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PTPN22 rs2476601 Polymorphism and Immunological Biomarkers in Systemic Lupus Erythematosus: A Case Control Study

Navami S[1], Prajna P Shetty[2], Jishamol K[3], Gargi R Nair[4]

[1,2,3]Department of Biochemistry, Srinivas Institute of Medical Sciences & Research Center, Mangalore, India
[4]Department of Pathology, St. Gregorios college of health science, Pathanamthitta, India

Corresponding author: Navami S. Email: snavami523@gmail.com

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ABSTRACT

Background:
Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disorder characterized by immune dysregulation, loss of self-tolerance, and chronic inflammation. Genetic variants, such as the PTPN22 polymorphism (rs2476601), have been associated with altered immune signaling and increased susceptibility to autoimmune diseases, though their impact differs among ethnic groups. Identifying such single nucleotide polymorphisms (SNPs) and comparing them with serological biomarkers including anti-dsDNA antibodies, complement components (C3, C4), and inflammatory markers provides valuable insight into disease pathogenesis, helps predict disease activity, and may contribute to personalized diagnostic and prognostic approaches in SLE.

Objectives:
This study aimed to investigate the presence of PTPN22 rs2476601 polymorphism in SLE patients compared with healthy controls and to evaluate the relationship between genetic findings and established serological biomarkers in an Indian cohort.

Methods:
A total of 50 samples were collected from clinically confirmed Systemic Lupus Erythematosus (SLE) patients and age- and sex-matched healthy controls after screening more than 1,000 symptomatic individuals at Holy Cross Hospital. Serological analyses included the measurement of anti-dsDNA antibodies by (enzyme-linked immunosorbent assay, ELISA), complement component C3 (Turbidimetric immunoassay), C-reactive protein (CRP) (Latex turbidimetric immunoassay), erythrocyte sedimentation rate (ESR) (Westergren method), and serum creatinine (Biochemical assay). All statistical analyses were performed using *GraphPad Prism version 7*. Group comparisons were conducted to evaluate intergroup differences, and Spearman's correlation analysis was applied to assess relationships between immunological and biochemical parameters.

Results:
SLE patients exhibited significantly elevated anti-dsDNA antibody levels and reduced C3 levels compared with controls ($p < 0.00001$). Both CRP and ESR were also significantly higher in the SLE group ($p < 0.00001$), while serum creatinine did not differ significantly ($p > 0.05$). No meaningful correlations were observed between biomarker levels and disease duration. Genotyping revealed that PTPN22 SNP-G and SNP-T alleles were more frequent among SLE patients, whereas controls predominantly carried the wild-type allele. Assay validation confirmed high specificity and reproducibility.

Conclusion:
The findings demonstrate that anti-dsDNA positivity and low C3 levels are reliable serological markers for SLE in this cohort, while PTPN22 rs2476601 polymorphism represents a potential genetic risk factor. Integration of biomarker profiling and genetic testing may enhance diagnostic precision and support risk stratification in SLE. Larger multi-ethnic studies are warranted to validate these results and explore their clinical utility.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic, multisystem autoimmune disease characterized by the production of autoantibodies and immune complex–mediated tissue damage. Its clinical course is highly variable, with manifestations ranging from mild cutaneous involvement to life-threatening renal and neurological disease. Despite advances in understanding disease mechanisms, the etiology of SLE remains incompletely defined, with both genetic predisposition and environmental triggers contributing to disease onset and progression [1].

Among genetic risk factors, the protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene has attracted considerable attention. A missense single nucleotide polymorphism (SNP), rs2476601 (R620W), results in an amino acid substitution that alters T-cell receptor signaling, thereby promoting autoimmunity [2]. This variant has been strongly associated with several autoimmune diseases, including rheumatoid arthritis, type 1 diabetes, and SLE, particularly in European and North American populations. However, the strength of association varies across ethnic groups, with inconsistent findings reported in Asian and Latin American cohorts. These differences highlight the importance of evaluating population-specific genetic contributions to autoimmune susceptibility [3].

