Clinical implication of ACE gene polymorphism in Systemic Lupus Erythematosus patients

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ABSTRACT

Systemic Lupus Erythematosus is a complex genetic disease, associated with environmental and genetic factors. Angiotensin-converting enzyme (ACE) plays an important role in the development of SLE, is expressed in a wide range of tissues including lung, vascular endothelium, kidney, cardiac muscles and testis. ACE gene I/D polymorphisms, particularly the DD genotype has been associated with lupus susceptibility and with risk of renal and cardiovascular disease. This study analysed the ACE gene polymorphic variants among the recruited 48 SLE patients (SLE was confirmed by Anti nuclear antibody (ANA) and dsDNA tests) associated with the cardiovascular and renal disease and 48 controls. The present study also analyzed the biochemical parameters (total serum cholesterol, HDL, LDL, VLDL, TGL) and C-reactive protein (CRP) in SLE patients and controls. Our study found that, SLE patients had higher levels of cholesterol (40%) than controls and the and CRP was found to be higher in SLE patients in comparison with controls and established normal reference range (<5mg/L). The distribution of genotype in SLE patients was DD 62.50%, ID 20.83% and II 16.66%. A significantly higher frequency of DD genotype (p<0.0004) was observed in SLE patients in comparison with controls.

Keywords: Systemic Lupus Erythematosus, ACE, CRP, Anti nuclear antibody, Cholesterol.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a complex, chronic, multi-system autoimmune disease which varies in prevalence and incidence depending on ethnicity (Lau et al., 2006; D’Cruz et al., 2007). While the precise etiology of SLE still remains vague, genetic predisposition, environmental and hormonal factors are deemed to play important roles in its pathogenesis. Severity, acquisition risk and clinical manifestations of this disease can vary by ethnicity, geography and sex, with a prevalence
that is higher in women during their childbearing ages and some non-European populations such as African Americans, Hispanics and Asians (Lau et al., 2006; Voskuhl, 2011). Genetic, hormonal, environmental and immunoregulatory factors contribute to the expression of the disease (Kammer et al, 1999).

Genetic studies have demonstrated a strong contribution of many gene variants to SLE incidence and the clinical manifestations of SLE (Wong and Tsao, 2006; Hom et al., 2008; Flesher et al., 2010).

SLE is a complex prototypic autoimmune disease that predominantly affects women; the hallmark of SLE is the generation of auto antibodies that react with self nuclear and cytoplasmic antigens, culminating in immunologic attacks to body organs (Petri, 2006). With very distinct forms of presentation, includes articular and mucocutaneous involvement, renal disease, hematological abnormalities and central nervous systems disease (Cervera et al., 1993). It is a multisystem disease with a variable course and a wide range of clinical manifestations (Gudmundsson and Steinsson, 1984). Among a range of factors that are thought to be contributing to the pathophysiology of SLE, chronic inflammation is thought to play a pivotal role in the pathogenesis of SLE (Alarcon-Segovia et al., 2005).

Several studies have shown that the C-reactive protein (CRP) levels in SLE patents are abnormally elevated both in the absence and presence of infection (Roy and Tan, 2001). The value of using CRP to monitor SLE disease activity has remained in practice. Serum lipids can be used to identify patients with SLE who are at increased risk of renal dysfunction (Font et al., 2001).

Rheumatoid factor (RF) and anti nuclear antibody (ANA) are associated with cardio vascular disease (CVD) events and overall mortality both in those with and without rheumatic diseases. Inflammation (as studied through elevations in CRP and/or Erythrocyte Sedimentation Rate (ESR)) contributes to increased risk of CVD, both in patients with autoimmune diseases (Danesh et al., 2004). ESR develops in 5–20 % of patients with SLE and renal involvement (Cortes-Hernandez et al., 2005).

Patients with SLE also develop arterial stiffness, but it is uncertain if arterial stiffness occurs independently of age, hypertension, renal function, or atherosclerosis (Yildiz et al., 2008). Renal injury in SLE is one of the most serious complications and its pathogenesis has not yet been completely clarified (Sprovieri and Sens, 2005). Angiotensin-converting enzyme (ACE) plays an important role in the development of SLE, expressed in a wide range of tissues including lung, vascular endothelium, kidney, cardiovascular and testis (Kaufman et al., 2001). ACE catalyses the conversion of Angiotensin I to Angiotensin II by its metalloproteinase enzymatic activity and plays a major role in the renin–angiotensin and kallikrein–kininogen systems. A major component of the renin-angiotensin system, ACE is up regulated in pressure overload–induced cardiac hypertrophy as well as heart failure (Schunkert et al., 1993). It is long recognized that autoimmune diseases such as SLE are associated with increased mortality and an increased risk of CVD, which is not explained by traditional CVD risk factors alone (Doria et al., 2005).

