

Report

Genetic endowment of proinflammatory cytokine tumor necrosis factor- α (–) 308 G > A polymorphism to *Parthenium hysterophorus*-induced airborne contact dermatitis in an Indian cohort

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Abstract

Background Parthenium dermatitis is a common airborne allergic health problem that induces a cell-mediated hypersensitivity immune response involving activated T lymphocytes, which culminates in injury to the skin. The disease is manifested as itchy erythematous papules and plaques and primarily affects the exposed areas and flexures. This study aimed to identify the role of tumor necrosis factor (TNF)- α (–) 308 G>A polymorphism in the pathogenesis of parthenium dermatitis.

Materials and methods A total of 120 subjects, including 60 patients exclusively diagnosed for parthenium dermatitis and 60 healthy individuals, were included in the study. The genotyping of the TNF- α (–) 308 G>A region was carried out by the amplification refractory mutational system.

Results In the present study, we demonstrated that polymorphism of the TNF- α (–) 308 position (A and/or G) was not statistically significant, and there was no difference in the distribution of any alleles of this locus in cases and controls.

Conclusion The present study suggests that there is a lack of association of potent proinflammatory cytokine TNF- α (–) 308 G>A polymorphism in parthenium dermatitis in the Indian cohort. It interprets genetically endowed transcriptional capacity due to this particular single nucleotide polymorphism but does not support the prevalence of high serum levels of TNF- α in parthenium-induced skin allergic inflammation.

Introduction

Parthenium hysterophorus is an alien weed and major cause of airborne contact dermatitis, which poses a serious problem with little mortality but distress and morbidity. Contact sensitization due to the parthenin antigen from this weed leads to a cell-mediated hypersensitivity immune response (delayed type hypersensitivity) involving activated T lymphocytes, which culminates in injury to the skin. The disease is manifested as itchy erythematous papules, plaques, and a classical pattern (diffuse dermatitis) that primarily affects the exposed areas and flexures. The classic airborne contact dermatitis may change to photodermatitis resembling chronic actinic dermatitis or mixed pattern dermatitis. The disease effects become worst during the summer and rainy season, when the plant grows more profusely.^{1–6} During pathogenesis of the disease, the allergen first provokes a sensitization phase and then, after exposure to the same compound, an

elicitation phase appears later.⁷ The immune response is mediated by a series of cellular and molecular mechanisms. Epidermal Langerhans cells and other cutaneous dendritic cells transport the allergen from the skin to regional lymph nodes, where it is presented to T lymphocytes.^{8–10} The reaction is characterized by infiltration of T lymphocytes into challenged skin sites and the development of a cutaneous inflammation.¹⁰

The immune responses are regulated by a complex and intricate regulatory network involving various control elements such as pro- and anti-inflammatory cytokines. There is a dynamic and ever shifting balance exists between the two.^{11,12} An allergenic stimulus leads to the polarization of T-helper (T_H) lymphocytes into functional T_H1 and T_H2 subsets that determine direction of shift in balance of the cytokine network and nature of immune response.¹³ Cytokines are soluble signals of paramount importance that mediate cell–cell communication during inflammatory and immune responses.

Our previous reports of parthenium dermatitis depict elevated circulating levels of proinflammatory cytokines such as tumor necrosis factor (TNF)- α , interferon (IFN)- γ , interleukin (IL)-6, IL-8, IL-17, and decreased levels of anti-inflammatory cytokines such as IL-10 in patients, which suggests a decreased level of IL-10 might be sufficient for downregulating the proinflammatory signals and lead to a hypersensitivity immune response.^{14,15}

Among various factors such as gene transcription stability, post-translational modification, protein intracellular stability, and export of cytokine to the extracellular environment, the genetic polymorphisms in these cytokine genes significantly affect the parameters, which influence interindividual differences in cytokine levels and determine the balance between these and direct the human immune response. In a recently published study, we analyzed IFN- γ (+) 874 A>T and IL-10 (–) 1082¹⁶ G>A and (–) 819 C>T single nucleotide polymorphisms (SNPs) in parthenium dermatitis cases versus controls. The study showed that genetically IFN- γ (+) 874 A>T SNP does not justify the high serum levels of IFN- γ in patients with parthenium dermatitis in comparison to healthy controls. Whereas the lower producing genotypes due to IL-10 (–) 1082 G>A & (–) 819 C>T SNPs remain in step with the prevalence of low serum IL-10 in patients with parthenium dermatitis compared to controls and these genotypes genetically predispose to this disease. The intermediate IL-10 producing genotypes due to these SNPs in IL-10 genes provide resistance to carriers of these genotypes for not developing the disease.¹⁶

