.g., major histo-compatibility complex class II genes and genes involved in tumor necrosis factor and interleukin regulation) [3]. The identified etiologic genes determine susceptibility, and no particular gene is necessary or sufficient for disease expression [2].

The dominant auto-antigen in SLE is the nucleosome and immune complexes involving nucleosomes are the major cause of tissue damage. Today, there is a mounting body of evidence that apoptotic cells are the source of auto-antigen in SLE, a defect in apoptosis regulation or apoptotic cell clearance has been suggested to be involved in lupus development [4].
The process of apoptosis contains a series of pathways leading to the regulated removal of unwanted cells and induction of tolerance for auto-antigens through the deletion of auto reactive T- and B-cells. This process is strictly regulated. In particular, the adequate removal of early apoptotic cells is of great importance. Since nuclear auto-antigens become clustered in apoptotic blebs, an impaired removal could lead to the release of nuclear structures which could then induce an immune response to these auto-antigens leading to the production of auto-antibodies [4]. Furthermore, phagocytosis of apoptotic cells may lead to a pro-inflammatory response in the presence of auto-antibodies. This may sustain inflammatory conditions and the pathology found in overt lupus [8]. Chromatin, a complex of proteins and double-stranded DNA, is an auto-antigen that is clustered in apoptotic blebs. Auto-antibodies against chromatin including anti ds-DNA antibodies are a hallmark of SLE. Moreover, the formation of anti-chromatin/chromatin complexes can lead to the binding of these complexes to basement membranes, including the glomerular basement membrane (GBM). This binding induces inflammation, which leads to lupus nephritis, the most serious manifestation of SLE [6].

The removal of DNA from nucleosomes released during cell death is thought to be mediated predominantly by DNase I, the major nuclease present in the serum and urine of both humans and mice, thus reduction in DNase I activity contributes to the accumulation of nucleosomal complexes, which in turn may become an antigenic stimulus. Anti ds-DNA antibodies are intimately associated with SLE and their presence has both diagnostic and prognostic significance [7]. This finding is not only important in understanding the pathogenesis of SLE, but also suggests that detection of high risk cases may be possible. Early diagnosis may also allow treatment with compounds containing DNase I as a useful adjunct to conventional treatment with immunosuppressive drugs [8].

The reasons for the reduced DNase I enzyme activity have not been determined, mutations in human DNase I may predispose individuals to SLE [9]. However, mutations affecting its function that have been described in several SLE patients seem to account for only a small proportion of the SLE subjects with very low DNase I activity [8,10,11]. Some studies suggest that DNase-inhibitory antibodies may be responsible for the decreased DNase I activity in SLE patients [12].

The DNase I gene maps to human chromosome 16 p13.3. This gene covers 46 298bp and is composed of 9 exons [13]. Common polymorphisms in DNase I affecting SLE susceptibility or phenotype had not been described until a study in Korean patients was carried out [7]. In this study the exon 8 +2373A>G SNP was associated with production of anti-ribonucleoprotein (RNP) and anti ds-DNA antibodies but not with SLE susceptibility. Other studies found this SNP not to be associated with decreased DNase I activity [14,15]. Therefore; it is unclear how this SNP could contribute to auto-antibody production [7].

So, the aim of this study was to investigate the association of serum DNase activity and +2373A>G SNP of DNase I gene with susceptibility to SLE and the production of anti ds-DNA antibodies. The relation between DNase activity and renal involvement in SLE patients was also investigated.

**Subjects and Methods**

A total of 42 female SLE patients were enrolled in the study, all fulfilled the revised criteria for SLE of the American College of Rheumatology (ACR) [16]. Their age ranged from 13-45 years (mean ± SD =26.11 ± 7.80). Patients were recruited from the outpatient clinics and inpatients of the departments of internal medicine and rheumatology & rehabilitation in Kasr El-Aini Hospital, Cairo University. A control group of 17 healthy individuals with matching age and sex distribution were included in the study (all females; mean age ± SD, 29.48 ± 8.70 years). Patients were subjected to thorough history taking, clinical examination and routine laboratory investigations. Systemic involvement was assessed clinically and confirmed by special tests when indicated. Patients with active urinary sediments or abnormal kidney functions were subjected to renal biopsy. SLE patients were divided into 2 groups (group with & group without lupus nephritis) 27 out of the 42 patients had lupus nephritis proved by renal biopsy [17].

