National Board of Examinations - Journal of Medical Sciences
Volume 4, Issue 4, Pages 512–525, April 2026
DOI 10.61770/NBEJMS.2026.v04.i04.008

ORIGINAL ARTICLE

Association of CTLA4 (Cytotoxic T-Lymphocyte Associated Antigen-4) Gene Single Nucleotide Polymorphism with Vasculitis

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Accepted: 16-March-2026 / Published Online: 3-April-2026

Abstract

Background: Vasculitis is a autoimmune disorders represented in heterogenous groups and it is characterized by inflammation and destruction of blood vessels. This leads to multi-organ involvement and substantial morbidity. Genetic susceptibility plays a very important key role in the pathogenesis of vasculitis disorders. Cytotoxic T-lymphocyte associated antigen-4 (CTLA4) has emerged as a critical immune checkpoint molecule regulating T-cell activation and maintaining peripheral immune tolerance. **Aim**: To evaluate the association between CTLA4 gene single nucleotide polymorphism (SNP) rs3087243 (CT60 G>A) and susceptibility to vasculitis in a South Indian population. **Materials and Methods:** This case-control study was conducted among patients who were diagnosed with systemic vasculitis and healthy controls. Peripheral blood samples were collected and genomic DNA was isolated. Genotyping of CTLA4 polymorphism rs3087243 was performed using polymerase chain reaction–based techniques. Allelic and genotypic frequencies were compared between cases and controls using appropriate statistical tests. **Results**: The distribution of CTLA4 polymorphism differed between vasculitis patients and healthy individuals, suggesting a possible association between CTLA4 genetic variation and susceptibility to vasculitic disorders. **Conclusion**: The CTLA4 rs3087243 polymorphism may contribute to genetic predisposition to vasculitis in the studied population. Further studies with larger sample sizes and multi-center cohorts are necessary to confirm these findings and clarify the role of CTLA4 in the immunopathogenesis of vasculitis.

Keywords: Vasculitis, CTLA4 polymorphism, Autoimmune disease, Gene polymorphism, Immune checkpoint

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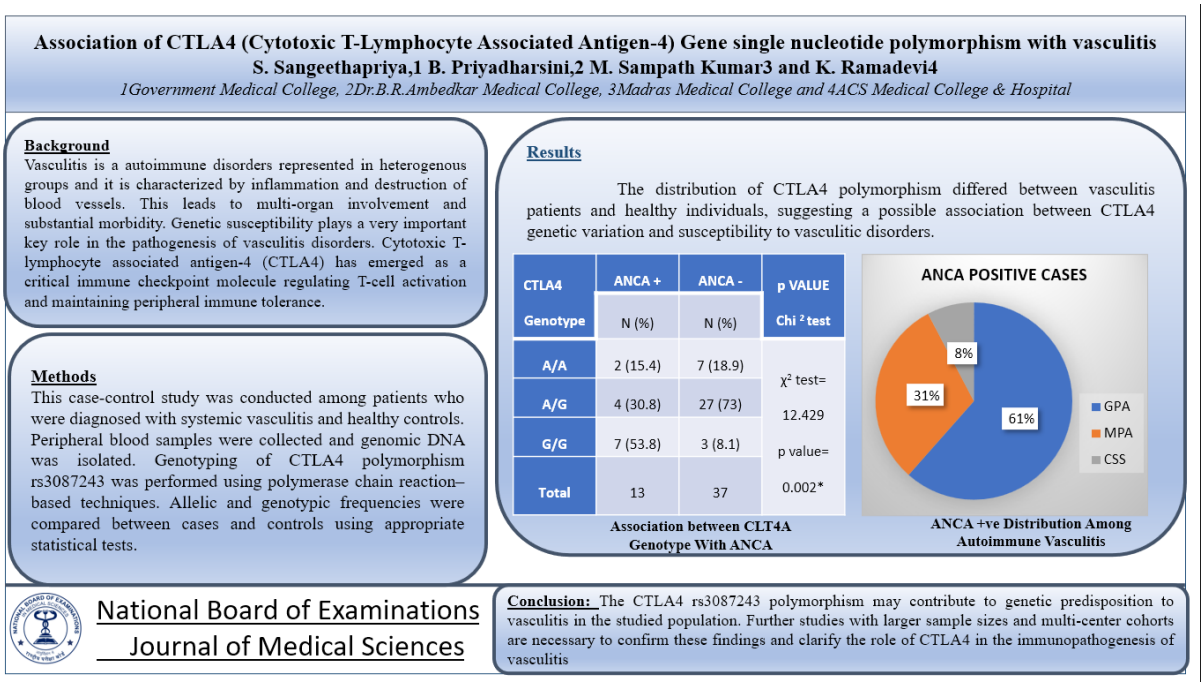
Graphical Abstract