In the complex regulatory circuit of cytokines, TNF- α is a potent proinflammatory cytokine and appears to be important in allergic contact dermatitis, as it works as a powerful mediator of the inflammatory reaction. It might affect an immune response either directly, as it interacts with a wide range of cell types, or indirectly by the activation of several other cells.¹⁷ In earlier reports, altered levels of TNF- α have been involved in the pathogenesis of psoriasis.¹⁸ There are numerous polymorphisms found in the TNF- α promoter region, which might modulate TNF- α expression. The SNP at position (–) 308 G/A has been found to be functional, and the genetic predisposition due to this polymorphism to various diseases such as liver cir-

rhosis, gastrointestinal tract inflammations such as an Andom and Crohn's disease, rheumatoid arthritis, fatal meningococcal disease, cerebral malaria, and inflammatory bowel disease has been analyzed. This has also been studied in asthma and other allergic diseases such as occupational chronic irritant contact dermatitis and psoriasis.¹⁹

There is no report of an association study of TNF- α cytokine gene polymorphisms with airborne parthenium dermatitis. The present study is a first attempt at evaluating the contribution of TNF- α (–) 308 G/A polymorphism in the pathogenesis of parthenium dermatitis or its susceptibility in an Indian cohort.

Materials and methods

Subject cohort

A total of 120 subjects, including 60 patients exclusively diagnosed with parthenium dermatitis and 60 normal controls as healthy individuals, were included in the study. The patients included 44 men and 16 women with mean age of 45.43 ± 7.146 (range 28–62) years, and in the healthy controls, there were 51 men and nine women with a mean age of 43.63 ± 9.294 (range 26–63) years (Table 1). The statistical significance for mean age in cases versus controls was $P < 0.237$ and for both sexes, $P = 0.116$ with RR (95% CI) of 0.724 (0.502–1.043), were not significant. Patients with clinical symptoms suspected of airborne contact dermatitis who did not have any respiratory allergy such as rhinitis or asthma and any eczema problem and were not on any immunosuppressive therapy before diagnosis were included in this study. In addition, in all the subjects, patients and healthy individuals, the level of IgE was tested and compared. In controls versus cases, there was no statistically significant difference in IgE levels. These particular patients were further subjected to patch testing on upper back skin to ascertain the exclusiveness of allergy due to parthenium sensitivity. All included patients were enrolled at a special parthenium dermatitis allergy clinic at the Department of Dermatology and Venereology, All India Institute of Medical Sciences (New Delhi, India). Sixty healthy individuals were also selected from different departments at the All India Institute of Medical Sciences. All subjects were informed in detail about this research project, and written consent was obtained before commencing blood collection. The study was approved by our institutional ethical committee. Five milliliters of venous blood was taken from all subjects. Blood samples were drawn into tubes coated with anticoagulant such as EDTA just after diagnostic patch test readings (4 days) and before any immunosuppressive treatment of patients with parthenium dermatitis.

Patch testing

All the patients were patch tested with the standardized aqueous extract of *P. hysterophorus* to confirm the diagnosis as described

Table 1 Sex of study group

Sex	Parthenium patients <i>n</i> = 60 (%)	Healthy controls <i>n</i> = 60 (%)	<i>P</i> value
Males	44 (73.3)	51 (85.0)	0.116 (NS)
Females	16 (26.7)	09 (15.0)	

NS, not significant.

by Sharma *et al.*²⁰ Patients were patch tested on upper back with 28 other allergens of Indian Standard Series, including aqueous extract of plant allergens of compositae family (Parthenium, Xanthium, and Chrysanthemum) by using Ven der Bend square chambers provided by Systopic Laboratories Ltd. (New Delhi, India) and evaluated for reaction development in the same way as for parthenium antigen.²¹ Patch test readings were taken on days 2 and 4, and reactions were evaluated as per International Contact Dermatitis Research Group Criteria recommendations. Only those reactions that persisted till day 4 were considered to be positive for parthenium dermatitis.²⁰

