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Original Article

Association of *MIF* gene polymorphisms with pemphigus vulgaris: a case-control study with comprehensive review of the literature

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Abstract: Background: Functional *macrophage migration inhibitory factor* (*MIF*) gene polymorphisms are associated with elevated serum levels of MIF and increased susceptibility to various autoimmune diseases. MIF levels in the sera of pemphigus vulgaris (PV) patients are increased; however, no definite association has been demonstrated between PV and *MIF* gene polymorphisms. The present study was conducted to ascertain any association between *MIF*-173*G-C and *MIF*-794*$CATT_{5-8}$ polymorphisms and PV. Methods: Seventy-five patients with PV and 252 healthy, unrelated, voluntary controls were enrolled randomly in the study. *MIF*-173*G-C polymorphism (rs755622) was genotyped using polymerase chain reaction (PCR) followed by restriction fragment length analysis, and *MIF*-794*$CATT_{5-8}$ (rs5844572) was genotyped using PCR followed by capillary gel electrophoresis. Subsequently, the allelic, genotype, and haplotype frequencies were determined and compared for both groups. Data were also analyzed with respect to sex, age at onset, type of disease, and duration of disease. Results: No significant association was observed in terms of allelic, genotype, and haplotype frequencies of MIF gene polymorphisms in PV patients. However, a significantly lower prevalence of the C allele (P=0.02) and $CATT_7$ allele (P=0.03) was seen in our patient population compared to healthy controls. Analysis of the effect of various factors such as gender, age at onset, type of disease, and disease duration revealed no significant association with the genetic variants. Conclusions: MIF-173*G-C and -794*$CATT_{5-8}$ polymorphisms are not associated with PV.

Keywords: Pemphigus vulgaris, macrophage migration inhibitory factor, macrophage migration inhibitory factor gene polymorphisms, *MIF*-173*G-C, *MIF*-794*CATT

Introduction

Pemphigus vulgaris (PV) is an autoimmune blistering skin disease mediated primarily through humoral immunity, in particular by B-cells that produce autoantibodies against a variety of keratinocyte antigens, such as desmoglein (Dsg) 1 and 3 and acetylcholine receptors [1].

Macrophage migration inhibitory factor (MIF), a multifunctional cytokine expressed by both immune and non-immune cells, is encoded by the human *MIF* gene at chromosome 22q11.2. Additionally, MIF has an already proven association with activation of B-cells [2, 3]. Similar to other inflammatory diseases, patients with PV reportedly have higher levels of MIF in their sera [4]. Therefore, there might be an important, though uncharacterized, role of MIF in the etiopathogenesis of this disease. In order to elucidate such a putative role, it is important to explore the association of PV with the *MIF* gene polymorphisms. Thus, we hypothesized that there might be an association between MIF and PV that could be relevant to our understanding of the pathophysiology of this autoantibody-mediated blistering disease.

Furthermore, it has been observed that mutations in the *MIF* gene are associated with diverse responses. Loss-of-function mutations of the *MIF* gene hamper the development of efficient innate host immune and inflammatory responses. Alternatively, gain of function *MIF*

mutations may predispose the host to more severe inflammatory and immune reactions [5, 6]. However, there is a paucity of literature on the association of *MIF* gene polymorphisms with PV. Hence, the present study was undertaken to determine the association of different alleles, genotypes, and haplotypes of *MIF* gene -173*G-C and -794*CATT polymorphisms with PV.

Materials and methods

This was a prospective case-control study conducted over 12 months. The study was approved by the Institute Ethics Committee (Approval number: PGI/IEC/2013/1729-30), and informed consent was obtained from all the enrolled cases and healthy controls.

Sample size calculation

The sample size was calculated using Open Epi, Version 3 (public domain web-based online size calculator). The sample size was calculated at an alpha error of 0.05 and power of 85% and the ratio of controls to cases 3:1, taking the expected odds ratio of 5.71 for *MIF* gene polymorphisms and assumption that proportion of controls and cases with MIF gene polymorphisms 85% and 97% respectively. The total sample size calculated (Fleiss with continuity correction) was 284 with 71 cases and 213 controls. It was decided to include extra subjects for possible dropouts, so the sample size was 75 cases and 252 healthy controls.

Inclusion criteria for cases

• All the patients with a clinical diagnosis of PV confirmed on histopathology and/or direct immunofluorescence (DIF), presenting to the dermatology outpatient clinic, were included as cases.

