

Toxic epidermal necrolysis and Steven Johnson syndrome in Sri Lanka; clinical course, HLA-B*1502 allele and its association to carbamazepine

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Abstract

Steven Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) occur frequently with carbamazepine among the carriers of HLA-B*1502 allele. The study evaluated the clinical course and strength of this association in Sri Lanka.

Twenty four consecutive patients with SJS/TEN from multiple dermatology centres were assessed for demographics, history and clinical course. Carbamazepine induced cases with a group of healthy control (10) were tested for HLA-B*1502 using multiplex PCR with sequence specific primers.

The sample had a mean age of 41.6 years; female: male ratio of 3:1. Antiepileptics were the aetiological cause in 12 (50%), amongst 9 (37.5%) taken carbamazepine. The HLA-B*1502 allele was positive only in one patient but none in the control group (10). The mucosal involvement was common (95.8%).

Carbamazepine is the commonest single therapeutic agent implicated in SJS/TEN, although the association with the HLA-B*1502 allele is weak in our sample. Early mucosal involvement is a reliable clinical sign useful for the diagnosis.

Key words: Steven Johnson syndrome, toxic epidermal necrolysis, HLA, carbamazepine, Sri Lanka

Introduction

Steven Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe mucocutaneous adverse drug reactions. These are idiosyncratic reactions characterized by skin necrosis, bullae formation and erosions. The new research evidence shows these are manifestations of the same clinical spectrum sharing a common pathogenesis.

Despite being uncommon (9.2 and 1.9 million/year in the USA for SJS and TEN respectively)¹, they carry high morbidity and mortality. The calculated mortality is 14.8% for TEN, 19.4% for SJS-TEN and 4.8% for SJS in the US population¹. Another study demonstrates 20% mortality in a Sri Lankan sample². Additionally, these can lead to profound disabilities like conjunctival scarring or blindness, disfiguring mucosal and skin scars, secondary infection, sepsis and thromboembolism.

A causative drug is found only in 75% of the cases³. According to the EuroSCAR/RegiSCAR, carbamazepine is a high-risk drug³. It was the highest multivariate risk factor in Europe in comparison to other antiepileptics causing SJS/TEN⁴. A landmark study done in 2004 among Han Chinese in Taiwan showed 100% association of the HLA-B* 1502 allele in the affected group in contrast to 3% in the controls⁵. Hence, in 2007 the United States Food and Drug Administration (FDA) released a recommendation to test HLA-B* 1502 before prescribing carbamazepine for Asian descent patients⁶. Similar recommendations were issued later in Hong Kong, Taiwan, Singapore and the Medicines and Healthcare Regulatory Agency (MHRA) in the UK. On the contrary, the specific allele was not commonly present in the European patients who developed SJS or TEN⁷. This highlights the ethnic variation in the genetic association of the disease to carbamazepine.

Sri Lanka is a South Asian island nation, ethnically distinct from East and South-East Asian communities. A previous community study of the HLA-B* 1502 allele showed a 4.3% prevalence⁸. However, the allele association to carbamazepine induced disease has never been tested in Sri Lanka.

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Methods

The study took place in four tertiary care hospitals in the Central, Western and Sabaragamuwa provinces of Sri Lanka (National Hospital of Sri Lanka, Colombo, Teaching Hospital, Kandy, District General Hospital Rathnapura and Base Hospital, Gampola).

The ethical approval was obtained from the Ethical Review Committee of the Teaching Hospital, Kandy. Written informed consent was taken after explaining the purpose of the study and the procedures.

All patients admitted with SJS, SJS-TEN (overlap) or TEN diagnosed by a consultant dermatologist that fulfils Roujeau's criteria⁹ were included. Three patients who had uncertain diagnosis or ambiguity in clinical presentation were excluded. Eventually, 24 suitable patients consented for the study. Ten carbamazepine tolerant patients were selected from the same study centres as controls. The controls were defined as patients taking only carbamazepine for more than six months without any cutaneous adverse effects.

The data collection had two arms; a clinical assessment and genomic testing.

A questionnaire-based interview was conducted by the principal investigator during the initial admission with the patient and or closest carer. The bed-head tickets archived at hospitals and the medical records were traced for accurate treatment history.

The structured questionnaire covered details on demographic data, drug history, disease characteristics, predisposing factors and family history. Age, gender, civil status and area of residence were considered in the demographics. Full drug history encompassed prescribed medicines, over the counter medicines, ayurvedic medicines and home remedies. The disease characteristics including duration from commencement of drugs to symptoms, the delay from the first symptom to admission, the sequence of body site involvement, the indication for the original prescription and other comorbidities were also documented.

