Molecular Vision 2022; 28:526-535 <http://www.molvis.org/molvis/v28/526>
Received 8 January 2022 | Accepted 29 December 2022 | Published 31 December 2022

Chronic ocular sequelae in Stevens–Johnson syndrome: a genetic association study

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Purpose: This study sought to investigate the association of molecular markers with chronic ocular sequelae in Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN).

Methods: One hundred SJS/TEN patients (200 eyes) with confirmed diagnosis were enrolled between July 2011 and July 2015 from a tertiary eye-care hospital, and their clinical histories were noted. Each eye was scored for severity of manifestation on a scale of 0–5. Peripheral blood samples were collected for DNA followed by screening for interleukins (IL-4, IL-13, IL-4R) polymorphisms, HLA-A locus allele typing, and sera to detect levels of the apoptotic markers granulysin and sFas L.

Results: Of the 100 enrolled patients (53 males/47 females; age range: 6–58 years), the incriminating drugs were non-steroidal anti-inflammatory (52%), antibiotics (10%), sulphonamides (8%), anti-epileptics (6%), and unknown (24%). Significant differences in the frequencies of IL-4R polymorphism, HLA-A*3301, HLA-A*02, and HLA-A*2402 alleles, and elevated levels of granulysin and sFas L were observed in patients compared to controls. The ocular complications of conjunctival keratinization (p=0.004) showed an association with IL-13 promoter region (IL-13a) genotypes.

Conclusions: The study highlights the possible association of interleukin-13 with severity-graded chronic sequelae and the role of HLA-A alleles- HLA-A*3301, HLA-A*02, and HLA-A*2402 in SJS/TEN causation and manifestation. Screening of these alleles may help caregivers to identify alleles associated with severe and lifelong ocular complications, and help in appropriate treatment and management of the condition.

Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) is a severe, acute, blistering reaction of the skin and mucous membranes. It is mainly ascribed to drugs and characterized by high mortality and morbidity, with widespread epidermal detachment ranging from mild (1%–10% of the total body surface area) in SJS to severe (>30%) in TEN and ocular complications resulting in serious lifelong sequelae in 20% to 70% of survivors. The acute phase is characterized by lesions of the skin and mucosa with active inflammation of the ocular surface, resulting in severe conjunctivitis and persistent epithelial defects of the cornea. The chronic phase involves healed skin lesions with persistent complications, such as severe dry eye, symblepharon, and ankyloblepharon, conjunctival invasion into the cornea, and partial or complete keratinization of the ocular surface, leading to visual debilitation or irreversible blindness.

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The chronic complications have a multifactorial origin due to differences in access and quality of acute care. Ethnicity and genetic variations also play a vital role in the causation and subsequent resolution of the condition. The hypersensitivity reactions are triggered by T-cell-mediated drug-specific immune response to a particular condition, which produces a variety of cytokines, including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-13 (IL-13), IL-6, and IL-10, leading to a specific clinical phenotype. Studies have documented the association of single nucleotide polymorphisms (SNPs) of several immune-related genes and human leukocyte antigen (HLA) alleles with severe ocular surface manifestations in SJS/TEN patients [1,2], which may thus be used as genetic markers for studying individual susceptibility as well as for pretreatment screening. The present study aimed to assess the genetic and biochemical markers associated with chronic ocular sequelae in SJS/TEN patients.

METHODS

The study was conducted between July 2011 and July 2015, in accordance with the tenets of Declaration of Helsinki and approved by the ethics committee of All India Institute of Medical Sciences (AIIMS). Informed consent was obtained from all subjects before enrollment.

The study included 100 patients recruited from a tertiary eye-care center after a confirmed diagnosis of SJS/TEN with ocular surface complications that persisted for at least one year from onset. Diagnosis was based on a history of acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least two mucosal sites, including the ocular surface.

All patients underwent detailed ocular examination and assessment using vision test charts, Schirmer's test, slit-lamp examination, and clinical photographs of the eyes. Chronic ocular surface complications were categorized into three groups based on their localization: 1) the cornea (loss of palisades of Vogt (POV), conjunctivalization, vascularization, keratinization, superficial punctate keratopathy (SPK), and opacification); 2) the conjunctiva (hyperemia, conjunctival keratinization, and symblepharon formation); and 3) the eyelids (with involvement of the meibomian gland, mucocutaneous junction, and puncta).