Introduction

Vasculitis is an autoimmune disorder that is caused due to inflammation and destruction of blood vessels, which results from leukocyte infiltration of the vascular wall. Inflammatory process can affect the arteries, veins, or capillaries of varying sizes and it may involve one or multiple organ systems simultaneously. In clinical aspect vasculitis represents a multisystem autoimmune disease which may lead to significant morbidity due to ischemia and tissue damage resulting from vascular injury [1].

Large-vessel vasculitis includes disorders such as giant cell arteritis and Takayasu arteritis. Medium-vessel vasculitis can be due to diseases such as Kawasaki disease and polyarteritis nodosa, whereas small-vessel vasculitis commonly includes antineutrophil cytoplasmic antibody (ANCA) associated vasculitis such as microscopic polyangiitis, granulomatosis with polyangiitis, and eosinophilic granulomatosis with polyangiitis [2,3].

Primary systemic vasculitis (PSV) is an uncommon disorder, with a global estimated incidence of approximately 15–20 cases per million population annually. Epidemiological studies reported in Europe between 1989 and 2003 mentioned an annual incidence of PSV of 19.6 cases per million population, with granulomatosis with polyangiitis accounting for approximately 10.2 cases per million, microscopic polyangiitis for 5.8 cases per million, and Churg–Strauss syndrome for about 4.2 cases per million [4]. The prevalence of vasculitis fiffers based on geographic location and it is higher rates among European populations when compared with non-European populations which may be due to genetic susceptibility [5].

From an adult cohort study in the United States the 5-year survival rate for vasculitis ranges from 45% to 75% in microscopic polyangiitis and

approximately 75–80% in polyarteritis nodosa and Churg–Strauss syndrome. Takayasu arteritis reported with a 10-year survival rate of nearly 87% [6]. Granulomatosis with polyangiitis is associated with significant morbidity, with approximately 11% of patients requiring mechanical ventilation or dialysis during the course of the disease, and a 5-year survival rate of around 75%. Kawasaki disease, although primarily affecting children, may lead to coronary artery complications with an acute mortality rate of approximately 0.12% and coronary artery involvement reported in 2–4% of cases [7].

Vasculitis pathogenesis is multifactorial and it involves a complex interaction between environmental triggers, immune dysregulation, and genetic predisposition. It was also reported that genetic variations affecting immune regulatory pathways contribute significantly to susceptibility to autoimmune diseases, including ANCA-associated vasculitis. Genome-wide association studies (GWAS) and candidate gene approaches have identified several genetic loci involved in immune regulation which includes polymorphisms in human leukocyte antigen (HLA) genes and non-HLA immune regulatory genes [8].