Manzi et al. (1997) established in the Pittsburgh cohort that certain risk factors were more common in SLE patients with cardiovascular events than in those without events, including older age at diagnosis, longer disease duration, hypercholesterolemia, postmenopausal status (Manzi et al., 1999).

ACE gene I/D polymorphisms, particularly DD genotype has been associated with lupus susceptibility and with risk of renal disease and cardiovascular disease (Kennon et al., 1999). Inhibition of ACE induces regression of cardiac hypertrophy independent of load (Linz et al. 1992) and prevents dilation and remodelling of the ventricle after myocardial infarction (Pfeffer et al., 1992). Inflammatory mediators and an array of inflammatory cells can induce a broad spectrum of clinical manifestation (Kyttaris et al., 2005).

The purpose of this study was to determine the relationship between the biochemical profile, autoantibodies, and ACE gene polymorphisms in relation to renal and cardiac disease.

**MATERIALS AND METHODS**

**Sample selection**

The study group comprised of 48 SLE female patients and equivalent number of healthy normal control subjects, matched for age (±5), gender, and socio-economic conditions. The samples were collected from SLE patients belonged to Coimbatore, Madurai, Erode districts in Tamil Nadu, South India. The work was...
followed and carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. After obtaining written informed consent from all participating subjects, information regarding health status, habit and occupation was recorded. Individuals who reported a history of chronic or acute diseases were excluded from the study. About 4 ml of peripheral blood sample was collected from SLE and control subjects in heparinized and EDTA coated vacutainers. The blood samples were brought to the laboratory within 24 hours for analysis of immunological, biochemical and genotypic analysis.

The immunological and biochemical parameters (Serum Cholesterol, ANA, RF, ESR and CRP) were analyzed through a commercial laboratory (Religare SRL Ranbaxy Mumbai India) and X-rays (for heart and lungs) and ECG (for the heart, pulmonary functions for the lungs) were done from Sugapriya Hospital Madurai, India.

Genotyping of ACE I/D Polymorphism
Genomic DNA was isolated according to the standard protocol (Miller et al, 1988). The specific segment of ACE gene was amplified by using the previously reported primers (Salem and Batzer, 2009). The primer sequences were as follows, Sense primer: 5’- CTTGAGACATCCATCTTCTTCT -3’ and antisense primer: 5’-GATGTGCCCATCACATTGTCGAT-3’. PCR reaction was performed in a final volume of 25 μl with 0.25μl of forward and 0.25μl of reverse primers, followed by 200ng of template DNA, 1.25units of Taq DNA polymerase, 2mM dNTPs in 2mM MgCl2. Thermal conditions consisted of an initial denaturation at 94°C for 5 minutes and 35 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 30 seconds, extension at 72°C for 1 minute and final extension at 72°C for 8 minutes. A 190bp amplicon was observed in case of homozygous DD genotype, 490bp in case of homozygous II genotype and heterozygous DI genotype.

All samples that were identified initially as a DD genotype were reanalyzed using an insertion-specific primer pair, as reported by Lindpaintner et al. (1995). Oligonucleotide sequences specific for I alleles primers were 5’-TTGGACCACAGCGCCCCCTACAG-3’ and 5’-TGCCACACCTCCATTG CCCATAA-3’. PCR was performed with an initial denaturation at 94°C for 1 minute and for 30 cycles of denaturation at 92°C for 40 seconds, annealing at 63°C for 40 seconds, extension at 72°C for 40 seconds and a final extension at 72°C for 5 minutes. The PCR products were separated by 8% polyacrylamide gel in 1X TBE buffer at 65V/cm for 1:45 hours. A 335 bp band was obtained only in the presence of the I allele and no bands were detected for samples with DD genotype.

Statistical Analyses
Statistical analysis was performed with SPSS 16.0 for windows. The distribution of genotypes in all groups was tested for deviation from Hardy–Weinberg equilibrium. The Odds ratio (OR) and 95% Confidence Intervals (95% CI) were calculated with statistical significance at p-value <0.05 (J Martin Bland and Douglas G Altman Statistics Notes: The odds ratio BMJ 2000; 320:1468). Chi-square analysis (χ² tests) was used to test the association between the polymorphic variants and biochemical parameters among the controls and experimental. p < 0.05 was used as the criterion of significance. The association between clinical manifestation, production of autoantibodies and polymorphism distribution in patients with SLE was determined by Fisher exact test.