The severity of each ocular complication was scored on a scale of 0–5 and recorded on a pre-designed proforma according to the modified grading system of Sotozono et al. [3,4]. The recorded data of the worse eye of all patients were chosen for analysis. The control group consisted of age- and sex-matched healthy volunteers without an individual or family history of any ocular disease or drug reactions and related manifestations. Detailed clinical and family histories were noted for all patients, pedigree charts were drawn, and peripheral blood samples were collected (7 ml).

Genomic DNA was isolated from 5 ml of peripheral blood using the standard protocol [5], and 2 ml of the blood was used for serum separation. DNA was subjected to PCR amplification of interleukins IL-4, IL-4R, and IL-13 promoter and coding regions using specific primers [6]. Reactions were performed using 100–120 ng DNA, 1.5 mM $MgCl_2$, 0.2 mM each of the dNTPs (Invitrogen, USA), 0.5 µM of primers, and 0.5 units of Taq polymerase (Invitrogen) in a 25-µl volume using an ABI 9700 thermocycler (Applied Biosystems).

The PCR products were purified using QIAamp gel extraction kits (Qiagen, Germany) and sequenced directly with BigDye Terminator Mix Version 3.1 (Applied Biosystems) according to the manufacturer's instructions and then analyzed using an ABI-3100 Genetic Analyzer (Applied Biosystems). Nucleotide sequences were compared with published cDNA sequences of the IL-4 (GeneBank accession number ENSG00000113520), IL-4R (GeneBank accession number ENSG00000077238), and IL-13 genes (GeneBank accession number ENSG00000169194).

HLA-A locus genotyping was performed using bead-based typing kits. Specifically, PCR amplification was followed by hybridization with sequence-specific oligonucleotide probes (PCR-SSO) using LABType SSO A Locus typing kits (One Lambda Inc., USA). The analysis and interpretation of results were performed using One Lambda HLA Fusion Software v 3.5. Quantitative estimation of apoptotic markers granulysin and sFas L was done for the 50 SJS/TEN patients and controls using a sandwich enzyme-linked immunosorbent assay (ELISA; USCN Life Science Inc., PRC) following the manufacturer's protocol. Statistical analysis was conducted using Stata IC/15, and data are shown as either median (interquartile range [IQR]/Range) or mean ± standard deviation (SD). The association of the SNPs of interleukins and HLA alleles was assessed using a Chi-square test or Fisher's exact test as appropriate. A Mann–Whitney U test was performed to compare the distributions of ocular manifestations across the interleukin genotypes and all the results were considered significant at $p<0.05$. Bonferroni correction of p-values was applied to adjust for multiple comparisons.

RESULTS

The study enrolled 100 patients with chronic ocular SJS/TEN (n=100, 198 eyes), including 53 males and 47 females with a median age of 22.0 (6.0–58.0) years, along with 100 healthy controls with a median age of 28.0 (15.0–77.0) years. The duration of disease onset was 7.0 (2.0–32.0) years.

Drugs were the most common triggering factors, primarily non-steroidal anti-inflammatory drugs (NSAIDs) (52% including antipyretics), followed by antibiotics (10%), sulphonamides (8%), and anti-epileptics (6%). In about 24% of patients, the nature of the offending drug could not be determined, but a history of febrile illness was reported before the onset of the condition.

The patients were evaluated for 12 different ocular sequelae of the cornea, conjunctiva, and eyelids and given a severity score from 0 to 5 (Table 1). Complications were documented, including active corneal ulcer or healed keratitis (27%), pyogenic granuloma (2%), cataract (6%), glaucoma (2%), lipid keratopathy (3%), anterior staphyloma (2%), and pterygium (1%).

The study screened the interleukin genes IL-4 and IL-13 and their common receptor IL-4Rα for the presence of

Table 1. Details of the chronic ocular sequelae in SJS/TEN patients (n=98 eyes).