The cytotoxic T-lymphocyte associated antigen-4 (CTLA4) gene has got an considerable attention and it is an inhibitory immune checkpoint molecule expressed on activated T lymphocytes that plays a crucial role in maintaining peripheral immune tolerance. T-cell activation normally occurs through the interaction between the T-cell receptor (TCR) and antigen presented by the major histocompatibility complex (MHC), along with co-stimulatory signaling mediated by CD28 and its ligands CD80 and CD86. CTLA4, a homolog of CD28, competes for binding to these ligands and transmits inhibitory signals that down-regulate T-cell activation [1]. Genetic variants in CTLA4 have been associated with several autoimmune conditions, including rheumatoid arthritis, systemic lupus erythematosus, and autoimmune thyroid disease [9].

It is important to understand the role of CTLA4 polymorphisms in vasculitis may provide important insights into the genetic mechanisms underlying disease susceptibility and pathogenesis. Thus, in the present study we intend to investigate the association between CTLA4 gene polymorphism rs3087243 (CT60 G>A) and vasculitis in South Indian population.

Aim and Objectives

Primary Objective

To determine the association between CTLA4 gene polymorphism rs3087243 (CT60 G>A) and vasculitis in a South Indian population.

Secondary Objectives

1. To determine the genotype distribution of CTLA4 polymorphism among patients with vasculitis.
2. To compare allele frequencies between vasculitis patients and healthy controls.
3. To evaluate the possible role of CTLA4 polymorphism in susceptibility to autoimmune vasculitic disorders.

Materials and Methods

Study Design and Study Setting

This study was a hospital-based case–control study that was carried out at the Institute of Biochemistry, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai.

The study involved departments of Biochemistry, Rheumatology, and Vascular Surgery to facilitate patient recruitment and sample collection. Ethical approval for the study was obtained from the Institutional Ethics Committee prior to initiation of the research work.

Study Population

Patients got diagnosed with vasculitis and healthy control individuals from the outpatient and inpatient services of the departments of Rheumatology and Vascular Surgery at Rajiv Gandhi Government General Hospital, Chennai were included in our study. Diagnosis of vasculitis was made by clinicians based on clinical presentation, laboratory investigations, and established diagnostic criteria. Control participants were healthy individuals selected from volunteers without any known history of autoimmune diseases or chronic inflammatory disorders, matched for age and gender.

Inclusion Criteria

Patients diagnosed with systemic vasculitis based on established clinical criteria were included in the study. Individuals aged above 18 years who were willing to participate and provide written informed consent were considered eligible for inclusion. Controls included healthy individuals with no personal or family history of vasculitis or other autoimmune diseases.

Exclusion Criteria

Individuals with secondary vasculitis due to infections, malignancies, drug-induced causes, or other systemic autoimmune diseases were excluded from the study. Patients with severe comorbid conditions that could interfere with interpretation of results were also excluded.

Sample Size and Sampling Method

A total of 100 participants, comprising 50 patients diagnosed with vasculitis as cases and 50 healthy individuals were included as controls. Participants were selected using a convenient sampling technique from the eligible population presenting to the hospital.

Collection of Clinical Data

Detailed clinical information of each participant was recorded using a structured proforma. Demographic variables such as age and sex were documented. Clinical details including type of vasculitis, presenting symptoms, organ involvement, laboratory findings, and relevant medical history were obtained from patient records and clinical examination.

Sample Collection

After obtaining informed consent, approximately 5 mL of peripheral venous blood was collected from each participant under aseptic conditions. Blood samples were drawn into ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes to prevent clotting. The samples were labeled appropriately and transported immediately to the Molecular Biology Laboratory of the Institute of Biochemistry for further processing and genetic analysis.

DNA Extraction

Genomic DNA was extracted from peripheral blood leukocytes using standard laboratory protocols. The collected blood samples were first subjected to cell lysis to release cellular components. Leukocytes

were separated and treated with lysis buffer containing detergents and proteinase enzymes to break down cellular membranes and proteins. The DNA was then precipitated, washed, and dissolved in appropriate buffer solution. The purity and concentration of the extracted DNA were assessed using spectrophotometric methods and agarose gel electrophoresis to ensure suitability for downstream molecular analysis.

