Mutational Analysis of DNAse I Gene in Pakistani SLE Patients

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Abstract
Systemic Lupus Erythematosus (SLE) is one of the classic examples of autoimmune diseases among human beings and is a rare disease in Pakistani population. Clinically, it is a quite diverse and complicated autoimmune disease in a sense that it involves multiple organs of the body and mimics other diseases as well. Mutational analysis of DNAse I gene exon 8 is performed because most of the mutations were reported in these regions with reference to SLE. Genes were amplified by Step-down PCR technique in order to get the bands of our interest. Extracted DNA bands were sequenced to analyze mutations. Thirteen SLE samples showed a missense mutation in DNAse I gene. The codon CAA changed to CGA (+2373 A>G) so the glutamine amino acid was replaced by arginine at position 244 of Deoxyribonuclease I protein.

Keywords: Systemic lupus erythematosus; Deoxyribonuclease gene I; Polymerase chain reaction

Introduction
Systemic Lupus Erythematosus (SLE) is an autoimmune disorder of unknown etiology with clinical presentations of almost all organs and tissues. Even some investigators referred it as a syndrome due to its heterogeneity and broad spectrum clinical pictures [1]. Deoxyribonuclease I gene (DNAse I), which is associated with the disposal of apoptotic nuclear debris, is located on the short arm of chromosome 16. This gene is 3.2 kb long with 9 exons separated by 8 introns. So it has been highlighted for its possible involvement in SLE pathogenesis. The defective enzyme production due to +2373 A to G mutation in exon 8 is considered to be a genetic risk factor for SLE patients in other populations [2]. The main objective of the present study was to analyze the mutations of Deoxyribonuclease I (DNAse I) gene exon 8 in Pakistani SLE patients.

Materials and Methods
A total 42 SLE patients and 42 healthy controls were included in this study. This study focused mainly the population of Lahore present in the Punjab province of Pakistan. A total of 42 SLE patients were selected on the basis of American Society of Rheumatology (ACR) criteria from different hospitals of Lahore. Most of the SLE patients fulfilled 5 of the 11 ACR criteria. DNA was isolated by phenol chloroform DNA extraction method [3]. Then PCR was performed to amplify the exon 8 region of DNAse I gene (Figure 1). PCR products were sequenced by dideoxy sequencing method. Bioinformatics (Chromas, EditSeq, BLAST) was applied to analyze mutations in the exon 8 region of DNAse I gene. DNAse I coding sequence (both mutated and non-mutated) was translated into protein using a DNA translator. Translated sequence was further analyzed by Phire ver 2 software [2].

Results
A total 42 SLE patients and 42 healthy controls were included in this study. 93% of the SLE patients were females while 7% were males, this may be because of the hormonal factors involved (Figure 2). Most of the SLE patients (49%) lie within the age group of 20-40 years (Figure 3). Most of the SLE patients fulfilled 5 of the 11 ACR criteria (Figure 4). In this study, 73% of the SLE patients were found to be positive for rheumatoid factor. SLE patients with positive rheumatoid factor have pain and swelling at lower and upper extremities which often limits the physical activity and interfere with rehabilitation activities such as transfer or movements. Photosensitivity was positive in 36% of the

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SLE patients. Discoid rash and malar rash were positive in 55% and 48% respectively. Hematological disorders were positive in 76% of SLE patients, but only 30% having neurological problems. 70% of the SLE patients with single missense mutation had arthralgia or arthritis and showed positive rheumatoid factor while 100% of such SLE patients had lupus nephritis (Figure 5).

Thirteen samples showed a missense mutation at position 3737 of DNAse I gene (Figures 6 and 7). This allelic variant is not present in the normal Pakistani population (Figure 8). A was replaced by G so the codon CAA changed to CGA. The glutamine (a polar amino acid) was replaced by Arginine (a charged amino acid) at position 244 of
Deoxyribonuclease I protein. Accession numbers of mutated samples are shown in Table 1. There is a change in the tertiary structure of mutated DNase I protein as compared to non-mutated DNase I protein. Figures 9 and 10 represents the “in silico” simulation of protein changes. By using PyMOL viewer software, glutamine shows five hydrogen bridges with five different amino acids but in the mutated DNase I protein, Arginine at position 244 showed a reduced number of hydrogen bridges, with only two interactions with two different amino acids.

**Table 1: Accession numbers of the SLE samples showing novel mutation.**

| Accession Numbers | Accession Numbers | Accession Numbers | Accession Numbers | Accession Numbers |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| KT037442          | KT037443          | KT037444          | KT037445          | KT037446          |
| KT037447          | KT037449          | KT037450          | KT037451          | KT037452          |
| KT037453          | KT037454          | KT037455          |                   |                   |

**Discussion**

In the present study, thirteen samples showed a missense mutation at position 3737 of DNAse I gene. The codon CAA changed to CGA (+2373 A>G) so the glutamine was replaced by arginine at position 244 of Deoxyribonuclease I protein. This novel mutation in DNAase I gene exon 8 may contribute to the pathogenicity of SLE as this type of allelic variant is not present in the Lahore healthy population. In this study, there is no evident consanguinity among the SLE patients carrying or not carrying
the mutation. Still there is need to characterize SLE patients according to ethnicity because Lahore population is relatively homogenous, cousin marriages are common in Muslims so genome must be very unique. Thus our future plan is to continue this research on SLE families.

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