Genotyping of tumor necrosis factor- α (-)308 G>A polymorphism

Genomic DNA was extracted using the commercial kit from Bioserve Biotechnologies (New Delhi, India), according to the manufacturer's instructions. The genotyping of the TNF- α (-) 308 G>A region was carried out by amplification refractory mutational system with a reaction for amplification of each allele (G or A) as described previously.²²

Statistical analysis

Genotypes and allele frequencies were compared between the study groups by χ^2 -test with Fisher's exact test when necessary. $P < 0.05$ was considered statistically significant.

Results

In this study, we analyzed the TNF- α (-) 308 G>A region by amplification refractory mutational system as shown in Figs. 1 and 2. The allelic and genotypic frequencies of both healthy controls and parthenium diseased individuals are shown in Tables 2 and 3. Both population, patient & control were in Hardy Weinberg equilibrium for the concern locus. Chi-squared test P values were 1.420 and 3.739 in controls and patients, which is still less than 3.84 (Table 4). In determining the polymorphism of TNF- α (-) 308 position (A and/or G alleles), the prevalent frequency of G allele in the control group

Table 2 Tumor necrosis factor- α (-) 308 G>A genotyping: allele frequency

	Parthenium patients <i>n</i> = 60 (%)	Healthy controls <i>n</i> = 60 (%)	<i>P</i> value	Odds ratio
G allele	99 (82.5)	104 (86.66)	NS	
A allele	21 (17.5)	16 (13.33)	0.371	0.73 (0.34–1.55)

NS, not significant.

Table 3 Tumor necrosis factor- α (-) 308 G>A genotyping: genotype frequency

Genotype	Parthenium patients <i>n</i> = 60 (%)	Healthy controls <i>n</i> = 60 (%)	<i>P</i> value	OR (95% CI)
GG	43 (71.66)	44 (73.33)	0.838	0.92 (0.38–2.21)
GA	13 (21.66)	16 (26.66)	0.522	0.76 (0.30–1.90)
AA	04 (6.67)	0 (0)	0.06	

was 86.66%, whereas in the patient group it was 82.5%. Allele A was present in controls at a frequency of 13.33% and in patients, 17.5%. Upon statistical comparison between cases versus controls, $P = 0.371$ with OR (95% CI) was 0.73 (0.34–1.55). The homozygous GG genotype was 71.66% in patients and 73.33% in healthy individuals, $P = 0.838$ with OR (95% CI) 0.92 (0.38–2.21). The heterozygous combination GA was 21.66% in patients and 26.66% of controls, $P = 0.522$ with OR 0.76 (0.30–1.90). The homozygous AA genotype was found 6.67% in patients and 0% of controls, $P = 0.06$. Thus, P values for either allele (G or A) and any genotypes of TNF- α (-) 308 G>A region were not significant between the groups. Upon comparison of GA genotypes with GG + AA combinations in cases and controls, the P value remained insignificant. Similarly, in the comparison of the GG carrier with the GA + AA combinations in

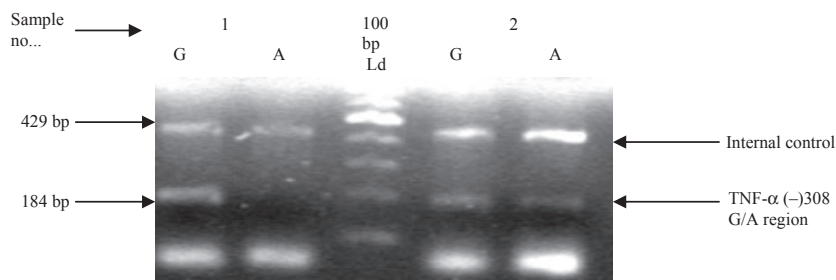
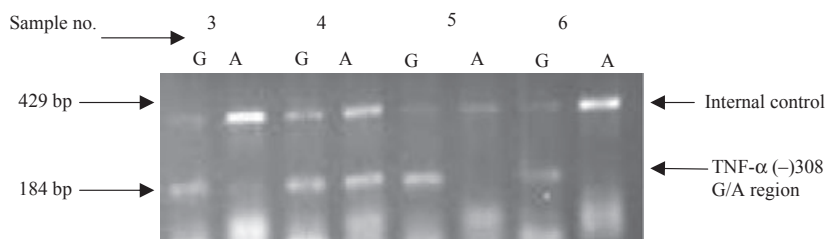