Genotyping of CTLA4 Gene Polymorphism

The CTLA4 gene polymorphism analyzed in this study was the single nucleotide polymorphism rs3087243 (CT60 G>A), located in the CTLA4 gene on chromosome 2q33. This polymorphism has been previously reported to influence immune regulatory mechanisms and susceptibility to autoimmune diseases. Genotyping was performed using polymerase chain reaction (PCR)–based molecular techniques.

Specific primers targeting the CTLA4 gene region containing the rs3087243 polymorphism were used for amplification of the DNA segment. The PCR reaction mixture consisted of genomic DNA template, forward and reverse primers, deoxynucleotide triphosphates (dNTPs), magnesium chloride, buffer solution, and Taq DNA polymerase. The amplification process was carried out in a thermal cycler using standard cycling conditions including initial denaturation, repeated cycles of denaturation, annealing, and extension, followed by a final extension step.

The amplified PCR products were then analyzed to determine the genotype of each participant. The resulting band patterns allowed identification of different genotypes corresponding to the CTLA4 polymorphic variants. To ensure accuracy and reliability of the molecular analysis, strict laboratory quality control measures were implemented. All reagents were prepared using sterile techniques, and negative controls were included in PCR reactions to detect contamination. DNA samples were handled carefully to prevent degradation. Selected samples were reanalyzed to confirm reproducibility of genotyping results.

Statistical Analysis

Statistical analysis of the data was performed using appropriate statistical software. Descriptive statistics were used to summarize demographic and clinical characteristics of the study population. Comparisons between cases and controls were performed using the chi-square test to determine whether there were significant differences in genotype and allele distributions. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate the strength of association between CTLA4 polymorphism and vasculitis susceptibility. A p-value of less than 0.05 was considered statistically significant.

Results

The present study included a total of 100 participants, comprising 50 clinically and radiologically confirmed cases of vasculitis and 50 healthy controls. The cases were diagnosed after excluding secondary causes such as rheumatoid arthritis, systemic lupus erythematosus, and peripheral vascular disease.

The mean age of vasculitis patients in our study was 33.46 ± 16.6 years, and in the control group it was 33.36 ± 9 years. The mean serum urea level was significantly higher in cases with 34.6 ±

13.8 mg/dL compared to controls with 27.1 ± 8.1 mg/dL. The mean serum creatinine level was higher among vasculitis patients was 1.1 ± 0.61 mg/dL than controls with 0.89 ± 0.3 mg/dL with a statistically significant difference. The erythrocyte sedimentation rate (ESR) was markedly elevated in cases 37.1 ± 21.1 mm/hr compared with controls it is 5.70 ± 2.7 mm/hr. The white blood cell count was also significantly higher in cases with 13.1 ± 15.9 $\times 10^3/\mu L$ compared to controls (7.6 ± 1.09 $\times 10^3/\mu L$) (p = 0.01). In contrast, the mean hemoglobin level was significantly lower among vasculitis patients 12.2 ± 2.0 g/dL compared with healthy controls it is 14.7 ± 1.65 g/dL as shown in Table 1.

Table 1. Mean comparison of variables

Variable	CASES n=50	CONTROLS (NORMAL) n=50	p value
	Mean± S.D	Mean± S.D	
AGE (years)	33.46 ± 16.6	33.36 ± 9	0.970
UREA (mg/dL)	34.6 ± 13.8	27.1 ± 8.1	0.00**
CREATININE (mg/dL)	1.1 ± 0.61	0.89 ± 0.3	0.01 **
ESR (mm/hr)	37.1 ± 21.1	5.70 ± 2.7	0.001**
WBC (*10^3 /µL)	13.1 ± 15.9	7.6 ± 1.09	0.01**
HB (gm%)	12.2 ± 2.0	14.7 ± 1.65	0.001**

*p<0.05- Statistically significant

The distribution of vasculitis subtypes among the cases showed that Takayasu arteritis was the most common form seen in 15 cases followed by non-specific vasculitis among 12 cases, Kawasaki disease in 10 cases, granulomatosis with polyangiitis in 8 cases, microscopic polyangiitis among 4 cases, and Churg–Strauss syndrome was observed in 1 case. A higher occurrence of vasculitis was observed among female patients, particularly in Takayasu arteritis, Kawasaki disease, and non-specific vasculitis as shown in Figure 1.

