Association of estrogen receptor 1 (ESR1) haplotypes with risk for systemic lupus erythematosus among South Indians

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Systemic lupus erythematosus (SLE) is a complex autoimmune disorder involving genetic, epigenetic and environmental factors and has higher incidence in women. In this study, we explored the association of estrogen receptor 1 (ESR1) rs2234693 (PvuII) and rs9340799 (XbaI) polymorphisms with susceptibility to SLE. PCR-RFLP and ELISA were used for genetic analysis and determination of specific autoantibodies, respectively. The univariate analysis showed no independent association of rs2234693 (OR: 1.14, 95% CI: 0.87 - 1.49, p = 0.36) and rs9340799 (OR: 0.87, 95% CI: 0.66-1.14, p = 0.34). The haplotype analysis using SHEsis platform revealed strong linkage disequilibrium between these two polymorphisms (D’: 0.81, r²: 0.55).

Among the four haplotype groups, the C-A haplotype (rs2234693-rs9340799) was strongly associated with the risk for SLE (OR: 2.10, 95% CI: 1.32 - 3.34, p = 0.001). The homozygous variant genotype of rs2234693 exhibited elevated TNF-α and depleted IFN-α, while the effects of rs9340799 were contradictory. The wild genotype of rs2234693 exhibited lower levels of IL-12 with number of rs9340799 variant alleles pronouncing this effect. From this study, it is concluded that the ESR1 haplotypes may influence the Th2 cytokine profile and susceptibility to SLE among the South Indians.

Keywords: Genetic polymorphisms, IFN-α, Interferons, Interleukins, IL-10, IL-12, TNF-α.

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by the presence of autoantibodies to self-antigens. With multisystem involvement, its incidence has been found to be elevated during puberty with more preponderance among the women. The etiology of SLE is complex involving genetic, epigenetic and environmental factors. Further, oxidative damage play a vital role in SLE pathogenesis.

Higher incidence of SLE in women has prompted attention of researchers on estrogen. The hyperinducibility of phase I enzymes e.g. cytochrome P450 1A1 (CYP1A1) m4 variant and defects in phase II (COMT) H108L and glutathione S-transferase (GST) T1 null are known to increase the catechol estrogen levels and impair methoxy estrogen levels. Since the action of estrogen on target cells is mediated through their binding to the estrogen receptors (ERs), several studies have been conducted on the ERs, encoded by the ER1 gene (ESR1), which is the main form of ER. Two intronic polymorphisms i.e. the ESR1 rs2234693 (PvuII T/C) and ESR1 rs9340799 (XbaI A/G) have been studied extensively for their possible association with the SLE susceptibility. However, the results of association were inconsistent due to the wide ethnic and population-level differences. A recent meta-analysis of the Asian studies showed the association of rs2234693 with SLE risk only in dominant model (CC/CT vs. TT).

Shajil et al. have observed the positive association of IL-10 and IL-4 mRNA with the ESR1 PpXx genotype; and an inverse association of IFN-γ and IL-2 mRNA with the ESR1 PpXx genotype. However, association of TNF-α, IFN-α and IL-12 with ESR1 haplotypes has not been established so far. One study has reported association of ESR1 genotypes with the age of SLE onset.

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Abbreviations: ACR, American College of Rheumatology; COMT, catecholamine-O-methyl transferase; CYP, cytochrome P450; ESR1, estrogen receptor 1; GST, glutathione-S-transferase; IFN, interferon; IL, interleukin; MDR, multifactor dimensionality reduction analysis; SLE, systemic lupus erythematosus.
and xx genotypes were reported to occur less frequently during the onset of childhood SLE than in the normal subjects. The PvulI C and XbaI G variant alleles have been reported to be associated with a milder form of SLE characterized by skin manifestations, later onset, and less organ damage.

In the current study, we focused to establish (i) the association of ESR1 rs2234693 and rs9340799 polymorphisms with the SLE; (ii) whether the risk could be attributed to individual genetic effects or due to the interactions with polymorphisms in estrogen metabolism; and (iii) whether these variants would influence the cytokines production.