Figure 1 Tumor necrosis factor (TNF)- α (-) 308 G>A amplification refractory mutational system. Lanes 1 and 2 depict products from internal controls Human growth hormone (HGH) and G and A allele-specific reactions for sample 1. Lane 3 shows 100 bp ladder. Lanes 4 and 5 shows G and A allele-specific reactions for sample 2

Table 4 Interpretation of Hardy and Weinbergs Law in subject group

Study group	Observed frequency			Expected frequency			<i>P</i> value <3.84	Hardy–Weinberg equilibrium present/absent
	GG	GA	AA	GG	GA	AA		
Healthy controls (<i>n</i> = 60)	0.8667	0.1333	0.1333	45.067	13.867	1.067	1.420	Present
Parthenium patients (<i>n</i> = 60)	0.8250	0.1750	0.1750	40.838	17.325	1.838	3.739	Present

P value for both control and patient groups remains less than 3.84, which means that both groups follow the Hardy–Weinberg law.

**Figure 2** Tumor necrosis factor (TNF)- α (-) 308 G>A amplification refractory mutational system product of internal control Humam growth hormone (HGH) and G & A allele-specific reactions for samples 3–6 in lanes 1–8

cases versus controls, *P* value was also statistically insignificant. Therefore, for alleles as well as for genotypes, there was no statistically significant difference in their distribution in cases and controls.

Discussion

The disease condition of parthenium dermatitis due to various reasons altered the regulatory network of pro- and anti-inflammatory cytokines leading to cell-mediated hypersensitivity. TNF- α is one such cytokine whose circulating level remains increased in patients with parthenium dermatitis in comparison to healthy controls,^{14,15} which could be due to various polymorphisms found in the TNF- α promoter region. In earlier reports, an SNP at (-) 308 position with G>A transition has been shown to influence TNF- α expression; presence of allele A at this locus has been reported with a six- to sevenfold increased transcriptional activity and higher constitutive and inducible level of TNF- α .²³ The association of the TNF- α (-) 308 G>A polymorphism has been analyzed in many diseases. The presence of TNF- α A allele shows an association with diseases such as fatal meningococcal disease, cerebral malaria, and inflammatory bowel disease and imposes high circulating levels of TNF- α in their diseased patients.¹⁹ In rheumatoid arthritis, TNF- α (-) 308 G>A alleles were not associated with the disease, but other polymorphisms in the TNF- α gene were found associated with severity of disease but not with initial susceptibility of disease.²⁴ In other inflammatory conditions, TNF- α (-)

308 A allele association is evident in a range of diseases, including liver diseases (primary sclerosing cholangitis and cirrhosis) as well as in gastrointestinal tract inflammations (Andom and Crohn's disease), which show a positive association with the (-) 308 A allele. By contrast, in ulcerative colitis the (-) 308 A allele seems to have a protective effect.¹⁹ In allergic asthma and skin inflammatory conditions such as psoriasis, TNF- α (-) 308 alleles and genotypes, there is no difference in distribution among the control and patient group; hence, it was not associated with disease pathogenesis or susceptibility.^{25,26} However, in occupational chronic irritant contact dermatitis, the presence of TNF- α (-) 308 A allele was increased in the less exposed patient group. It suggests that in patients with high susceptibility, dermatitis appears at a lower exposure level compared to patients with normal susceptibility.²⁷ In the present study, the higher TNF- α producing AA genotypes compared to the other two types were least prevalent in 6.67% of patients but totally absent in healthy controls. The *P* value remained statistically insignificant. From previous reports, geographical variation is consistent with the frequency of TNF- α allele. It is present in approximately 5% of the population of South Africa compared with 30% in white Caucasians in the United Kingdom. Thus, the TNF- α A allele is less frequently found in the Indian and South African cohort.²⁸ In our population, the AA genotypes were least prevalent compared to the other two types of genotypes, and in controls they were even absent. When comparing the cases versus controls for these genotypes, the *P* value was