Ocular complications Severity grade (0–5)	Severity Grade, N(%)					
	Absent (0)	Mild (1)	Moderate (2)	Severe (3)	Very severe (4)	Severe most (5)
Loss of palisades of Vogt (POV)	12(12.2)	16(16.3)	16(16.3)	28(28.6)	20(20.4)	6(6.2)
Conjunctivalization (CONC)	12(12.2)	15(15.3)	17(17.4)	28(28.6)	19(19.4)	7(7.1)
Vascularization (VASC)	11(11.2)	18(18.4)	30(30.6)	25(25.5)	10(10.2)	4(4.1)
Keratinization (KERT)	59(60.2)	12(12.2)	7(7.1)	5(5.1)	11(11.2)	4(4.2)
Superficial punctate keratopathy (SPK)	77(78.6)	12(12.2)	6(6.1)	3(3.1)	-	-
Opacification (OPAC)	10(10.2)	15(15.3)	24(24.5)	27(27.6)	17(17.4)	5(5.1)
Meibomian gland involvement (MGI)	12(12.2)	40(40.8)	28(28.6)	18(18.4)	-	-
MucocutaneousJunction involvement (MCI)	9(9.2)	22(22.5)	36(36.7)	19(19.4)	9(9.2)	3(3.1)
Punctal involvement (PUNC)	38(38.8)	-	60(61.2)	-	-	-
Hyperaemia (HYPER)	23(23.5)	35(35.7)	29(29.6)	8(8.2)	1(1.0)	2(2.0)
Conjunctival keratinization (CKERT)	61(62.2)	7(7.1)	10(10.2)	10(10.2)	7(7.2)	3(3.1)
Symblepharon formation (SYMB)	46(46.9)	30(30.6)	9(9.2)	5(5.1)	6(6.1)	2(2.1)

Patient's eyes were graded for twelve different ocular complications (of cornea, conjunctiva and eyelids) between severity score of 0 to 5. Data shown here is for worse eye (2 eyes with phthisis bulbi were excluded).

polymorphisms. All the screened SNPs were in Hardy–Weinberg equilibrium (p>0.01), and the minor allele frequencies (MAFs) for different populations are shown in Table 2.

Screening identified a significant association of IL-4R SNP rs1801275 (p=0.02) with chronic ocular SJS/TEN, while the IL-4 promoter rs2243250, IL-13 rs20541 and rs1800925 (promoter region) SNPs did not show any significant association. The frequency of allele A of IL-4R SNP rs1801275 was higher in SJS cases (82.5%) than in the controls (73.7%; p=0.03, odds ratio=1.7).

The dominant and recessive genetic SNP models were analyzed to better understand how the genes work in individuals and affect the phenotype. A significant association was found between IL-4R SNP rs1801275 and chronic

Table 2. Genotype frequencies of interleukins in SJS/TEN patients and controls.

Gene	Genotype Patient (%) Control (%) Patient n=100 n=131	p-value	Minor Allele Frequency (MAF)					
			Present study	All	American	East Asian	European	South Asian
IL-4 promoter (rs2243250)	CC 65 (65) 77 (58.8) CT 23 (23) 52 (39.7) TT 12 (12) 2 (1.5)	0.59	0.21	0.47	0.37	0.22	0.17	0.18
IL-4R coding (rs1801275)	AA 70 (70) 66 (50.4) AG 25 (25) 61 (46.6) GG 5 (5) 4 (3.1)	**0.02***	0.26	0.38	0.32	0.17	0.21	0.24
IL-13 promoter (rs1800925)	CC 69 (69) 87 (66.4) CT 29 (29) 38 (29.0) TT 2 (2) 6 (4.6)	0.47	0.19	0.25	0.23	0.18	0.18	0.20
IL-13 coding (rs20541)	GG 58 (58) 83 (63.4) GA 34 (34) 41 (31.3) AA 8 (8) 7 (5.3)	0.31	0.21	0.27	0.38	0.36	0.21	0.27

Screening for polymorphisms of interleukins showed C/T change for IL-4 promoter region, A/G change for IL-4R coding region, C/T change for IL-13 promoter region and G/A polymorphism for IL-13 coding region. Minor allele frequency is shown for different populations. *p<0.05.

Table 3. Genotype Frequencies for Interleukin SNPs and SJS/TEN susceptibility.