In addition to genetic predisposition, serological biomarkers remain central to SLE diagnosis and disease monitoring. Autoantibodies such as antinuclear antibodies (ANA) and anti-double stranded DNA (anti-dsDNA), along with complement components (C3, C4), are widely used to support diagnosis and assess disease activity [4]. Markers of systemic inflammation, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), are often elevated but lack disease specificity. Serum creatinine is routinely evaluated to monitor renal involvement, one of the most severe complications of SLE [5].

Given the interplay between genetic polymorphisms and immune dysregulation, evaluating both PTPN22 variants and serological biomarkers in SLE may provide a more comprehensive understanding of disease mechanisms and potential diagnostic strategies. To date, no study has systematically examined this relationship in an Indian cohort. Therefore, the present study aimed to investigate the prevalence of PTPN22 rs2476601 polymorphism using quantitative PCR (qPCR) and to assess its correlation with established serological biomarkers in SLE patients compared to healthy controls.

2. Materials and Methods

2.1. Patients and Healthy Controls

A total of 1,000 patients attending the outpatient and inpatient departments at Holy Cross Hospital were screened for systemic lupus erythematosus (SLE) using antinuclear antibody (ANA) testing. Of these, 25 clinically confirmed SLE patients were recruited according to the 2019 EULAR/ACR classification criteria, with mandatory ANA and anti-dsDNA positivity. Twenty-five age- and sex-matched healthy controls with no autoimmune disease history and normal inflammatory markers (CRP and ESR) were included. Venous blood was collected

aseptically from all participants after informed consent. The study was conducted following ethical guidelines, with approval from the Ethical Committee under protocol number 29/AHS/2023.

Inclusion criteria: SLE patients aged 15-44 years. Only Females were considered to maintain age and sex matched criteria. .
Exclusion criteria: Individuals who were pregnant, had malignancies, other autoimmune disorders, a history of prior immunosuppressive therapy, or were unable to provide informed consent were excluded from the study.

2.2. Genotyping

Genomic DNA was extracted from EDTA-treated blood samples using a magnetic bead–based DNA extraction kit (Zymag). DNA quality and concentration were evaluated by UV spectrophotometry (A260/A280) and agarose gel electrophoresis.

The PTPN22 R620W (rs2476601) polymorphism was targeted. Primers were designed using Primer-BLAST and checked for specificity, GC content, and self-complementarity. Allele-specific TaqMan probes (wild-type, SNP-G, SNP-T) were synthesized (BioDeskINDIA Labs). RNase P was used as a housekeeping gene.

PCR standardization was performed using DNA from 5 SLE patients and 5 controls. Validation included agarose gel electrophoresis and sequencing of the PTPN22 partial region (878 bp).

2.4. Quantitative PCR (qPCR)

Allelic discrimination for PTPN22 polymorphism was performed using real-time quantitative PCR (qPCR). The assay included.

Forward primer: CCAGCTTCCTCAACCACAATA
Reverse primer: TGGATAGCAACTGCTCCAAG
Probe A (wild allele): FAM-CCCTCCACTTCCTGTATGGACACC-BHQ1
Probe G (SNP-G): FAM-TCAGGTGTCCGTACAGGAAGTGGA-BHQ1
Probe T (SNP-T): FAM-AGGTGTCCTTACAGGAAGTGGAGGG-BHQ1

Reaction conditions were optimized for annealing temperature, Mg^{2+} concentration, and cycle parameters. RNase P amplification confirmed DNA quality and served as an internal control. Amplification curves and cycle threshold (Ct) values were analyzed to distinguish wild-type, heterozygous, and homozygous SNP genotypes.