Materials and Methods

Recruitment of study subjects— All the subjects with SLE were recruited during the period of January 2011-March 2014 at the Department of Rheumatology, Nizam’s Institute of Medical Sciences (NIMS), Hyderabad, India. A total of 219 cases, diagnosed along the criteria of the American College of Rheumatology (ACR) for SLE, were included in the study. Cases with allergic inflammatory disorders other than SLE were excluded. Eligible control subjects with no history of any allergic inflammatory disorders other than SLE were recruited during the period of January 2011-March 2014 at the Department of Rheumatology, Nizam’s Institute of Medical Sciences (NIMS), Hyderabad, India. A total of 219 cases, diagnosed along the criteria of the American College of Rheumatology (ACR) for SLE, were included in the study. Cases with allergic inflammatory disorders other than SLE were excluded. Eligible control subjects with no history of any allergic inflammatory disorder were enrolled from a group of 267 healthy volunteers. Their recruitment was done at the NIMS, Hyderabad after the careful evaluation of their personal health and family history. The current study was approved by the Institutional Ethical Committee of the Nizam’s Institute of Medical Sciences (NIMS), Hyderabad, India (EC/NIMS/1225/2010, dated 09.06.2010).

The informed consent was obtained from all the subjects (both controls and cases) before the enrolment in the study. The demographic details included age, gender, body mass index (BMI), smoking habits, alcohol intake, food frequency, different environmental exposure and the medical history were obtained. The mean age was 27.6±9.97 yr. The cases were comprised of 209 women and 10 men.

All the clinical parameters of the cases were obtained from the case records of patients and the follow-up information provided by the rheumatology registry. The clinical features such as skin symptoms, renal and pulmonary involvement, myositis, peripheral and central nervous involvement, oral ulcers, malar rash, discoid lupus, pericarditis, endocarditis, antiphospholipid syndrome, hematological features like anemia, lymphopenia, leucopenia, and thrombocytopenia, pulmonary hypertension, seizures, and psychosis were recorded.

Collection of blood samples— Blood samples were collected in 5 ml EDTA tube and also in 5 ml in plain vacutainer for the collection of serum. Serum was separated after centrifugation at 3000 rpm for 10 min and stored as aliquots at −20 °C until analysis. Genomic DNA was extracted from lymphocytes using the phenol-chloroform extraction method following proteinase K digestion.

Analysis of ESR1 polymorphisms— PCR using specific primers 5'-CTGCCACCCTATCTGTATCTTT TCTATTTCTCC-3' and 5'-TCTTTCTCTGACCACCT GCCTCGATTATCTGA-3' resulted in an amplicon of 1374 bp, which was subjected to restriction digestion with PvulI and XbaI restriction enzymes. In the presence of T-allele, PvulI restriction site was created resulting in the cleavage of 1374 bp into 936 bp and 438 bp. Similarly, in the presence of A-allele, XbaI site was created resulting in the cleavage of 1374 bp into 981 and 383 bp.

Estimation of cytokine levels— Serum levels of interferon-α, TNF-α, IL-12 and IL-10 were determined by the commercial-ELISA kits (M/s Boster Biological Tech Ltd, USA).

Statistical analysis— Accordance of genotype distribution in cases and controls with the Hardy-Weinberg equilibrium was tested using the χ² statistics. All the genetic variables were computed as 2 × 2 contingency table based on the absence or presence of variant allele in cases and controls and were subjected to the Fisher exact test to obtain odds ratios (ORs), 95% confidence intervals (CIs) and P values. For all the above statistical analyses, the statistical web page “www.statpages.org” was used. Construction of haplotypes, linkage disequilibrium testing and haplotype association analysis were performed using the SHEsis web-based platform. For all the associations, a P value of <0.05 was considered statistically significant. The multifactor dimensionality reduction (version 3.0.2) (MDR) was used to explore gene-gene interactions between the ESR1 and estrogen metabolic pathway that modulate susceptibility to SLE.

Results

As shown in Table 1, both the polymorphisms in ESR1 were distributed in cases and controls in accordance with the Hardy-Weinberg equilibrium. The frequency of rs2234693 C-allele was higher in SLE cases compared to controls (42.9% vs. 38.8%). The frequency of rs9340799 G-allele was lower in the SLE cases as compared to controls (34.5% vs. 37.6%). However, both polymorphisms showed no independent association with the SLE risk (OR: 1.14, 95% CI: 0.87-1.49 and OR: 0.87, 95% CI: 0.66-1.14).
As shown in Table 2, haplotype analysis using the SHEsis platform revealed strong linkage disequilibrium between these two polymorphisms (D’: 0.81, r^2: 0.55). Among the four haplotype groups, the C-A haplotype (rs2234693-rs9340799) was strongly associated with the risk for SLE (OR: 2.10, 95% CI: 1.32-3.34, p = 0.001). However, no association was observed with other haplotypes with the risk of SLE.