Exclusion criteria for cases

• Patients with coexistent infections, including hepatitis B, hepatitis C, human immunodeficiency virus, active tuberculosis or sepsis; abnormal liver and renal function tests; known cardiac arrhythmias or conduction abnormalities were excluded.

• Patients without a histopathologic and/or DIF confirmation of the PV diagnosis were excluded.

• All patients with blistering autoimmune diseases other than PV were excluded.

Inclusion criteria for controls

• Age and sex-matched healthy volunteers (almost thrice the number of cases) were enrolled as controls.

Sample collection

Under aseptic precautions, peripheral blood samples were obtained consecutively from patients presenting to the dermatology outpatient clinics, with histopathology and/or direct immunofluorescence proven PV and from healthy unrelated voluntary controls [7].

Clinical details

The following demographic details were recorded for all patients: sex, age, age at onset, total duration of illness, severity, and status of illness at the time of sampling. The disease severity was evaluated using the autoimmune bullous severity index score (ABSIS) system for cutaneous and mucous disease separately.

Sample processing

Sera were separated and stored at -20°C until further processing.

DNA extraction

Genomic DNA was extracted manually using the phenol-chloroform-isoamyl alcohol (PCI) method. The first step included cell and nuclear membrane lysis and protein denaturation using proteinase K, ammonium acetate, and sodium dodecyl sulphate. This was followed by separating DNA from the precipitated proteins and lysed cell debris using a mixture of phenol, chloroform, and isoamyl alcohol. This step separated the DNA into the aqueous phase. The next step was purifying the separated DNA using isoamyl alcohol and then its precipitation using chilled ethanol. The purified and precipitated DNA was then dissolved in Tris-ethylenediamine tetra acetic acid buffer. Subsequently, the concentration of this extracted DNA was estimated using a UV spectrophotometer.

Genotyping of MIF gene polymorphisms

We assessed the prevalence of the following *MIF* gene polymorphisms:

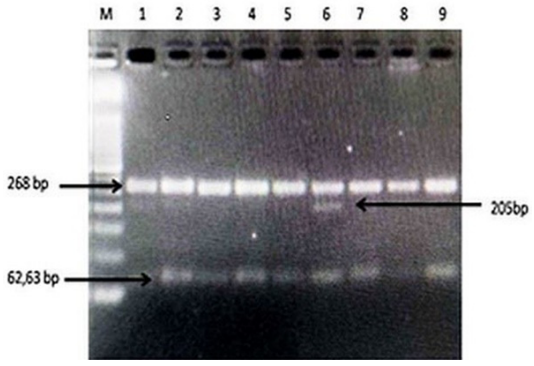

Figure 1. Representative image of 3% agarose gel subjected to electrophoresis showing PCR-RFLP amplified products. Lane 1 to 9 shows PCR-RFLP amplified products in patient samples. Lane 1-5 and 7-9 shows PCR-RFLP amplified products in homozygous patients with GG genotype (268 bp, 62 bp) and lane 6-shows heterozygous individual with GC genotype confirmed by presence of digested products of 268 bp, 205 bp, 63 bp, and 62 bp.

1. *MIF*-173*G-C polymorphism.

2. *MIF*-794*CATT repeat polymorphism.

*MIF-173*G-C polymorphism*

This polymorphism was detected using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). The sequence of primers was taken from the published literature [8]. The following primers were used: Forward primer: 5' ACTAAGAAAGACCCGAGG 3'; Reverse primer: 5' TGGCAGAAGGACCAGGAGAC 3'.

PCR cycling conditions consisted of 34 cycles of 98°C for 30 seconds, 58°C for 20 seconds, and 72°C for 30 seconds each, preceded by a single cycle of 98°C for 5 minutes and followed by a single cycle of 72°C for 8 minutes. The PCR product (330 bp) was digested with AluI (NEB) restriction enzyme at 37°C, overnight. Subsequently, the digested products were electrophoresed using 3% agarose gel followed by visualization under UV transillumination. The 330 bp PCR products have a consistent restriction site resulting in 62 and 268 bp fragments. The GG genotype does not have a second cutting site for AluI. The CC genotype has a second cutting site, resulting in three fragments of sizes 205, 62, and 63 bp. The heterozygous GC genotype was characterized by four bands: 268, 205, 62, and 63 bp [**Figure 1**]. Thus, one of the following RFLP patterns could be observed:

• GG: 2 bands corresponding to 268 and 62 bp.

• GC: 4 bands corresponding to 268, 205, 63, and 62 bp.