2.5. Immunological and inflammatory profiling

Serological assays were performed for both SLE patients and healthy controls to evaluate immunological and inflammatory status. Antinuclear antibodies (ANA) were detected using the indirect immunofluorescence assay (IFA) with HEp-2 cell substrates, while anti-double-stranded DNA (anti-dsDNA) antibodies were quantified by enzyme-linked immunosorbent assay (ELISA), with cut-off values defined according to the manufacturer's instructions. Complement

component C3 levels were measured using a turbidimetric immunoassay, with a reference range of 90–180 mg/dL. Inflammatory markers included C-reactive protein (CRP), determined by a turbidimetric latex immunoassay (reference: <6 mg/L), and erythrocyte sedimentation rate (ESR), assessed by the standard Westergren method. Serum creatinine levels were analyzed to assess renal function. All assays were carried out with appropriate positive and negative controls, and strict quality assurance protocols were followed at each stage to ensure accuracy and reproducibility.

2.6. Statistical analysis

Data were analyzed using 'GraphpadPrism-7' Continuous variables were expressed as mean ± standard deviation (SD) or median (range), and categorical data as frequencies. Group comparisons between SLE patients and controls were made using the Kruskal–Wallis test. Correlations between disease duration and biomarkers were assessed using Spearman's rank correlation coefficient. A p-value < 0.05 was considered statistically significant.

3.1. Demographic and clinical characteristics of the study population

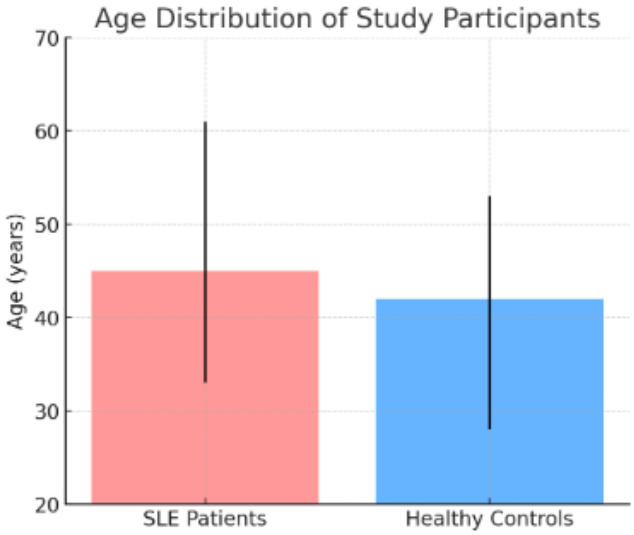

Figure 1: Demographic and clinical characteristics of the study population

A total of 25 female patients with systemic lupus erythematosus (SLE) and 25 age- and sex-matched healthy controls were included in the study. Two male patients presenting with lupus-like symptoms were excluded due to ANA negativity. The mean age of the SLE group was 45 years (range: 33–61 years), while that of the control group was 42 years (range: 28–53 years). The mean disease duration among SLE patients was 9.0 years (range: 5–15 years).

The figure 1 shows demographic and clinical characteristics of the study population

3.2. Immunological and serological biomarkers in SLE patients and healthy controls

Serological analysis revealed significant differences between systemic lupus erythematosus (SLE) patients and healthy controls. Anti-dsDNA antibody levels were

markedly elevated in SLE patients compared with controls ($p < 0.00001$), confirming its role as a highly disease-specific marker. Complement component C3 levels were significantly reduced in the SLE group relative to controls ($p < 0.00001$), reflecting complement consumption due to disease activity.