Blood samples were obtained from both patients and controls; sera were stored in aliquots at –20°C for up to 1 month. EDTA samples were obtained for DNase I Genotyping. Extracted DNA was stored at –20°C until analyzed for no more than 2 months before analysis.

Anti ds-DNA antibody level was measured by an enzyme-linked immunosorbent assay (ELISA; QUANTA Lite, INOVA diagnostics, San Diego, USA), with values <200IU/ml were considered negative, according to the instructions of the manufacturer [18].
Serum DNase I activity was also measured by an ELISA (Orgentec Diagnostica GmbH, Mainz, Germany). Briefly, DNase enzyme from patients’ sera was allowed to react with the specific substrate coated onto the plate during incubation at 37 °C for an hour, followed by the addition of horseradish peroxidase-conjugated antibodies to the residual DNase substrate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is inversely proportional to the DNase activity. A standard series was included with the kit. DNase activity was measured as percent of activity reduction (%AR) [10].

Genotype analysis:

Genomic DNA was extracted from peripheral blood leukocytes by standard Salting out technique [19]. The +2373 A→G SNP was genotyped by polymerase chain reaction (PCR)–restriction fragment length polymorphism analysis [20]. Primers were designed to include two potential restriction sites in the amplified fragment: The site with the SNP and an invariant site, which was used as an internal control for the enzymatic digestion. Amplification was carried out with the primer pair 5’-TCCAAGGGCACGCGTGACCTC-3’ (forward) and 5’-TCCTGTCGAGGATGGGTCC -3’ (reverse). The PCRs were performed in a total volume of 10 µL containing 50 ng of genomic DNA, 200 mM of dNTPs, 0.1 mM of each primer, 1 U of Taq DNA polymerase, 1.5 mM MgCl2 and 1x PCR buffer. Cycling conditions were as follows: Initial denaturation at 94°C for 4min followed by 30 cycles of denaturation at 94 °C for 1min, annealing at 64 °C for 1 min and extension at 72 °C for 1 min. Final elongation was done at 72°C for 10min. The digestions were done with 3.5 U of StyI and the fragments were separated by electrophoresis in 3% agarose gels in TBE. The size of PCR fragment was 629bp. After digestion, we obtained two fragments 166, 196 & 267bp in the allele A.

Statistical methods:

The SPSS 10.0 for windows was used for data management and analysis and the Microsoft power point for charts. Quantitative data were presented as mean ± SD, non parametric quantitative data were expressed as median (quartile range). Mann-whitney test was used for comparison of medians. Qualitative data was expressed as frequency and percentage. Spearman correlation coefficient (r) was used to correlate between quantitative vari-
ables. Risk estimate was done by odds ratio. p value was considered significant at 0.05.

Results

The results showed a significant decrease in DNase I enzyme activity in the sera of SLE patients compared to the healthy individuals. Median DNaseI %AR was 23.15 [interquartile range (IQR) 15.73-28.85] in SLE patients, which was significantly higher than that observed in healthy controls (median 10.40, IQR 9.15-13.85; p=0.000). Anti ds-DNA antibody level was significantly higher in SLE patients compared to control group (median 310.35, IQR 130.73-1251.75 Vs 25.80, IQR 022.70-35.30; p=0.000) (Table 2). There was a statistically significant positive correlation between DNase enzyme (%AR) & the anti ds-DNA antibody level (r=0.596, p=0.000) (Fig. 1). Comparing the results of lupus patients with and without nephritis revealed an increase in both DNase enzyme %AR & the level of Anti ds-DNA antibody in the nephritis group but the difference was not statistically significant with p=0.396, 0.102 respectively (Table 3).