Genomic studies

Two millilitres of blood were drawn to an EDTA tube and transferred to the laboratory within 4 hours of collection. The genomic DNA samples were extracted using the Wizard® (Promega, USA), genomic DNA isolation kit.

The HLA-B* 1502 allele detection method was modified from the methods described by Man *et al*¹⁰ and Gunathilake *et al*⁸.

A multiplex polymerase chain reaction (PCR) using sequence specific (SS) primers was used to detect the allele HLA-B* 1502 specifically. This included four sets of HLA specific primers and two sets of control primers as shown in Table 1.

Four separate master mixtures were prepared for PCR analysis. Each master mixture contained an internal control primer and one HLA specific primer. The control primer C1 was used in master mixtures containing HLA specific primers 2, 3 and 4, while C2 was used in master mixture 1 as shown in Table 2.

The optimized reaction mixture was prepared as described by Gunathilake *et al*⁸. This consists of 1 X PCR buffer (Promega, USA), 0.2 mM dNTPs, 2 mM MgCl₂, 0.05 units of Taq polymerase (GoTaq® Promega, USA), 2 µl of the extracted DNA from subjects and 0.19 µM of HLA specific primer set 1, 4, control primers or 0.27 µM of HLA specific primer 2,3 (IDT, USA). The total volume was the master mixture was brought up to 26 µl. The PCR conditions were 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s.

The PCR product analysis was done by a gel-based electrophoresis on 2% agarose with ethidium bromide, followed by direct visualization by an ultraviolet transilluminator. The positivity of HLA-b* 1502 was defined only when all four HLA specific PCR products and internal controls were present.

Data analysis

The demographic data were expressed in percentages. A Chi-square test with Fisher's exact was used to determine the association of HLA-B* 1502 among patients taking carbamazepine and carbamazepine tolerant patients. To reduce bias in the odds ratio, whenever a zero-count cell is encountered, 0.5 was added to all cells in a 2 × 2 table (Haldane-Anscombe correction)¹¹. A p≤0.05 was considered statistically significant.

Results

Out of the twenty-four patients in the test group, the majority were females (females: male, 3:1). The average age was 41.6 years in the range of 16-78 years. Twelve patients (50%) had TEN, 7 (29.2%) had SJS-TEN while 5 (20.8%) had SJS only. The symptoms began in a mucosal surface in 23 (95.8%) patients.

Table 1. Sequence specific primers of HLA-B* 1502 and the controls

HLA specific primers		
Set 1	Forward (F1)	5' -CGAGAGAGCCTGCGGAAC-3'
	Reverse (R1)	5' -GCCCACTTCTGGAAGGTTCT-3'
Set 2	Forward (F2)	5' -CGCGAGTCCGAGGATGGC-3'
	Reverse (R2)	5' -GCAGGTTCGCGAGGCTCT-3'
Set 3	Forward (F3)	5' -ACCGGAACACACAGATCTC-3'
	Reverse (R3)	5' -GAGCCACTC- CACGCACAGT-3'
Set 4	Forward (F4)	5' -GGAGTATTGGGACCGGAAC-3'
	Reverse (R4)	5' -GCCATACATCCTCTGGATGA -3'
Control primers		
Set 1	Forward (FC1)	5' -TGCCAAGTGGAGCACCCAA-3'
	Reverse (RC1)	5' - GCATCTTGCTCTGTGCAGAT-3'
Set 2	Forward (FC2)	5' -ATGATGTTGACCT TTCCAGGG-3'
	Reverse (RC2)	5' -TTCTGTAACITTTTCATCAGTTFC-3'

Table 2. Sequence specific multiplex PCR master mixtures

Master mixture	HLA specific primer	Control primer
Mix 1	Set 1	Set 2
Mix 2	Set 2	Set 1
Mix 3	Set 3	Set 1
Mix 4	Set 3	Set 1

The usual order of involvement was conjunctival>oral>genital mucosa before emanating to the skin. The symptoms started most frequently on day 10 (range 4-17) since the first dose of the culprit drug. Patients had an average delay of 2.7 days before seeking medical care. There was only one mortality in the sample. Another patient developed severe corneal scarring. Anti epileptics were the most common group of drugs (50%), while carbamazepine was the most frequent drug (37.5%): Figure 1.

Only one patient from the test group tested positive for HLA-B* 1502 gene and none of the control group had the allele positivity. No association between the

presence of the gene and SJS/TEN in patients who had carbamazepine (Fishers exact test, $p=0.47$ (>0.05)).

The strength of association of HLA-B* 1502 to carbamazepine induced SJS/TEN is low in our sample. A possible explanation could be related to the biochemical function of HLA protein.