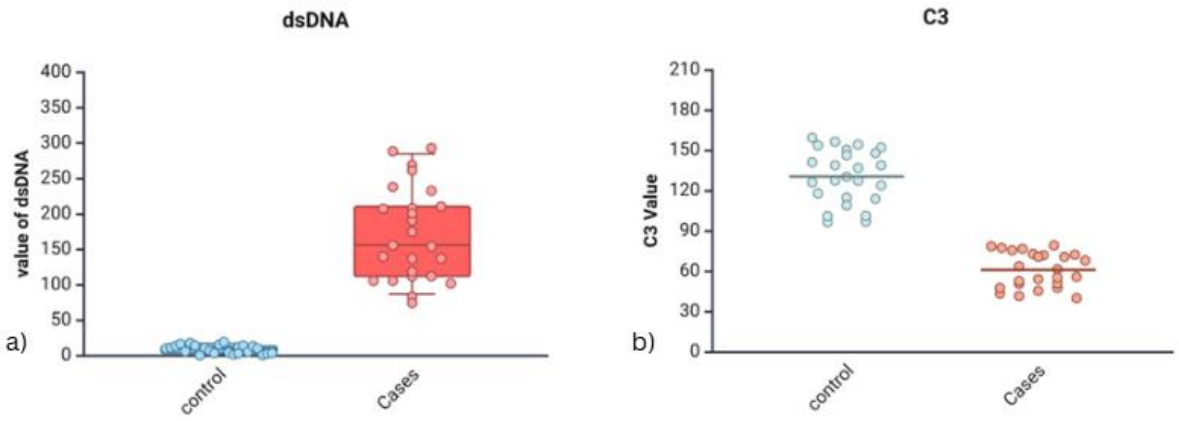

Figure 2: a) dsDNA in SLE Patients and Healthy Controls b) C3 Value Comparison Between SLE Cases and Healthy Controls

Inflammatory markers demonstrated a similar trend: both C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were significantly higher in patients than in controls ($p < 0.00001$), indicating systemic inflammation. In contrast, serum creatinine values did not differ significantly between the two groups ($p > 0.05$), suggesting that renal function remained largely preserved in the study cohort.

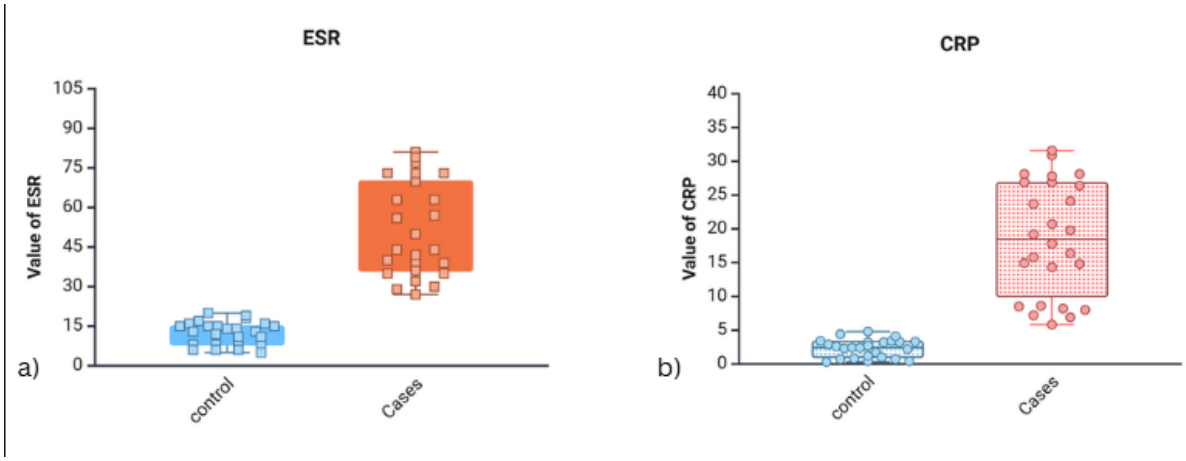

Figure 3: a) ESR Value Comparison Between SLE Cases and Healthy Controls b) CRP levels in cases and controls

3.3. Correlation between biomarkers and disease duration

The relationship between biomarker levels and disease duration was evaluated among SLE patients. Weak correlation was observed between anti-dsDNA antibody titre and disease duration (Spearman's r = 0.10, p = 0.46). Similarly, C3 levels showed very weak association with disease duration (r = 0.08, p = 0.70).