Gene with Genotype	Patient (%) n=100	Control (%) n=131	Allele 1 versus Allele 2 p OR (95% CI)	Dominant Model 11 versus 12+22 p OR (95% CI)	Recessive Model 11+12 versus 22 p OR (95% CI)
IL-4 promoter (rs2243250)			0.65	0.34	**0.001***
11 CC	65 (65)	77 (58.8)	-	-	-
12 CT	23 (23)	52 (39.7)			
22 TT	12 (12)	2 (1.5)			
IL-4R coding (rs1801275)					
11 AA	70 (70)	66 (50.4)	**0.03***	**0.003***	0.51
12 AG	25 (25)	61 (46.6)	1.7 (1.1–2.7)	2.3 (1.3–4.0)	-
22 GG	5 (5)	4 (3.1)			
IL-13 promoter (rs1800925)					
11 CC	69 (69)	87 (66.4)	0.54	0.78	0.47
12 CT	29 (29)	38 (29.0)	-	-	2.3 (0.5–17.2)
22 TT	2 (2)	6 (4.6)			
IL-13 coding (rs20541)					
11 GG	58 (58)	83 (63.4)	0.32	0.42	0.43
12 GA	34 (34)	41 (31.3)	-	-	-
22 AA	8 (8)	7 (5.3)			

The Recessive/Dominant model (whichever applicable) were employed for interleukin SNP to check disease susceptibility. *p<0.05.

ocular SJS/TEN under the dominant model (AA versus AG + GG, p=0.003; odds ratio=2.3), whereas the IL-4 promoter SNP rs2243250 showed a significant relationship under the recessive model (p=0.001; Table 3). Linkage disequilibrium between the markers was assessed using the confidence interval method of Gabriel et al. [7] with the Haploview v4.2 program, but no linkage disequilibrium was seen.

The frequency of HLA-A locus alleles in the patients and controls was as follows: HLA-A*3301 (32%, 0.5%), HLA-A*02 (22%, 5.8%), and HLA-A*2402 (11%, 1.7%). The frequency of HLA-A*02 was higher in patients than in the controls and showed the following sub-alleles: HLA-A*0201 (7%), HLA-A*0206 (5%), HLA-A*0211 (7%), and HLA-A*0203 (3%). The analysis showed that HLA-A*3301 (p<0.00001; OR, 84.24; 95% CI, 11.29–628.57), HLA-A*02 (p=0.0002; OR, 4.79; 95% CI, 2.04–11.28) and HLA-A*2402 (p=0.001; OR, 7.29; 95% CI, 1.98–26.8) were associated with SJS/TEN (Table 4). Limited resources constrained our HLA-A typing of the controls; therefore, control data of ethnicity matched north Indians were taken from the Allele Frequency Net Database (AFND) for analysis and comparison.

Biochemical analysis showed that granulysin levels in patients and controls were in the range of 1.89 (0.47, 3.19) and 1.75 (0.23, 2.68) (median [IQR]), respectively (p=0.87), while the sFas L levels differed significantly between patients 13.3 (7.71, 89.6) and controls 7.71 (6.19, 10.76; p=0.0001).

Genotype–phenotype analysis of graded ocular manifestations of the cornea, conjunctiva, and eye lids and the interleukin genotypes of IL-4, IL-13 (promoter and coding region) and IL-4R was done. Initial analysis showed an association between punctal involvement and IL-4 genotype; SPK and symblepharon formation with IL-13 coding region (13b); and SPK, the meibomian gland, and keratinization of the cornea and conjunctiva with the IL-13 promoter region (IL-13a). After Bonferroni correction, a significant association between the IL-13 promoter region (IL-13a) and conjunctival keratinization (CKERT; p=0.004) was seen, where the TT genotypes of the IL-13 promoter region were significantly different in comparison to the CC and CT genotypes (Table 5).

DISCUSSION

SJS/TEN is a life-threatening condition that is classified into acute and chronic phases based on the onset of symptoms and has an overall fatality rate of 12.95% [8]. Previous studies have shown that early treatment increases patient survival rates [8,9]. Although there is scarcity of large-scale epidemiological data from India [8], Devi et al. [10] reported that approximately 68% of patients are between 20 and 50 years of age, while Patel et al. [8] found that the majority were in the age group of 21–40 years. Kannabiran et al. [11] reported the highest prevalence in Indian patients aged 14–42 years.

In accordance with these studies, we also found a younger age of symptom onset of 23–34 years. Yamane et al. [9] and Sotozono et al. [3], both from Japan, reported a mean age of 45.7 years and 47.9±18.9 years, respectively, while Roujeau et al. [12] observed a mean age of 46.8±25.5 years in France.