RESULTS AND DISCUSSION

The mean age of SLE patients and controls was 45.11 ± 11.50 and 44.18±11.50 respectively. In this study 40% of the SLE patients had increased total cholesterol levels and were classified into two groups based on cholesterol level such as SLE high (>200 mg/dL) and SLE low (<200 mg/dL) (Table 1). In Patients with high and low cholesterol levels, high TGL levels showed corresponding high levels of LDL and VLDL suggesting a correlation between these levels but HDL levels in both high and low level patients remained unaffected by total cholesterol level.

RF and ESR level were analysed in the SLE and control subjects and found that, in SLE subjects ESR 53.45±10.32 and RF 119±199.19 were significantly (P<0.05) increased as compared to control subjects (Table 1 and 2). CRP levels as expected were higher in the SLE patients than the normal reference range (<5 mg/dL) (Table 2). ANA score was 4+ in severe SLE patients and 1+ in low severity patients (Table 2). The patients in SLE high and SLE low groups had insignificant difference in the levels of CRP and ESR, but had high and low levels of RF in 4+ and 1+ patient respectively which points towards a positive correlation of ANA and RF.
The antinuclear antibody test is positive in almost all individuals with SLE (97%) and in the present study it was found to be elevated in all the SLE patients as compared to controls (Table 2).

Chi square analysis revealed that there was significant difference in the distribution of DD genotype between controls and SLE patients (Table 3). The patients with DD genotype had high total cholesterol levels suggesting a relationship between the two. Gel electrophoresis of amplified PCR products showed 490 bp and 190 bp products, corresponding to the PCR amplification of I and D alleles, respectively (Figure 2). The distribution of ACE genotypes in the study population was as follows 62% DD, 20.83% ID and 16.66% II. The frequency of allele D was 0.72 and I allele was 0.27.

### Table 1: Immunological and biochemical profile in control and SLE subjects

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Age (mm at 1 hr)</th>
<th>ESR (35-60 mg/dL)</th>
<th>HDL&lt;100 mg/dL</th>
<th>LDL&lt;70.35 mg/dL</th>
<th>VLDL&lt;30-200 mg/dL</th>
<th>TGL&lt;30-200 mg/dL</th>
<th>Total Cholesterol&lt;30-200 mg/dL</th>
<th>CRP&lt;5 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>44.18 ±1.55</td>
<td>15.95 ±4.23</td>
<td>85.10 ±18.57</td>
<td>24.73 ±7.75b</td>
<td>135.75 ±50.49</td>
<td>146.90 ±40.45</td>
<td>6.039 ±5.55</td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>46.77 ±1.25</td>
<td>53.45 ±41.04</td>
<td>129.49 ±28.14bc</td>
<td>40.67 ±22.55abc</td>
<td>184.09 ±83.35bc</td>
<td>233.95 ±33.99abc</td>
<td>10.66 ±7.24bc</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>43.46 ±12.58</td>
<td>62.38 ±43.57</td>
<td>88.95 ±24.10</td>
<td>24.13 ±6.08</td>
<td>127.31 ±33.48</td>
<td>151.15 ±129.02</td>
<td>6.59 ±4.40</td>
<td></td>
</tr>
<tr>
<td>SLE Low</td>
<td>41.28 ±4.2abc</td>
<td>62.38 ±43.57</td>
<td>88.95 ±24.10</td>
<td>24.13 ±6.08</td>
<td>127.31 ±33.48</td>
<td>151.15 ±129.02</td>
<td>6.59 ±4.40</td>
<td></td>
</tr>
</tbody>
</table>

* Homogeneity Variances are significant compared to the controls and low level of cholesterol in the experimental subjects (ANOVA)

### Table 2: Immunological and biochemical profiles in control and SLE subjects

<table>
<thead>
<tr>
<th>Particulars</th>
<th>RF (IU/ml)&lt;40</th>
<th>ANA,AB- IFA,HEP2 (U/ml)&lt;15.0</th>
<th>Anti CCP antibodies (U/ml)</th>
<th>DsDNA Test IU/ml</th>
<th>CRP&lt;0.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>34.52 ±20.62</td>
<td>N</td>
<td>14.65 ±9.4</td>
<td>2.37±2.12</td>
<td>5.26±3.98</td>
</tr>
<tr>
<td>SLE High</td>
<td>119.35 ±199.19</td>
<td>4+</td>
<td>32.48 ±63.73</td>
<td>46.29±25.44</td>
<td>21.78±2097</td>
</tr>
<tr>
<td>SLE Low</td>
<td>191.21 ±23.4</td>
<td>2+</td>
<td>26.98 ±51.89</td>
<td>15.25±12.15</td>
<td>45.52±47.78</td>
</tr>
</tbody>
</table>