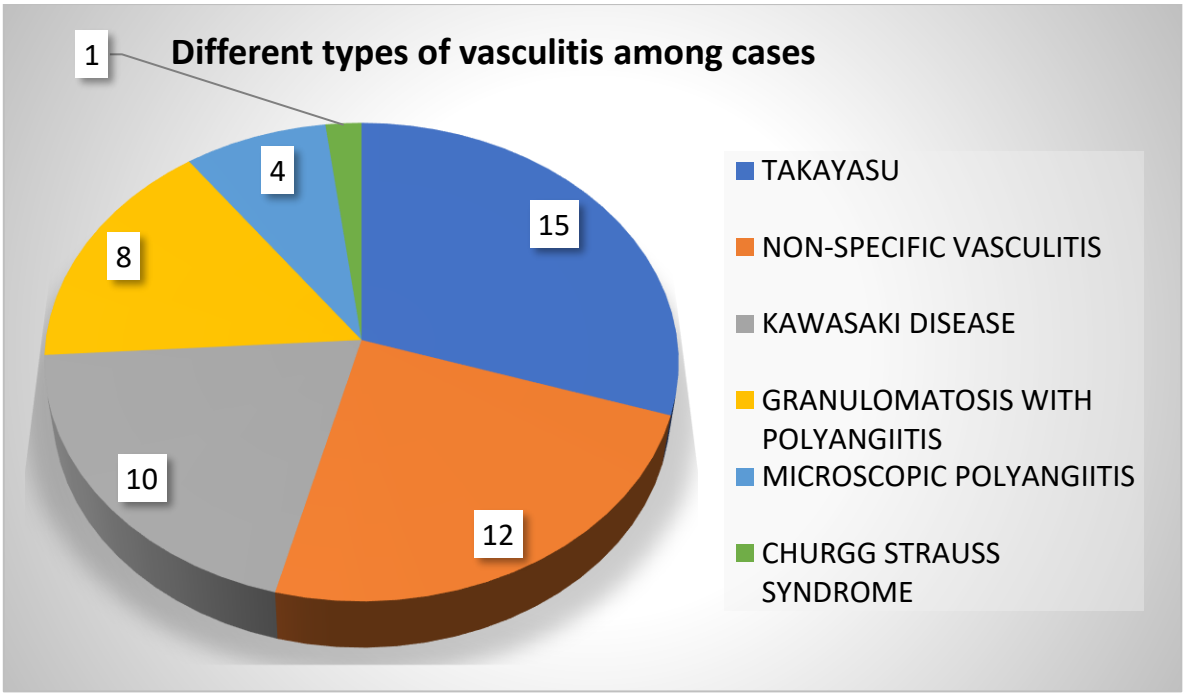


Figure 1. Different Type of Vasculitis Among Cases

Evaluation of antineutrophil cytoplasmic antibody (ANCA) status revealed that 13 patients (26%) were ANCA positive, of which 8 patients had granulomatosis with polyangiitis, 4 had microscopic polyangiitis, and 1 had Churg–Strauss syndrome, indicating that ANCA positivity was predominantly associated with small-vessel vasculitis in the study population as shown in Table 2 and Figure 2.