• CC: 3 bands corresponding to 205, 63, and 62 bp.

*MIF-794*CATT repeat polymorphism*

A polymerase chain reaction followed by capillary gel electrophoresis was performed. The sequence of primers was taken from the published literature [8]. The forward primer was pre-labeled with a FAM fluorescent dye. The following primers were used to amplify a 207 bp ($CATT_5$) segment.

Forward primer: 5' TTG CAC CTA TCA GAG ACC 3'; Reverse primer: 5' TCC ACT AAT GGT AAA CTC G 3'.

PCR cycling conditions consisted of 34 cycles of 98°C for 30 seconds, 59.1°C for 30 seconds, and 72°C for 35 seconds each, preceded by a single cycle of 98°C for 5 minutes and followed by a single cycle of 72°C for 8 minutes. Finally, the amplified product was pooled with the GeneScan™ 500 LIZ™ dye Size Standard (Applied Biosystems™). Electrophoresis was performed on a 50 cm capillary array on the 3130xl Genetic analyzer (Applied Biosystems™, ThermoFisher Scientific, MA, USA). The electrophoresis was done at 15 kV for 30 minutes. Semi-automated genotyping was carried out using Gene Mapper Software v4.0 and later was manually rechecked.

Statistical analysis

The Hardy Weinberg equilibrium (HWE) was analyzed for each MIF gene polymorphism by an online HWE calculator (http://www.oege.org/software/hwe-mr-calc.shtml). The strength of association between alleles or genotypes of MIF gene polymorphism and different groups was estimated using odds ratios (OR) and 95% confidence intervals (CI). All analyses were carried out using SPSS software (IBM Corp. Released 2013. IBM SPSS, Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.)

Table 1. Demographics and clinical features of patients with pemphigus vulgaris (n=75)

Variable	Frequency
Gender (men:women ratio)	29:46
Mean age ± *standard deviation* (years)	46.68±12.9
Age range (years)	18-75
Mean age of onset ± *standard deviation*; range (years)	43.2±13.2; 7-72.5
Clinical type of pemphigus vulgaris	
Mucocutaneous	52 (70%)
Cutaneous	13 (17.5%)
Mucosal	10 (12.5%)
Severity of disease*	
ABSIS score, cutaneous (Mean ± standard deviation, range)	12.9±14.1, 0-60
ABSIS score, mucosal (Mean ± standard deviation)	3.85±3.23, 0-10
Total duration of illness *(Mean ± standard deviation, range)*	29.03 months, 2 months-5 years
Status of disease at the time of sampling	
Active disease	42 (56.5%)
Partial remission	12 (16.3%)
Complete remission	21 (27.2%)

*The Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) scores were available in 40 patients only.

and Graph Pad Prism® software (v4.0; GraphPad Inc., La Jolla, CA, USA). All the numerical data are presented as the mean ± standard error. *P*-value <0.05 was considered significant. Chi-Square test was used to compare the allele and genotype frequencies between patients and healthy controls. The haplotype analysis was performed by PHASE, version 2.1 (http://www.stat.washington.edu/stephens/software.html). The association between different independent and dependent variables was determined using Pearson's chi-square test (Fisher's exact test in appropriate cases). Independent variables that were statistically significant were considered for bivariate analysis to calculate unadjusted odds-ratios that were then used for logistic regression modeling to determine the significant predictors.

Results

The study group comprised 75 PV patients and 252 ethnically related and age-matched healthy controls. The demographic and clinical characteristics of patients are summarized in **Table 1**. PV patients showed a significant female predominance with 30 (40.0%) males and 45 (60.0%) females in comparison to the healthy control population (P=0.001). The mean age of patients at the time of presentation was 46.68±12.9 years. The maximum number of patients (23; 30.7%) were in 41-50 year age-group; 37 patients (49.3%) were <45 years. The mean age of onset for pemphigus was 43 years. The most common clinical subtype of PV was the mucocutaneous type (52 patients; 70%). Forty-two patients (56%) presented with active disease at the time of enrollment.

The ABSIS scores were not available for some patients (n=35). This was because ABSIS scoring was adopted as a routine procedure after around six months of starting the sample collection for this study. In the remaining 40 patients wherever it was available, the mean ABSIS score for the cutaneous disease was 12.9±14.1, ranging from 0 to 60, and for mucosal disease, it was 3.85±3.23, ranging from 0 to 10.