Both DNase +2373A>G genotypes and alleles were not associated with SLE susceptibility, OR= 2.10 and 1.87; 95% CI 0.67-6.6 and 0.78-4.45 with p=0.200 and 0.157 respectively (Table 4). SLE patients with +2373 GG genotype showed significant increase in both DNase enzyme %AR & the anti ds-DNA antibody level than those with AG & AA genotypes (Figs. 2,3).

Table (1): Demographic, clinical and laboratory characteristics of SLE group (n=42).

| Age (years) | 26.11±7.80 |
| Malar rash | 31/42 (73.8%) |
| Photosensitivity | 27/42 (64.3%) |
| Oral ulcers | 25/42 (59.5%) |
| Arthritis | 37/42 (88.1%) |
| Serositis | 26/42 (61.9%) |
| Fever | 27/42 (64.3%) |
| Cerebritis | 6/42 (14.3%) |
| Nephritis | 27/42 (64.3%) |
| Hb g/dl | 9.21±1.96 |
| Ptt 10^3/mm³ | 253.60±104.88 |
| TLC 10^3/mm³ | 7.87±4.9 |
| ESR mm/hr | 91.5±36.89 |

Values are given as mean ± SD or as frequency (percentage).

Table (2): Comparison of DNase (%AR) & Anti ds-DNA antibodies between SLE patients and control group.

<table>
<thead>
<tr>
<th></th>
<th>SLE group (n=42)</th>
<th>Control group (n=17)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNase (%AR)</td>
<td>23.15 (15.73-28.85)</td>
<td>10.40 (9.15-13.85)</td>
<td>0.000</td>
</tr>
<tr>
<td>Anti ds-DNA (IU/ml)</td>
<td>310.35 (130.73-1251.75)</td>
<td>25.80 (22.70-35.30)</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Table (3): Comparison of DNAse (%AR) & Anti ds-DNA antibodies between SLE patients with and without nephritis.

<table>
<thead>
<tr>
<th></th>
<th>SLE patients with nephritis (n=27)</th>
<th>SLE patients without nephritis (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNase (%AR)</td>
<td>23.15 (18-29.2)</td>
<td>20.25 (15.2-30.6)</td>
<td>0.396</td>
</tr>
<tr>
<td>Anti ds-DNA (IU/ml)</td>
<td>374.25 (190.87-1515.25)</td>
<td>224.5 (102.4-572.9)</td>
<td>0.102</td>
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</tbody>
</table>

Table (4): Genotype and allele frequencies of the +2373 A→G SNP in SLE patients and controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SLE (n=42)</th>
<th>Controls (n=17)</th>
<th>Odds ratio (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>25 (59.5%)</td>
<td>7 (41.18%)</td>
<td>2.10 (0.67-6.6)*</td>
<td>0.200</td>
</tr>
<tr>
<td>AG</td>
<td>15 (35.7%)</td>
<td>8 (47.06%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>2 (4.8%)</td>
<td>2 (11.76%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>65 (77.4%)</td>
<td>22 (64.7%)</td>
<td>1.87 (0.78-4.45)</td>
<td>0.157</td>
</tr>
<tr>
<td>A</td>
<td>19 (22.6%)</td>
<td>12 (35.3%)</td>
<td></td>
<td></td>
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</table>

*GG Vs AA & AG.

Discussion

Systemic lupus erythematosus is an autoimmune disease of unknown etiology with a complex genetic basis. The formation of anti-nuclear autoantibodies is the main feature of the autoimmune disease systemic lupus erythematosus (SLE). DNase 1 has been implicated in the pathophysiology of SLE since the 1950s. The importance of DNase 1 has grown up since the description that apoptotic cells can be the source of self-antigens in SLE. The enzyme DNase 1 plays a role in the clearance of apoptotic debris and is therefore of capital interest in this process [21]. Several studies point to defective DNase I as an important player in development of SLE and lupus nephritis [22,23].