not significant. Therefore, the higher TNF- α producing homozygous AA combinations do not appear to be associated with parthenium-induced skin allergy. Although TNF- α is a potent proinflammatory cytokine and contributes to chronic inflammatory conditions in various diseases and in parthenium dermatitis, skin inflammation is also a prominent attribute, but this is genetically not conferred by higher TNF- α producing genotypes of the TNF- α allele. Under certain circumstances, where increased levels of TNF- α are triggered and if there is already a susceptibility to the disease through genetic endowment, then it might lead to more severe inflammatory conditions. Thus, it suggests that genetically endowed capacity does not support parthenium inflammation for TNF- α (-) 308 G>A locus. Concerning analysis of other genotypes, we found that homozygous combinations for TNF- α G allele in cases versus controls were 71.66 and 73.33%, respectively, and remained insignificant ($P = 0.838$) when compared with other genotypes. Whereas, the heterozygous combinations of GA were 21.66 and 26.66% in cases and controls respectively, upon comparison with other genotypes they also remained statistically insignificant ($P = 0.522$). This shows that homozygous combinations for the TNF- α G allele are more common in both the groups. In addition, heterozygous genotypes for both the TNF- α alleles are less prevalent in both the groups and do not appear to provide a protective role to individuals in preventing parthenium dermatitis.

In previous reports of inflammatory diseases such as rheumatoid arthritis the TNF- α alleles were not associated in many studies. However, in a Japanese study, TNF- α allele was increased in patients with RA by being dependent on the linkage to DRB1. An Australian study reported increased TNF- α alleles in patients with RA, whereas a Taiwan and Sweden study reported increased TNF- α alleles in patients with RA.²⁹ Studies conducted about different diseases in Indian cohorts show a similar frequency distribution of TNF alleles but different from that of Caucasians, Latin American, and Southeast Asian populations. In the Indian population, the TNF- α allele has a low frequency (4–7%) compared with a Dutch population where it is as high as 43.1%. In a case-control study of RA conducted within a north Indian cohort, the TNF- α allele was more prevalent in healthy individuals than patients and contributed a protective role.³⁰ In asthmatic conditions, there are mixed results of associations of TNF- α allele, whereas the Australian population shows an association of TNF- α alleles with asthma, as was the case in RA. Extensive studies suggest that extended haplotypes to the TNF locus account for an association of TNF SNPs with asthma.²⁹ Regarding leprosy in the Indian cohort, the TNF- α allele has been found associated with multibacillary leprosy, whereas in the Brazilian population

it was associated with resistance.³⁰ In Asian Indian patients with sarcoidosis, TNF- α (-) 308 G>A polymorphism was found to affect serum TNF- α levels. Higher TNF- α levels in patients were associated with TNF- α G alleles, whereas in controls no such association was found. Thus high TNF- α levels in GG homozygous patients could be under any other influence than the effect of concerned polymorphism. In addition, there was no association of this polymorphism with the disease in this cohort.³¹

Although TNF- α plays a pivotal role in the inflammatory response, it is only one component of the entire pro- and anti-inflammatory network. Its production is regulated by other cytokines such as IFN- γ , IL-12, IL-10, and transforming growth factor- β . In functional terms, the active TNF- α amount matters not the total one, and its activity is modulated by soluble TNF receptors that inhibit its activity but increase its stability and persistence. The active fraction of TNF- α then triggers a cascade of other immunological events. Thus, soluble TNF receptors contribute in deciding the actual active TNF- α amount in understanding a given disease.¹⁹ Therefore, polymorphisms in TNF receptors also might contribute to causing the disease.

Conclusion

A thorough analysis is required to understand the role of TNF genetics in the pathogenesis of parthenium dermatitis. The present study concludes that there was no genetic endowment of TNF- α (-) 308 G>A polymorphism in the pathogenesis/susceptibility of parthenium dermatitis in the Indian cohort under study.

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