Genotype analysis for *MIF* polymorphisms revealed no deviation of observed genotype frequencies from the Hardy-Weinberg equilibrium in either the PV patients or the healthy controls (P>0.05). The most common allele for *MIF*-173*G-C polymorphism amongst the patients and the controls was G, with a frequency of 83.33% and 74.60%, respectively. However, the C allele was found to be significantly less in patients (16.67%) as compared to the control population (25.39%) (P=0.02). Among the genotypes, the GG genotype was most commonly encountered in both patients (68.00%) and controls (55.15%). GC and CC genotypes were

Table 2. Distribution of alleles and genotypes of MIF-173*G-C polymorphism among pemphigus vulgaris patients and healthy controls

Alleles MIF-173*G-C	Patients (%) (n=150)	Controls (%) (n=504)	Odds Ratio (95% Confidence intervals)	*P* value
G	125 (83.33)	376 (74.60)	Reference	Reference
C	25 (16.67)	128 (25.39)	0.58 (0.36-0.94)	0.02
Genotypes MIF-173*G-C	Patients (%) (n=75)	Controls (%) (n=252)	Odds Ratio (95% Confidence intervals)	*P* value
G/G	51 (68.00)	139 (55.15)	Reference	Reference
G/C	23 (30.67)	98 (38.89)	0.64 (0.36-1.11)	0.11
C/C	1 (1.33)	15 (5.95)	0.18 (0.02-1.41)	0.06
G/C+C/C	24 (32.00)	113 (44.84)	0.57 (0.33-0.99)	0.04

MIF: Macrophage migration inhibitory factor.

Table 3. Distribution of alleles and genotypes of MIF-794*CATT repeat polymorphism among patients with pemphigus vulgaris and healthy controls

Alleles MIF-794*CATT	Patients (%) (n=150)	Controls (%) (n=504)	Odds Ratio (95% Confidence intervals)	*P* value
5	35 (23.33)	109 (21.62)	Reference	Reference
6	102 (68.00)	308 (61.11)	1.03 (0.66-1.60)	1.00
7	13 (8.67)	87 (17.26)	0.46 (0.23-0.93)	0.03
8	-	-	NA	NA
Genotypes MIF-794*CATT	Patients (%) (n=75)	Controls (%) (n=252)	Odds Ratio (95% Confidence intervals)	*P* value
5/5	3 (4.00)	11 (4.36)	Reference	Reference
5/6	26 (34.67)	63 (25.00)	1.51 (0.38-5.80)	0.75
5/7	3 (4.00)	24 (9.52)	0.45 (0.08-2.64)	0.39
5/8	-	-	NA	NA
6/6	33 (44.00)	100 (39.68)	1.2 (0.31-4.60)	1.00
6/7	10 (13.33)	45 (17.85)	0.81 (0.19-3.47)	0.71
6/8	-	-	NA	NA
7/7	-	9 (3.57)	NA	0.12
7/8	-	-	NA	NA

MIF: Macrophage migration inhibitory factor; NA: not available.

less commonly seen in the patients (30.67% and 1.33%, respectively) than in the control group (38.89% and 5.95%, respectively). However, comparing *MIF*-173 genotype frequencies between patients and controls showed no significant difference ($P>0.05$). Allelic and genotype frequencies for the *MIF*-173*G-C polymorphism in PV patients and controls are shown in **Table 2**.

For *MIF*-794*CATT polymorphism, the most commonly observed allele was $CATT_6$ in both the cases and the control group. An important observation was that the $CATT_7$ allele was significantly less frequent among patients with PV (8.67%), in contrast to healthy controls (17.26%) (P=0.03). The most commonly observed genotype among PV and control groups was 6/6. The frequencies of genotypes 5/7, 6/7, and 7/7 were higher among controls than the patients; however, the difference was not significant ($P>0.05$). Hence, no significant association was found between any of the CATT genotypes and susceptibility to PV. Allelic and genotype frequencies for the *MIF*-794*CATT repeat polymorphism in PV patients and controls are shown in **Table 3**.