Drugs are the single most frequent cause of SJS/TEN. A systematic review of SJS/TEN in an Indian population reported that antimicrobials were the leading cause, followed by anti-epileptics and NSAIDs, although there were regional differences between the south, west, and northern parts of India [8]. The present study found NSAIDs (antipyretics), antibiotics, sulphonamides and anti-epileptics to be the most common drugs leading to SJS/TEN, which is in agreement with Roujeau et al. [12]. The main causative drugs may differ across populations, possibly due to ethnic/genetic variations or different prescribing patterns [1,2]. Exposure rate disparities have been reported in different countries as well, as patients often take analgesics and antipyretics to treat the early signs of adverse reaction or infection [13]. In concordance, the present study also documented an increased risk of SJS/TEN for individuals who took "over-the-counter" medicines for general malaise. Hirapara et al. [14] reported about 33.2% cases of SJS/TEN were due to antipyretic drugs and highlighted the risk of over-the-counter medicines, especially paracetamol, in developing countries [8]. A recent survey in the United States reported that acetaminophen was the most common over-the-counter medicine associated with SJS/TEN, followed by ibuprofen [15].

The ocular surface is the most common site of serious long-term sequelae, ranging from mild conjunctival infection to severe corneal scarring and vascularization, often leading to permanent visual loss [3,16-18]. Chronic ocular sequelae, including symblepharon, entropion, trichiasis, tear film abnormalities, corneal opacity, keratinization, and corneal neovascularization, occur in up to 35% of long-term survivors [19].

Studies from India reported visual disturbance due to involvement of the cornea and conjunctiva in SJS/TEN patients [17,18]. Using a revised scoring system [4], our study documented more severe cases with a loss of POV, conjunctivalization, vascularization, opacification, and mucocutaneous junction involvement as ocular manifestations compared to Sotozono et al. [3], who found a loss of POV and meibomian gland involvement to be the most common ocular complications. The differences could be attributed to ethnic/genetic variations, improper management of the acute disease stage, delay in seeking medical care, and younger age of the patients. We also found moderate-to-severe keratinization, meibomian gland involvement, and symblepharon formation in our patients and note that such chronic sequelae may result in corneal erosions, ulcers, scarring, and persistent inflammation and thus the progressive corneal melting and perforation seen in our patients, which is in agreement with previous reports. Acute manisfestations of the SJS/TEN eyes lead to chronic ocular sequelae in about 30% of the cases, causing debilitating pain, threatening vision and leading to late corneal blindness [16]. A study reported that about 35% of SJS/TEN patients experienced chronic ocular sequelae due

Table 4. HLA-A locus allele association with chronic SJS/TEN patients.

HLA	Allele/Gene Frequency n (%)			
	Patients (n=100)	Controls[a] (n=144)[a]	OR (95% CI)	P value[b]
A*3301	32 (32)	1(0.5)	84.24 (11.29 - 628.57)	**<0.00001***
A*02	22 (22)	8 (5.8)	4.79 (2.04 – 11.28)	**0.0002***
A*2402	11 (11)	3 (1.7)	7.29 (1.98 – 26.8)	**0.001***
A*1101/22	9 (9)	18(12.5)	0.69 (0.3 – 1.61)	0.42
A*0101	8 (8)	17 (11.5)	0.65 (0.27 – 1.57)	0.40
A*6801/03	7 (7)	17 (9.4)	0.72 (0.29 – 1.8)	0.66
A*2601	3 (3)	3 (1.9)	1.45 (0.29 – 7.35)	0.69
A*0301	3 (3)	8 (5.8)	0.53 (0.14 – 2.03)	0.53
A*3201	3 (3)	8 (5.8)	0.53 (0.14 – 2.03)	0.53
A*2301	1 (1)	2 (1.0)	0.72 (0.06 – 8.02)	1.00

SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; HLA, human leukocyte antigen; n=total number of alleles; OR,odds ratio; [a]Allele Frequency Net Database (AFND); [b]Fisher exact test; *p<0.05.

Table 5. Evaluation of IL-4, IL-4R, IL-13a & IL-13b genotype association with the graded and ocular surface complications of chronic SJS/TEN patients.