### Table 3: ACE Gene polymorphisms identified in controls and SLE subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No</th>
<th>Genotypes</th>
<th>P&lt;0.05</th>
<th>I allele n (%)</th>
<th>D allele n (%)</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE patients Controls</td>
<td>48</td>
<td>8(16.66)</td>
<td>10(20.83)</td>
<td>30(62.50)</td>
<td>26(27.08)</td>
<td>3.15(1.67-5.77)</td>
</tr>
<tr>
<td>SLE and controls Genotypic association and the risk ratio</td>
<td>II/DD RR 1.79 (1.19-2.87) Odds ratio 4.77 (1.57-14.48) (( \chi^2 )) P&lt;0.004 (F test) P&lt;0.005</td>
<td>II/ID RR 0.89 (0.55-1.44) Odds ratio 0.76 (0.24-2.38) Pearson P=0.63 Log odds -0.27</td>
<td>ID/DD RR 2.31 (1.38-3.89) Odds ratio 6.27 (2.27-17.29) (( \chi^2 )) (F test, Pearson test) P&lt;0.0002</td>
<td>P&lt;0.0001 (F test and Pearson)</td>
<td>Risk ratio 1.55 (1.21-1.98) Odds ratio 3.05 (1.67-5.77) ( \chi^2 ) P&lt;0.0004, Pearson P&lt;0.0002 F test p&lt;0.0001</td>
<td></td>
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</table>
In our study, high cholesterol levels observed in SLE patients were accompanied by an increase in CRP and ESR levels. These results are consistent with a study by Font et al. (2001) where SLE patients had higher total cholesterol levels which was associated with renal deterioration. RF is an antibody that is not usually present in normal individual. Our study found that, SLE patients had RF present in their body which is in total agreement with a previous study (Steiner and Smolen, 2002) which reported presence of RF in SLE disease condition. SLE is often accompanied by a number of biochemical, hematological and immunological abnormalities, though several of these abnormalities are non-specific (Dubois et al., 1987).

SLE is associated with high prevalence of dyslipidemia, endothelial dysfunction, hypertension and vascular stiffness (Avalos et al., 2007). The true prevalence of vascular disease in women with SLE is unknown, but could certainly be higher than that defined by cardiovascular events alone (Manzi et al., 1997). With improved corticosteroid and immunosuppressive SLE therapy, there is a growing pool of women at increased risk of developing cardiovascular disease, which is now one of the leading causes of death. In a large series, Gladman and Urowitz reported a 9% incidence of angina pectoris and/or myocardial infarction that occurred on average 89 months after the onset of SLE (Gladmann and Urowitz, 1987).

Jonsson et al. (1989) reported that patients with SLE who developed myocardial infarction had significantly longer duration of the disease. Previous data has suggested that ESR levels in SLE patients can be used to assess disease activity as ESR is an inexpensive diagnostic tool (Luis et al., 2005). SLE is an immune...
complex mediated disease, which is more common in women. LDL (especially in its oxidized form) may be an important mediator of nephrosclerosis. A high triglyceride level in women and a low HDL cholesterol level in men predicted the decline of renal function (Ettinger et al., 1987).

Font et al. (2001) showed link between hypercholesterolemia with renal failure in a smaller cohort of patients with SLE. In our study, SLE patients had significantly raised triglyceride and LDL cholesterol levels, and significantly lower HDL cholesterol levels in comparison with controls. As women comprise as much as 90% of most SLE cohorts, it would be interesting to investigate whether the triglyceride level is also predictive of renal deterioration in patients with lupus. The present study found DD genotype overrepresented in SLE patients than controls. In a previous study, the D allele was found to be significantly higher in the SLE patients versus non-related controls (Mattei et al., 1989). In another study DD allele frequency was higher in the SLE patients compared to the controls (Kaufman et al., 2001). Angiotensin-converting enzyme is an attractive candidate to play a role in the development of vascular pathological states. Evidence suggests that the presence of the DD genotype increases susceptibility for coronary heart disease, myocardial infarction and both diabetic and non-diabetic renal disease (Kennon et al., 1999). Recently two studies have identified an association with the insertion/deletion in the ACE gene with systemic lupus Erythematosus (Pullmann et al., 2002). In addition to several of the variables used in this study, previously reported prognosticators of poor renal function in patients with SLE include the presence of antibodies. Renal function can be evaluated effectively in patients with SLE, by measuring serum total cholesterol levels patients with SLE, who commonly suffer from both kidney dysfunctions.

CONCLUSION

The abnormal elevation pattern of CRP in SLE patients provided the first clinical clue that variation in the CRP may contribute to the pathogenesis of SLE. The study again reemphasizes that cholesterol levels may play a role in SLE patients and may increase risk of cardiovascular and renal diseases. Also DD genotype of ACE may increase the risk of renal and CVD in such patients. Altogether taking into account an array of investigations, SLE patients may be more susceptible to CVD and renal disease risk with ACE polymorphisms having a significant role.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES


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