We also studied the combined effects of the -794*CATT and the -173*G-C polymorphisms in our cohort of 75 patients with PV. We observed a total of 6 haplotypes in our study population. The haplotype analysis along with respective odds ratios and *P*-values is presented in **Table 4**. We observed that 6G was the most frequent haplotype while 5C was the least frequent, both among patients and healthy controls. However, no significant difference was observed in the distribution of haplotypes amongst the patients and controls (P>0.05). Additionally, no significant differences were observed in the distribution of various

Table 4. Haplotype frequencies of MIF gene polymorphisms among patients with pemphigus vulgaris and healthy controls

Haplotype MIF	Patients (%) (n=150)	Controls (%) (n=504)	Odds Ratio (95% Confidence intervals)	*P* value
5G	33 (22.00)	103 (20.43)	Reference	Reference
5C	1 (0.66)	4 (0.79)	NA	NA
6G	89 (59.33)	255 (50.59)	1.08 (0.68-1.72)	0.81
6C	13 (8.66)	54 (10.71)	0.75 (0.36-1.54)	0.48
7G	3 (2.00)	17 (3.37)	NA	NA
7C	11 (7.33)	71 (14.08)	0.48 (0.22-1.02)	0.05

MIF: Macrophage migration inhibitory factor; NA: not available.

genotypes of both the MIF gene polymorphisms studied in terms of sex distribution, duration of disease, the onset of disease, or type of disease ($P>0.05$).

Discussion

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that plays diverse roles, including the enhancement of TLR4 expression, phagocytosis, intracellular killing, and the production of nitric oxide, H_2O_2, and TNF-α in macrophages [3]. It also acts as a potent counter-regulator of the anti-inflammatory and immunosuppressive actions of corticosteroids [9]. Gore et al. have shown that MIF regulates the adaptive immune response by inducing B-cell survival, maintaining the mature B-cell population [4]. MIF has been recently characterized as a novel B-cell chemokine comparable to CXCL12 and CXCL13 (classical B-cell chemokines) in extent and kinetics [5].

Four polymorphisms of the human *MIF* gene have been reported to date, including the 5-8-CATT tetranucleotide repeat at position -794 (-794*$CATT_{5-8}$) and 3 single-nucleotide polymorphisms (SNPs) at positions: -173 (-173*G-C), +254 (+254*T-C) and +656 (+656*C-G). The +254 and +656 SNPs are positioned in introns and, thus, do not affect the coding sequence of the *MIF* gene [10, 11]. Of these, two functional polymorphisms of the human *MIF* gene have been implicated in different diseases: -794*$CATT_{5-8}$ microsatellite tetranucleotide repeat and the -173*G-C polymorphism, which are associated with increased susceptibility and increased severity of juvenile idiopathic and adult rheumatoid arthritis, ulcerative colitis, psoriasis, multiple sclerosis, systemic sclerosis, glomerulonephritis, and sarcoidosis.

A comprehensive search of the literature to date revealed only a single study wherein the association of *MIF*-173*G-C polymorphism with PV has been studied [12]. The authors did not find any significant difference between patients and controls regarding the allelic and genotype frequencies. The authors, however, did not study the association of MIF-794*CATT polymorphism with PV. To the best of our knowledge, ours is the first study that has assessed the association of the two most common functional *MIF* gene polymorphisms with PV. We observed no significant association between any alleles, genotypes, or haplotypes concerning increased susceptibility to develop PV.

Due to the unavailability of sera from subjects, resources, and technical limitations, we could not measure the serum levels of MIF in our study population. Hence, we could not compare these levels with the polymorphisms, nor could we assess the functional influence of *MIF*-173*G-C transition (rs755622) and -794*CATT 5-8 repeats (rs5844572). If serum MIF levels had been available, some conclusive remarks could have been made to distinguish cases from controls based on particular polymorphic sites and their influence, either in disease causation or outcome. The reported mutations in our studied population might be producing a non-functional protein or leading to a defective downstream signal transduction pathway. This is a significant limitation of our study as the absence of these data has limited the complete elucidation of the role of *MIF* in the pathogenesis of PV. Another important limitation of our study is that the association of disease severity with genetic variants of *MIF* could not be studied due to the lack of ABSIS scores in 35 patients.

Table 5. Review of literature showing distribution of allele and genotype frequencies of MIF-173*G-C and -794*CATT repeat polymorphisms in healthy controls among different populations across the world