Graded ocular complications (0–5)	IL-4 genotypes			IL-4R genotypes			IL-13a genotypes			IL-13b genotypes		
	CC (n=64)	CT (n=22)	TT (n=12)	AA (n=69)	AG (n=25)	GG (n=4)	CC (n=68)	CT (n=28)	TT (n=2)	GG (n=57)	GA (n=33)	AA (n=8)
POV (0–5)	3(0,5)	2(0,5)	3.5(0,5)	3(0,5)	3(0,5)	2.5(1,4)	3(0,5)	3(0,5)	3(3,3)	3(0,5)	3(0,5)	3.5(0,5)
CONC (0–5)	3(0,5)	2(0,5)	3.5(0,5)	3(0,5)	3(0,5)	2.5(1,4)	3(0,5)	3(0,5)	3(3,3)	3(0,5)	3(0,5)	3.5(0,5)
VASC (0–5)	2(0,5)	2(0,4)	2.5(0,5)	2(0,5)	2(0,5)	2.5(1,4)	2(0,5)	2.5(0,5)	1.5(1,2)	2(0,5)	2(0,5)	3(0,5)
KERT (0–5)	0(0,5)	0(0,4)	0.5(0,5)	0.0(0,5)	0.0(0,5)	0.0(0,4)	0(0,5)	0(0,5)	3.5(3,4)	0(0,5)	0(0,5)	2(0,5)
SPK (0–3)	0(0,3)	0(0,2)	0(0,2)	0.0(0,3)	0.0(0,2)	0.0(0,0)	0(0,2)	0(0,3)	1.5(0,3)	0(0,2)	0(0,3)	0(0,3)
OPAC (0–5)	2.5(0,5)	2(0,5)	3(0,5)	2(0,5)	3(0,5)	2(1,4)	2(0,5)	3(0,5)	3.5(3,4)	2(0,5)	2(0,5)	3.5(0,5)
MGI (0–3)	1(0,3)	2(0,3)	1.5(0,3)	1(0,3)	2(0,3)	1.5(1,2)	1(0,3)	1(0,3)	1.5(1,2)	1(0,3)	2(0,3)	1(0,3)
MCI (0–5)	2(0,5)	2(0,5)	2(1,5)	2(0,5)	2(0,5)	2(2,3)	2(0,5)	2(0,5)	2(1,3)	2(0,5)	2(0,4)	2.5(0,5)
PUNC (0–2)	2(0,2)	1(0,2)	2(0,2)	2(0,2)	2(0,2)	2(0,2)	2(0,2)	2(0,2)	2(2,2)	2(0,2)	2(0,2)	2(0,2)
HYPER (0–5)	1(0,5)	1(0,3)	1.5(0,5)	1(0,5)	1(0,5)	1(1,2)	1(0,5)	1(0,3)	1(0,2)	1(0,5)	1(0,3)	1(0,2)
CKERT (0–5)	0(0,5)	0(0,5)	0(0,5)	0(0,5)	0(0,5)	0(0,3)	**0(0,5)**	**0(0,4)**	**3(3,3)***	0(0,5)	0(0,4)	1(0,4)
SYMB (0–5)	1(0,5)	0(0,4)	1(0,4)	1(0,5)	1(0,4)	0.5(0,1)	0.5(0,5)	1(0,4)	1.5(1,2)	1(0,5)	1(0,4)	2(0,4)

Number of patients with specific interleukin genotypes are shown column wise and the related graded ocular complications are shown row wise. Data are shown as median (min, max) values. Genotype-phenotype analysis revealed no significant link between IL-4, IL-4R, IL-13b genotypes and ocular complications except IL-13a genotypes for conjunctival keratinization (CKERT) where * shows 'p'=0.004 as compared to CC and CT genotypes of IL-13a on Bonferroni correction. IL-4R,interleukin-4 receptor, IL-13a-interleukin-13 promoter region; IL-13b-interleukin-13 coding region; (0–5),severity score 0, 1,2,3,4,5; POV-loss of palisades of vogt; CONC- conjunctivalization; VASC-vascularization; KERT-keratinization; SPK-,superficial punctate keratopathy; OPAC-opacification; MGI-Meibomian gland involvement; MCI-mucocutaneous junction involvement; PUNC-punctal involvement; HYPER-Hyperemia; CKERT-conjunctival keratinization; SYMB-symblepharon formation.

to prolonged inflammation and ulcers along with cicatricial complications [19]. Other studies have described end-stage disease as a dry and keratinized ocular surface having poor outcome of ocular surface surgeries and a very low survival rate of corneal or limbal cell transplantation [16,20], suggesting that the compromised ocular surface has detrimental effects on visual acuity.