Table 2. ANCA Distribution of Vasculitis Among Cases

ANCA	**Cases n (%)**
ANCA +	13 (26%)
ANCA -	37 (74%)
Total	50

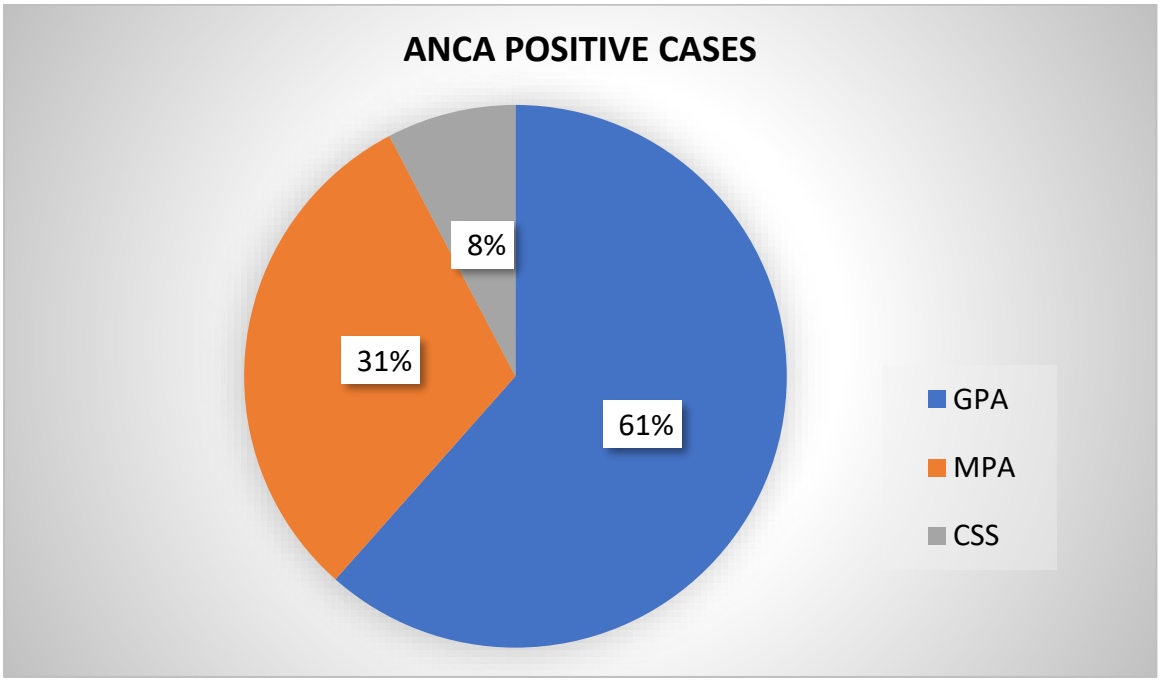


Figure 2. ANCA Positive Distribution Among Autoimmune Vasculitis

The genotype frequencies of the CTLA4 rs3087243 (CT60) polymorphism were analyzed among cases and controls. The distribution of genotypes among vasculitis patients was AA – 18%, AG – 62%, and GG – 20%, whereas in the control group the frequencies were AA – 14%, AG – 78%, and GG – 8%, with no significant difference in genotype distribution between cases and controls (p = 0.154) as shown in Table 3.

Table 3. Distribution of CTLA4 genotype

CTLA4	**CASES**	**CONTROLS (NORMAL)**	**p VALUE Chi ^{2} test**
	n (%)	**n (%)**	
A/A	9 (18)	7 (14)	
A/G	31 (62)	39 (78)	0.154 – NS
G/G	10 (20)	4 (8)	
Total	50	50	

Allele frequency analysis in our study reported that there is no statistically significant difference between the vasculitis group and controls. The G allele frequency was 82% among cases and 86% among controls, whereas the A allele frequency was 18% among cases and 14% among controls, with a p value of 0.584, indicating that CTLA4 rs3087243 polymorphism was not significantly associated with overall vasculitis susceptibility in the study population as shown in Table 4.