Anti-dsDNA demonstrated a moderate positive correlation with serum creatinine (Rs = 0.69, p < 0.01), highlighting its relevance in lupus nephritis and kidney dysfunction. This suggests that rising anti-dsDNA levels might precede renal damage, with creatinine levels reflecting established kidney dysfunction. However, no significant correlations were found between anti-dsDNA and C3 (Rs = 0.1387, p > 0.50), CRP (Rs = 0.0752, p > 0.50), or ESR (Rs = 0.059, p > 0.50), implying that anti-dsDNA is not closely linked to general inflammation or immune activity in SLE, but rather more specifically associated with disease flares and renal involvement.

Complement C3 showed weak negative correlations with CRP (Rs = -0.1638, p > 0.50), ESR (Rs = -0.1458, p > 0.50), and serum creatinine (Rs = -0.0194, p > 0.50), suggesting that C3 depletion, which occurs due to immune complex deposition, may be associated with increased inflammation and renal stress. However, the weak associations limit the strength of these conclusions. This indicates that as disease progresses, C3 levels decrease, potentially reflecting immune complex formation and systemic inflammation. The observed trend, where C3 decreases while other markers (such as CRP, ESR, and creatinine) increase, supports the concept that C3 depletion is linked to heightened immune activation and renal stress.

CRP showed a weak positive correlation with ESR (Rs = 0.3096, p = 0.20), suggesting that these two inflammatory markers tend to rise together, but the correlation is not strong enough to be statistically significant. Additionally, CRP showed a very weak correlation with serum creatinine (Rs = 0.0469, p > 0.50), indicating that CRP is not a reliable marker for renal function in SLE, as it primarily reflects systemic inflammation.

Overall, the results underscore the complex nature of SLE, with different biomarkers serving distinct roles in reflecting immune activity, inflammation, and renal function. These findings highlight the necessity of a comprehensive, multiparametric approach in diagnosis, disease monitoring, and management of SLE, as no single biomarker can fully capture the complexity of the disease.

3.4. Genotyping of PTPN22 polymorphism by qPCR

Allelic discrimination of the PTPN22 R620W (rs2476601) polymorphism was performed using real-time qPCR with allele-specific TaqMan probes. Distinct amplification curves were obtained for the wild-type allele, SNP-G, and SNP-T variants, confirming the assay's ability to differentiate genotypes.

The analysis of the rs2476601 polymorphism in the *PTPN22* gene revealed that the G allele represents the wild-type variant, which was predominantly observed in healthy control samples, indicating its protective role

against autoimmunity. In contrast, the A allele corresponds to the mutant variant, which was more frequently detected among SLE patients, suggesting a possible association between this allele and increased susceptibility to Systemic Lupus Erythematosus. The amplification of the G allele in most control samples and the detection of the A allele in a subset of patient samples (7 out of 25) support the hypothesis that the substitution of guanine (G) with adenine (A) at the rs2476601 locus may alter immune regulatory pathways, contributing to autoimmune disease development. No amplification of the T allele was observed in either group, confirming its absence or extremely low prevalence in the studied population. Overall, these results indicate that individuals carrying the mutant A allele may possess a higher genetic risk for SLE compared to those with the wild-type G allele.

4. Discussion

In this study, we evaluated the association of PTPN22 gene polymorphism (rs2476601) and serological biomarkers in patients with systemic lupus erythematosus (SLE) compared with healthy controls. Our results demonstrated that anti-dsDNA antibody positivity and reduced complement C3 levels were prominent serological differentiators, while CRP and ESR reflected systemic inflammation. Importantly, we observed a higher prevalence of PTPN22 polymorphisms in SLE patients compared to controls, suggesting a potential role of this variant in disease susceptibility.

These findings are consistent with prior studies that have linked PTPN22 polymorphisms to autoimmunity. For instance, Menchaca-Tapia et al. (2023) investigated rs2476601 along with other variants in primary Sjögren's syndrome and reported no significant genotypic associations, but observed markedly elevated PTPN22 mRNA expression, highlighting a possible functional role of this gene in autoimmunity beyond direct SNP associations. Our study similarly supports the importance of PTPN22, although in SLE we observed a clearer genotypic distinction between cases and controls [6].