Population	Sample size	MIF-173*G-C					MIF-794*CATT													
		G	C	GG	GC	CC	5	6	7	8	5/5	5/6	5/7	5/8	6/6	6/7	6/8	7/7	7/8	8/8
US, 2002 [11]	159	-	-	-	-	-	27.7	60.7	11	0.6	-	-	-	-	-	-	-	-	-	-
Spain, 2002 [13]	122	85.0	15.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Germany, 2004 [14]	390	79.0	21.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Japan, 2004 [15]	750	80.7	19.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Japan, 2004 [16]	155	77.7	22.3	-	-	-	39.4	42.6	17.4	0.6	-	-	-	-	-	-	-	-	-	-
UK, 2004 [17]	342	88.0	12.0	-	-	-	25.3	65.6	8.8	0.3	-	-	-	-	-	-	-	-	-	-
US, 2005 [18]	270	83.9	16.1	-	-	-	23.5	63.9	11.9	0.7	-	-	-	-	-	-	-	-	-	-
Spain, 2006 [19]	755	86.8	13.2	-	-	-	26.6	64.3	9.0	0.1	-	-	-	-	-	-	-	-	-	-
China, 2007 [20]	496	77.7	22.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Columbia, 2007 [21]	235	74.9	25.1	-	-	-	23.4	57.4	19.2	0	-	-	-	-	-	-	-	-	-	-
Spain, 2007 [22]	886	85.0	15.0	-	-	-	27.0	65.0	8.0	0	-	-	-	-	-	-	-	-	-	-
UK, 2008 [23]	378	87.1	12.9	-	-	-	25.4	65.5	9.1	0	-	-	-	-	-	-	-	-	-	-
China, 2009 [8]	263	79.1	20.9	61.3	35.7	3.0	42.2	41.3	16.5	0.2	17.5	36.4	13.1	0	16.0	14.3	0	2.3	0	0
India, 2013 [24]	164	-	-	-	-	-	28.5	65.5	5.8	0	12.1	32.9	0	0	43.9	10.3	0	0.6	0	0
Iran, 2013 [12]	100	74.5	25.5	59.0	31.0	10.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
China, 2016 [25]	603	77.9	22.1	60.9	34.0	5.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saudi Arabia, 2016 [26]	202	76.0	24.0	60.3	31.6	7.9	28.0	62.0	7.0	2.0	9.4	31.6	3.4	1.9	40.6	8.9	2.9	1.0	0	0
China, 2017 [27]	40	86.2	13.75	75.0	22.5	2.5	40.0	52.5	7.5	-	10.0	55.0	5.0	-	20.0	10.0	-	-	-	-
China, 2017 [28]	186	-	-	-	-	-	35.5	51.1	13.2	0.27	17.6	31.8	13.6	-	17.0	14.8	0.54	4.0	-	-
Japan, 2017 [29]	728	-	-	-	-	-	38.2	45.3	16.5	-	14.5	34.5	13.0	-	21.7	12.8	-	3.6	-	-
Turkey, 2017 [30]	100	78.0	22.0	58.0	40.0	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
India, [Present study and 7]	252	74.6	25.4	55.1	38.9	5.9	21.6	61.1	17.2	0	4.3	25.0	9.5	0	39.7	17.8	0	3.6	0	0

The reported allelic and genotype frequencies of these two polymorphisms in cohorts of healthy controls from different countries across the world have been summarized in **Table 5** [13-30]. Of all the alleles, the frequency of the $CATT_8$ allele is the lowest (<1%) in all the ethnic groups. We did not encounter the $CATT_8$ allele in our patients or in controls [7]. This probably indicates an ethnic variation in the prevalence of these alleles. In all populations studied, the -173*G allele (75-90%) was far more common than the -173*C allele. Of note, the frequency of the -173*C allele was almost twice as high in Asian countries' studies compared to those from western countries (12-16%). Similarly, the frequency of the $CATT_7$ allele was higher in healthy controls in studies from China, Japan, and Columbia compared to western countries [**Table 5**]. Our data are in concordance with these reports.

Although many studies have suggested a positive association between specific *MIF* alleles and increased susceptibility to certain immune-mediated diseases, a few studies negate an association between MIF alleles and some autoimmune diseases, thereby indicating the role of *MIF* polymorphisms in different diseases is highly pleiotropic [12, 26, 31, 32]. In the present study, we did not observe any significant association of PV with the known *MIF* gene polymorphisms. Nevertheless, we believe that the role of MIF in the pathogenesis of PV cannot be entirely excluded although no significant association was noted between *MIF* polymorphisms and PV in our study. Probably, some alternative mechanisms exist that contribute to the elevation of MIF levels in the sera of PV patients; however, these mechanisms are not yet well-elucidated.

Conclusions

The present study showed no significant association between PV and *MIF* gene polymorphisms. A significantly higher prevalence of *MIF*-173*C allele and $CATT_7$ allele was noted in our healthy controls and healthy control populations from other continents, as reported in the literature. Our observations, since this was a pilot study, need validation by more extensive population-based studies combined with functional analyses.

Disclosure of conflict of interest

None.

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