Table 4. Allele Distribution Among Cases and Controls

CTLA4	**CASES**	**CONTROLS (NORMAL)**	**p VALUE Chi ^{2} test**
	n (%)	**n (%)**	
G+	41 (82)	43 (86)	
G -	9 (18)	7 (14)	0.584
Total	50	50	

The subgroup analysis showed that among ANCA-positive patients, the genotype distribution was AA – 15.4%, AG – 30.8%, and GG – 53.8%, indicating a significantly higher frequency of the GG genotype compared with ANCA-negative patients (8.1%), with p VALUE <0.05 as shown in Table 5.

Table 5. Association between CTLA4 Genotype With ANCA

CTLA4 Genotype	**ANCA +**	**ANCA -**	**p VALUE Chi ^{2} test**
	n (%)	**n (%)**	
A/A	2 (15.4)	7 (18.9)	χ^2 test=
A/G	4 (30.8)	27 (73)	12.429
G/G	7 (53.8)	3 (8.1)	p value=
Total	13	37	0.002*

*p<0.05- Statistically significant

Our present study findings reports that the CTLA4 rs3087243 polymorphism does not show a significant association with general vasculitis susceptibility, the G allele may contribute to genetic susceptibility in ANCA-associated vasculitis, highlighting the possible role of immune regulatory gene polymorphisms in specific vasculitis subtypes.

Discussion

The present study evaluated the association between CTLA4 gene polymorphism and vasculitis in a cohort consisting of 50 patients with vasculitis and 50 healthy controls. Among the vasculitis cases, Takayasu arteritis was the most common subtype seen in 30% followed by non-specific vasculitis among 24%, Kawasaki disease among 20%, granulomatosis with polyangiitis in 16%, microscopic polyangiitis and Churg–Strauss syndrome in 10% of the study participants. This pattern reflects the epidemiological trend observed in Asian populations, where large-vessel vasculitis such as Takayasu arteritis is relatively more common compared with Western populations. Hospital-based epidemiological studies in India have also demonstrated a similar distribution of vasculitis subtypes, highlighting the relatively higher prevalence of Takayasu arteritis and other large-vessel vasculitides in the Indian subcontinent compared with European cohorts [2].

Evaluation of antineutrophil cytoplasmic antibody (ANCA) status in our study revealed that 13 of the 50 vasculitis patients (26%) were ANCA positive, including 8 patients with granulomatosis with polyangiitis, 4 with microscopic polyangiitis, and 1 with Churg–Strauss syndrome. This observation is consistent with the established role of ANCA in the pathogenesis of small-vessel vasculitis. ANCA antibodies, directed primarily against proteinase-3 (PR3) and myeloperoxidase (MPO), are known to activate neutrophils and trigger inflammatory cascades that lead to vascular injury. Previous studies have demonstrated that ANCA-mediated neutrophil activation and the formation of neutrophil extracellular traps (NETs) play a crucial role in endothelial damage and the development of ANCA-associated vasculitis [7-14].

The primary objective of the present study was to determine whether CTLA4 gene polymorphism rs3087243 (CT60 G>A) is associated with susceptibility to vasculitis. Analysis of genotype distribution showed that among vasculitis patients the frequencies were AA – 18%, AG – 62%, and GG – 20%, whereas among healthy controls the frequencies were AA – 14%, AG – 78%, and GG – 8%. Statistical comparison demonstrated no significant difference in genotype distribution between cases and controls ($p = 0.154$). These findings suggest that CTLA4 rs3087243 polymorphism may not be directly associated with overall susceptibility to vasculitis in the studied population. However, CTLA4 plays a well-recognized role in immune regulation by inhibiting T-cell activation through interaction with CD80 and CD86 ligands, thereby maintaining immune tolerance and preventing excessive immune responses [15-17].

Allele frequency analysis further demonstrated that the G allele frequency was 82% among vasculitis cases and 86% among controls, whereas the A allele frequency was 18% among cases and 14% among controls, and this difference was not

statistically significant (p = 0.584). These findings indicate that the presence of the G allele alone may not confer increased risk of vasculitis in the overall population studied. Similar variations in allele frequencies have been reported across different ethnic groups, and studies have shown that the G allele frequency may vary significantly among populations. Previous genetic studies have also highlighted population-specific differences in CTLA4 polymorphism frequencies, particularly in South Asian populations [18-21].