SJS/TEN is considered the outcome of the additive/epistatic effects of variations in numerous genes. Ueta et al. [6] found a significant association between the IL-4R and IL-13 coding region SNPs and SJS/TEN in Japanese patients, while a weak or no association was seen for the IL-4 and IL-13 promoter region SNPs. Our study found no significant difference between the IL-13 coding, IL-4 and IL-13 promoter region SNPs, while for IL-4R SNP rs1801275 we found a significant association between allele frequency (A/G, p=0.03; odds ratio=1.7) and the dominant model (AA versus AG + GG, p=0.003; odds ratio=2.3), in alignment with previous reports on SJS/TEN [6]. For the IL-4R Gln551Arg polymorphism, a significantly higher frequency of the Arg551 allele was seen in atopy compared to a higher frequency of the Gln551 allele in SJS/TEN [6]. A study using next-generation sequencing (NGS) explored the role of relevant genes in drug metabolism and disease pathogenesis to understand the molecular basis of this disease, emphasizing the complex combinations of frequently occurring and rare variants involved in molecular drug metabolism cascades for particular phenotypic presentations [21].

Considering the complex network of genes and respective phenotypes, our study investigated the association of graded chronic ocular manifestations with interleukins IL-13 and IL-4 and observed an association of the interleukin IL-4 genotype with puncta; the IL-13 coding region with SPK and symblepharon formation; and the IL-13 promoter region with SPK, the meibomian gland, keratinization of the cornea, and conjunctiva. Bonferroni's correction revealed a significant association of only the IL-13 promoter genotype with chronic sequelae of conjunctival keratinization. The onset of keratinization indicates the presence of complete dryness in itself, and these two conditions together lead to the formation of symblepharon and finally affect visual acuity partially or wholly.

Di Pascuale et al. [22] emphasized that keratinization of the lid margin in chronic SJS/TEN leads to significant long-term corneal compromise and subsequent progressive visual loss. Keratinized lid margin has also been reported as the primary cause of end-stage corneal blindness in SJS/TEN [23]. These reports show that the sequelae outcome is end-stage blindness due to chronic inflammation and functional impairment of the sensitive, dry, and photophobic ocular surface and lid margins. Our study also found changes/inflammation of lid margins with involvement of the meibomian gland, mucocutaneous junction, and puncta, which may contribute to a keratinized and compromised ocular surface with inflamed and keratinized eye lid margins, leading to visual disturbances and/or complete loss of sight.

The unknown pathology of the disease suggests individual susceptibility to drug exposure, and studies highlighted that individuals of specific ethnicities or with HLA alleles are at increased risk of developing SJS/TEN ocular sequelae [1,4,11]. Ueta et al. [1] associated about 80% of reactions to cold medicine (CM) and the development of CM-SJS/TEN with severe ocular complications (SOCs) in Japanese patients. A positive association was found with HLA-A*0206 and an inverse association with HLA-A*1101 [24]. Studies reported additive effects of HLA-A*0206 with polymorphisms of immune-related genes in SJS/TEN with SOCs [1,25]. In agreement with this, our study noted a higher frequency of HLA-A*0206 in SJS/TEN ocular sequelae but did not assess the additive effects of specific immune genes. Another study associated HLA-A*0206 with CM-SJS/TEN with SOCs but not with CM-SJS/TEN without SOCs [1]. Our patient cohort with the HLA-A*02 allele had chronic sequelae of SJS/TEN and mainly took NSAIDs, but categorization of patients as "with SOC" or "without SOC" was not possible since we only recruited chronic cases. However, Roujeau et al. [26] disagreed with the causal role of CMs (antipyretics, analgesics, and NSAIDs) and suggested the term "idiopathic" rather than "cold medicine" for SOCs, as such medicines are frequently used and are available without a prescription to treat general symptoms. Another study pointed toward the difficulty in determining the main culprit drug due to the usage of multiple drugs by patients [27]. Similarly, our study is inconclusive regarding the role of CM in SOCs, as the majority of the patients took NSAIDs along with other drugs. Additionally, studies based on in silico molecular docking approaches have revealed that CMs have more binding affinity to HLA-A*0206 protein molecules, which trigger the molecular cascades in SJS/TEN with SOCs [28,29].

A Korean study showed a significant association between HLA-A*2402 and lamotrigine-induced maculopapular exanthema (MPE) [30]. A higher frequency of this allele in carbamazepine (CBZ)-induced MPE was also reported in north India [31]. Similarly, other studies showed a significant association of the allele with mild MPE [13,32] as well as with cutaneous adverse drug reactions (cADRs) [32]. Our study found a higher frequency of HLA-A*2402 in SJS/TEN

patients who took antipyretics, suggesting it is a risk factor for severe cADRs.