Furthermore, studies in different ethnic populations suggest variable effects of PTPN22 variants. In North American cohorts, rs2476601 has been strongly associated with SLE and rheumatoid arthritis, supporting its pathogenic relevance [7,8]. In contrast, Mexican and Asian studies have sometimes failed to replicate these associations, likely due to ethnic genetic background and allele frequency differences [6,9]. Our findings in an Indian cohort add to the global evidence and emphasize the need for population-specific evaluations when considering genetic markers for autoimmune risk.

From a biomarker perspective, our observation that low C3 and high anti-dsDNA antibody levels correlate with disease activity aligns with established reports that these markers serve as reliable indices of disease flares and renal involvement in SLE [10,11]. Interestingly, while CRP is typically elevated in infection rather than SLE flares, we observed mild increases, which may indicate overlapping inflammatory processes rather than lupus-

specific activity. Similarly, serum creatinine remained largely within normal limits, consistent with the fact that not all SLE patients develop renal impairment at early disease stages.

In integrating both genetic and serological markers, our study underscores that while serological biomarkers remain central for disease monitoring, genetic testing of PTPN22 polymorphisms could serve as an adjunct tool in identifying individuals at risk. This dual approach has been suggested by recent studies exploring multi-marker predictive models in autoimmune diseases [6].

This study was limited by sample size, which may affect the power to detect less frequent alleles. Additionally, functional assays to directly measure PTPN22 expression were not performed, which could provide mechanistic insights. Future research should expand cohort size, include diverse populations, and investigate gene–environment interactions to fully elucidate the role of PTPN22 in SLE pathogenesis.

The qPCR-based allelic discrimination revealed that the G allele was predominantly present in healthy controls, which aligns with the known protective role of this allele against autoimmunity. On the other hand, the A allele, which is associated with increased susceptibility to autoimmune conditions, was more frequently detected in SLE patients, with 7 out of 25 patient samples showing amplification for the A allele. This suggests a potential genetic risk for SLE associated with the A allele at the rs2476601 locus. Interestingly, no amplification of the T allele was observed in either the patient or control groups, indicating that this variant may either be absent or present at extremely low frequencies in the Indian population.

From a serological perspective, the study evaluated key inflammatory markers that are widely used for SLE diagnosis and monitoring disease activity. The results demonstrated elevated anti-dsDNA and reduced C3 levels in SLE patients, confirming the central role of these markers in disease pathology. Additionally, the study revealed weak correlations between anti-dsDNA and C3, CRP, and ESR, indicating that while these markers reflect different aspects of immune activation and inflammation, their relationships are complex and not always directly aligned.

CRP and ESR were significantly elevated in SLE patients, highlighting the systemic inflammatory burden of the disease. However, these markers showed weak correlations with anti-dsDNA, serum creatinine, and C3, reinforcing the notion that these biomarkers have limited specificity and should be interpreted in conjunction with other clinical findings. The study also demonstrated a moderate correlation between anti-dsDNA and serum creatinine ($Rs = 0.69$, $p < 0.01$), suggesting that anti-dsDNA may play a key role in lupus nephritis and kidney dysfunction, which is one of the most serious complications of SLE.

The findings also confirmed that C3 depletion is associated with immune complex formation, which leads to increased inflammation and renal stress, as indicated by the weak negative correlations with CRP, ESR, and serum creatinine. Although these associations were statistically not strong, they reflect the broader pathophysiological processes in

SLE where C3 consumption is linked to immune dysregulation and renal involvement.

Conclusion

In conclusion, this study highlights the genetic predisposition of the A allele of rs2476601 in the PTPN22 gene to SLE susceptibility in the Indian population. The findings underscore the importance of a multidimensional approach in the diagnosis and management of SLE, where genetic markers like PTPN22 rs2476601 and serological markers such as anti-dsDNA, C3, CRP, ESR, and serum creatinine are used together to understand the complexities of the disease. While further validation in larger cohorts is necessary, the results of this study contribute to the growing body of knowledge on the genetic and immunological factors that influence SLE and its clinical manifestations.

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