The subgroup analysis based on ANCA status revealed a significant association between CTLA4 genotype and ANCA-positive vasculitis. Among ANCA-positive patients, the genotype distribution was AA – 15.4%, AG – 30.8%, and GG – 53.8%, whereas among ANCA-negative patients the GG genotype was observed in only 8.1% of cases, with a statistically significant p value of 0.002. This suggests that the GG genotype and G allele of CTLA4 rs3087243 may be associated with susceptibility to ANCA-associated vasculitis. Previous studies investigating CTLA4 polymorphisms in autoimmune diseases have reported similar findings. For instance, Bonatti et al. demonstrated that the G allele of CTLA4 CT60 polymorphism was associated with increased susceptibility to autoimmune disorders including vasculitis [1]. Kamesh et al. reported a significant association between CTLA4 polymorphism and ANCA-associated vasculitis in a large cohort of British patients, suggesting that genetic variation in CTLA4 may influence immune dysregulation and susceptibility to small-vessel vasculitis [22].

The findings of our study indicate that while CTLA4 rs3087243 polymorphism does not appear to influence the overall risk of vasculitis, it may play a role in ANCA-associated vasculitis, particularly through the increased prevalence of the GG genotype among ANCA-positive patients. This observation supports the hypothesis that genetic variations in immune regulatory genes such as CTLA4 may contribute to the pathogenesis of specific vasculitis subtypes rather than vasculitis as a single disease entity.

Conclusion

The present study included 50 cases of clinically and radiologically proven vasculitis patients and 50 healthy controls.

We evaluated the association of CTLA4 rs3087243 single nucleotide polymorphism with vasculitis and found that 26% of cases were ANCA positive and 37% of cases were ANCA negative. There is no significant difference in frequency of genotype among vasculitis group and controls. There is an increased frequency of patients homozygous for CTLA4 CT60 (rs3087243) single nucleotide polymorphism in ANCA associated vasculitis (53.8%). The G allele of CT60 indicated a positive role in susceptibility to autoimmune vasculitis. Present results need to be confirmed by investigation of large cohorts to show differences between the subgroups.

Limitations of Study

The present study has certain limitations that should be considered while interpreting the findings. The relatively modest sample size may have limited the statistical power to detect subtle associations between CTLA4 polymorphism and vasculitis susceptibility. In addition, the subgroup analysis of ANCA-positive vasculitis included a small

number of cases (n = 13), and therefore the observed association between the GG genotype and ANCA positivity should be interpreted cautiously due to the potential risk of type I error. The functional implications of this polymorphism, particularly the relationship with soluble CTLA4 isoform levels, were not evaluated in this study. Furthermore, as one of the few studies conducted in a Southern Indian population, the findings should be considered preliminary and warrant validation through larger multicentric studies with detailed subgroup analyses of vasculitis.

Statements and Declarations

Conflicts of interest

The authors declare that they do not have conflict of interest.

Funding

No funding was received for conducting this study.

Ethical approval

This study has been approved by the Institution Ethics Committee of Madras Medical College carrying certificate number 27082018 dt:07.08.2018 Written informed consent was obtained from all participants after explaining the study procedures, potential risks and benefits. Consent covered both participation and publication of anonymised findings, with assurance of confidentiality and data privacy.

Acknowledgements

Use of AI: The authors declare the usage of AI tool (ClaudeAI) for language moderation. After using this tool, the authors reviewed and edited the content and took full responsibility for the contents of this article.

Data availability statement

The datasets generated and analysed in this study are available from the corresponding author on reasonable request. They are not publicly shared because they contain sensitive information that could indirectly identify participants.

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