Conversely, a Japanese study showed an inverse association between HLA-A*2402 and CM-SJS/TEN with SOCs [1], while another study highlighted HLA-A*2402 as a protective variant against SJS/TEN in Asians and labeled it a controversial genetic factor [2]. HLA-A*2402 was also identified as an additional contributing factor in CBZ-induced SJS/TEN and recommended for pretreatment screening in a southern China population [32].

The HLA-A*3303 and HLA-A*3301 alleles belong to the same family and share 99.5% sequence homology [33]. An Indian study showed their frequencies to be 43.7% for HLA-A*3303 and 4.3% for HLA-A*3301 [34]. Another north Indian study documented a higher frequency of the HLA-A*3301 allele (20%) in MPE cases compared to levetiracetam-tolerant controls (2%), although the association was not significant [35]. An association between HLA-A*3303 and acetaminophen-related SJS/TEN with SOCs was found in a Thai population [36]. The present study found the HLA-A*3301 allele in 32% of SJS/TEN patients of north Indian origin with chronic ocular sequelae, the majority of whom took NSAIDs to alleviate cold-like symptoms, while a study from south India suggested that HLA-A*3303 is a risk allele for SJS/TEN with SOCs [11]. These differences may be related to race and diversity, as north Indians are an admixture of European and ancient Indian races [37]. There is a clear need for multicentric studies on Indian communities/races based on registries to ascertain the exact incidence and prevalence of HLA alleles related to SJS/TEN [38].

Keratinocyte apoptosis occurs through the T-cell mediated Fas-Fas ligand (Fas L), perforin/granzyme B, and granulysin pathways in SJS/TEN [39]. The blister fluid of cells showed high levels of granulysin in the acute phase, while the serum granulysin levels were higher in SJS/TEN and normal in non-blistering adverse drug reactions [39]. Our study found increased granulysin serum levels in chronic ocular cases compared to controls, although the difference not significant. The functional significance of granulysin was demonstrated in mouse experiments, where the removal of blister fluid resulted in reduced cytotoxicity [39], but when the same blister fluid was injected under the mouse skin, it resulted in changes similar to SJS/TEN [40]. Molecular studies of granulysin revealed several non-synonymous sequence variants contributing to the pathophysiology of SJS-TEN. Functional assays of the mutant GNLY proteins demonstrated that they were localized in the nuclear compartment, resulting in toxicity [39,41], based on which the authors concluded that granulysin constitutes a potential clinical biomarker of SJS-TEN.

Studies have revealed elevated soluble Fas L levels in the sera of TEN patients [42], and serial serum sFas L levels were increased between 24 and 48 h after the onset of skin damage in an acute TEN patient [43]. In vitro stimulation of isolated blood cells from SJS/TEN patients with a causal drug resulted in increased sFas L concentrations [44]. In agreement, our study also found significantly elevated sFas L levels in chronic ocular SJS/TEN compared to controls. sFas L levels may be linked to persistent ocular inflammation, and the sFas/sFas L ratio acts as an index of inflammation in chronic diseases, while matrix metalloproteinases play a pivotal regulatory role [45]. These metalloproteinases cleave the cell surface of Fas L into sFas L and a TNF-like molecule, thus increasing sFas L levels [40].

To conclude, this is the first study from north India that explores the possible role of genetic markers in SJS/TEN with chronic ocular sequelae. The results indicate NSAIDs to be the main offending drugs, highlight the significant involvement of IL-13 promoter, higher frequency of HLA-A*3301, HLA-A*02, and HLA-A*2402 alleles and increased granulysin and sFas L levels in patients with chronic sequelae. These markers may indicate severe long-term chronic ocular sequelae, thus alerting caregivers and supporting better management of the condition. The results of this preliminary study highlight the need to conduct more multi-centric studies on large patient samples to substantiate and validate the association between the marker genotypes and ocular complications of SJS/TEN.

ACKNOWLEDGMENTS

The study was funded by an extramural grant (Code no. I723) from the Indian Council of Medical Research (ICMR), New Delhi, India. SKS (first author) was supported by the Council of Scientific and Industrial Research (CSIR), New Delhi, India. The funding organizations had no role in the design or conduct of this research. The authors acknowledge the assistance provided for HLA work by Dr. U Kanga (AIIMS, New Delhi) and A Shekhar (Biogenuix Medsystems), New Delhi, India.

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 31 December 2022. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.