The results of this study showed that DNase I enzyme activity is significantly decreased (higher %AR) in the sera of SLE patients compared to those of healthy individuals (p=0.000). This confirms previous reports [11,23-26], which reported that serum DNase I activity is decreased in patients with SLE. The reasons for the reduced enzyme activity have not been determined. Mutation in the DNase1 gene has been suggested but discrepancy between the results was great [10]. While many DNase I single-nucleotide polymorphisms (SNPs) were found, there was no evidence suggesting a functional role for them [20]. Also, there are conflicting reports on an inhibitor responsible for the reduced DNase activity, some suggested the presence [12] and others suggest the absence [24,27] of such a factor, thought to be an antibody [12].

The anti ds-DNA antibody level was significantly higher in SLE patients and showed a statis-
tically significant positive correlation with the %AR of DNase enzyme activity ($r=0.596, p=0.000$) such a result supports the etiological role of decreased DNase activity in the autoantibody production, this is in agreement with the results of Yasutomo et al. [10], Bodaño et al. [11] and Sallai et al. [25] who reported a significant negative correlation of anti ds-DNA antibodies with DNase enzyme activity in SLE patients.

Because the antibodies to dsDNA were associated with renal manifestation of SLE and anti ds-DNA antibodies have been eluted from affected glomeruli [28] we compared the DNase enzyme (%AR) in the SLE patients with & without nephritis and we found a non-significant increase in DNase enzyme (%AR) in the nephritis group. The previous results were contradictory, with the study done by Napirei et al., who suggested that the prevalence of glomerulonephritis is increased in DNase1-deficient mice [9] while Sallai et al. [25] stated that the DNase activity in the group of SLE patients without renal involvement did not differ significantly from those in individuals with active or with a history of glomerulonephritis.

As regards DNase $+2373A>G$ SNP in DNase I gene, (we found that both genotypes & alleles are not) associated with SLE susceptibility, OR= 2.10 and 1.87; 95% CI 0.67-6.6 and 0.78-4.45 with $p=0.200$ and 0.157 respectively. Similar results were reported by shin et al. [7]. In contrast, other studies reported that some common polymorphism of DNaseI (such as the $+2373A>G$ SNP) are associated with SLE susceptibility [20,23].

In this study, SLE patients with $+2373 GG$ genotype showed significant increase in both DNase %AR & anti ds-DNA antibody level than those with AG & AA genotypes. In partial agreement with our results, Shin et al. [7] reported that this SNP was significantly associated with an increased risk of the production of anti-RNP and anti ds-DNA antibodies but not with decreased DNase I activity among SLE patients. In another study the $+2373A>G$ genotype did not correlate with DNase I activity in sera from SLE patients or influence auto-antibody titres significantly [20]. Previous studies with DNase I electrophoresis phenotypes also indicated that the $+2373A>G$ SNP is irrelevant for enzymatic activity [14,15].

The observed discrepancy between different studies could be related either to heterogeneity between the populations or to technological differences in antibody determination; ELISA was used to determine antibodies in some studies [20,25]. whereas Crithidia luciliae and double immunodiffusion in others [7]. Indeed, clinical heterogeneity of SLE between different ethnic groups is a well-known phenomenon [7,20].

In conclusion, the results of this study contribute to establishing the deficiency in DNase I activity as one of the defects in the clearance of cellular debris involved in the pathogenesis of SLE. Also significant association of $+2373A>G$ SNP of DNase I gene with DNase I activity and production of anti ds-DNA antibodies was found, but not with SLE susceptibility. However the etiology of diminished serum DNase I activity in SLE is complex and multifactorial and cannot be accounted for simply by gene mutations alone. DNase activity may be influenced by post-translational or tissue-specific mechanisms. Further work up for better understanding of the role of DNase I in SLE and its relation to lupus nephritis is recommended to allow for proper evaluation of DNase I in therapeutic strategies or as a prognostic indicator for the development of SLE.

References


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50