The SLE cases exhibited higher levels of cytokines than the healthy controls irrespective of genotypes (Fig. 1). TNF-α levels were found significantly higher in the SLE cases with the ESR1 rs2234693 CC-genotype and the ESR1 rs9340799 G-allele exhibiting dose-dependent inverse association. IFN-α values were significantly lower in the SLE cases and controls having the ppXX and ppXx genotypes. IL-10 values showed no significant variation across genotypes. The PPXX, PPXx and PPxx genotypes showed lower IL-12 values, which was dose-dependent on number of x-alleles. (Fig. 1)

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Discussion

This is the first study conducted on the SLE subjects in India demonstrating the association of ESR1 haplotypes with the SLE. ESR1 rs2234693 and rs9340799 that showed a strong linkage disequilibrium. The ESR1 C-A haplotype has been shown to be an independent risk factor for SLE. In our earlier case-control study, we observed the cytochrome P 450 1A1 (CYP1A1) m4 (OR: 4.93, 95% CI: 1.31-18.49), catecholamine-o-methyl transferase (COMT) H108L (OR: 1.39, 95% CI: 1.03-1.88) and glutathione-S-transferase (GST) T1 null (OR: 1.83, 95% CI: 1.11-3.01) variants showed association with the SLE risk. In the current study, ESR1 rs2234693 has been shown to interact with genetic variants of the estrogen metabolism i.e., CYP1A1 m2, COMT H108L and GSTT1 null in inflating the risk for the SLE as confirmed by MDR analysis. The SLE cases exhibited elevated levels of TNF-α, IFN-α, IL-10 and IL-12 irrespective of the ESR1 genotypes. The IL-10 levels showed no variation according to the ESR1 genotypes. Homozygous variant genotype of rs2234693 exhibited elevated TNF-α and depleted IFN-α levels while the effects of rs9340799 were contradictory. The wild genotype of rs2234693 exhibited lower levels of IL-12 with number of rs9340799 variant alleles pronouncing this effect.

The frequency of ESR1 rs2234693 C-variant as observed in this study (38.8%) corroborated with the frequency of this variant among the North Indians (39.7%) suggesting no marked deviations in baseline frequencies among the South and North Indians. Our results are contradictory to the meta-analysis by Li Cai et al., suggesting that even in Asians, distinct ethnic variations do exist that contribute to genetic diversity. Although, many studies focused on the ESR1 genotypes, none of them explored the haplotype based association that might have possibly contributed to disparities in association.

The estrogen receptor (ER) is inactive in the absence of estrogen as it is bound to a multiprotein complex. An upsurge in estrogens during puberty or exogenous estrogen analogues induce a conformational change in the ER resulting in its release from the bound proteins and enhances homodimerization of the ER subtypes followed by high affinity binding to consensus estrogen responsive elements located in the regulatory region of target genes. Alternatively, the ER can interact with other transcription factors such as the AP-1 (via protein-protein interactions) and modulation of the binding of AP-1 to AP-1 binding sites onto DNA (AP-1 responsive elements), thus regulating the AP-1 depending gene transcription. Herrington et al. have demonstrated that as a result of the T→C transition (rs2234693), the ESR1 can produce a binding site for the B-Myb transcriptional factor, which could enhance the ability to upregulate the downstream receptor structures.

Our current results showing elevation of serum IL-10 levels in SLE patients irrespective of the genotypes corroborated the findings of Doria et al. suggesting that IL-10 elevation is constitutive rather than modulated by estradiol. Further, elevated levels of TNF-α and IFN-α in the sera of SLE patients as observed in this study is in alignment with the observations of Weckerle et al. Similarly, elevated levels of IL-12 were consistent with the findings of Tokano et al. The positive association of rs2234693 with TNF-α and IL-12 probably might be mediated through the B-Myb. The strong linkage disequilibrium between the rs2234693 and rs9340799 reported in the current study was consistent with the findings reported elsewhere.

The MDR results were consistent with the published reports suggesting the role of catechol estrogen-DNA adducts as better epitopes for the binding of autoantibodies. The hyper inducible CYP1A1 (m2) and labile COMT (H108L) impair the conversion of catechol estrogen to methoxy estrogen. Salama et al. reported that TNF-α also can enhance the oxidative metabolism of estrogen to catechol estrogen.

Overall, from the current study, it is concluded that the C-A haplotype of ESR1 influences the Th-2 cytokine profile and susceptibility to the SLE. The ESR1 rs2234693 exhibits synergistic interactions with the CYP1A1 m2, COMT H108L and GSTT1 to further inflate the SLE risk.

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References