The pathogenesis of SJS and TEN is complex and not fully understood. Nevertheless, the end result is cell apoptosis due to a cytotoxic T cell mediated Type IV hypersensitivity reaction against keratinocytes expressing a foreign antigen^{13,14}.

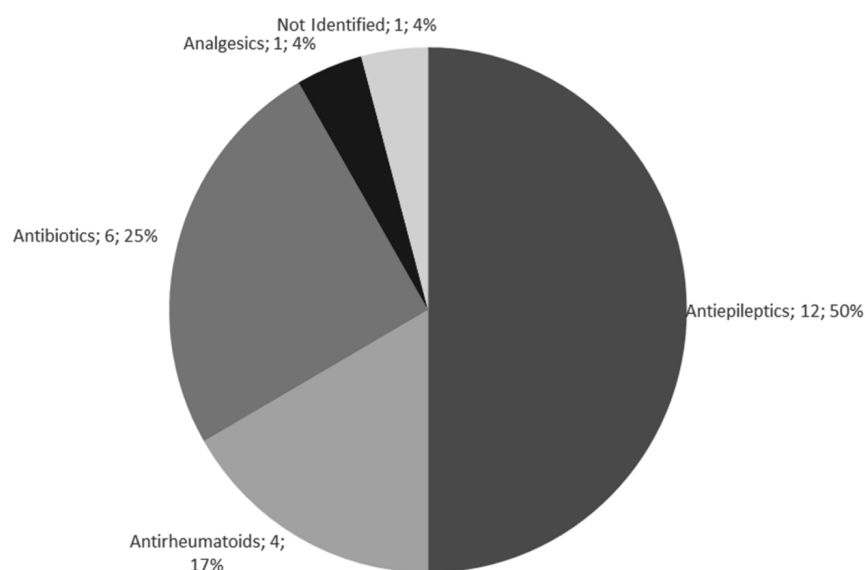


Figure 1. The causative drug classes and the frequencies.

HLA molecules are highly polymorphic. They are classified into different supertypes by their functional binding properties. HLA-B* 1502 is a type of the HLA-B62 super type. The main function of HLA molecule is to present peptides processed and derived from intra and extracellular proteins. Given that most drugs responsible for SJS/TEN are non-peptide molecules, it is likely that some drug molecules may have bound to tissue proteins forming haptens. In the patients who developed SJS/TEN without the HLA-B* 1502, it can be hypothesised that, different HLA molecules coded by different alleles in the same locus are involved in antigen (hapten) presentation. Presumably, it is this allele variation that decides ethnic variation of SJS/TEM susceptibility.

Another reason for low positive HLA-B* 1502 allele in this study is explained by the relative infrequency of the allele in the population. Allele prevalence in Sri Lanka can be considered moderate to that of extremely low prevalence (<1%) countries and the high prevalence countries (8-15%)¹⁵. The community prevalence rates in India varies from 0- 6%^{16,17,18} where the population shares some similarity to Sri Lanka.

The early onset and consistent involvement of mucosal symptoms are important clinical presentations heralding the diagnosis. Mucosal involvement is common to all entities with varying degrees of skin involvement. We were able to demonstrate mucosal involvement as the earliest sign to herald the onset and progression which is often overlooked. The

conjunctival involvement in the form of redness, irritation, gritty feeling is a common and serious complication. Extensive oral ulceration is painful and often affects feeding and nutrition.

Conclusion

Carbamazepine is the single most frequent drug responsible for SJS/TEN our study. Antiepileptics contribute to SJS/TEN more than any other drug category and is as high as 50% which is similar to world literature¹².

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Conflicts of interest

The authors have no conflicts of interests to declare.

References

1. Hsu D, Brieva J, Silverberg N, Silverberg J. Morbidity and Mortality of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in United States Adults. *J Invest Dermatol.* 2016; **136**(7): 1387-97.

2. Wickramanayake A, Dilrukshi P, Maddumarachchi C, *et al.* Toxic Epidermal Necrolysis - a retrospective study of 10 adults. *Proceedings of the 25th Annual Academic Sessions of Sri Lanka College of Dermatologists and joint meeting with Dowling Club UK*; 2017 May 4-6 Colombo Sri Lanka.
3. Mockenhaupt M. The current understanding of Stevens Johnson syndrome and toxic epidermal necrolysis. *Expert Rev Immunol* 2011; **7**(6): 803-15.
4. Raujeou J, Kelly J, Naldi L, *et al.* Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. *N Engl J Med.* 1995; (333): 1600-7.
5. Chung W, Hung S, Hong H, *et al.* A marker for Stevens-Johnson syndrome. *Nature.* 2004; **428**(6982): 486-6.
6. Accessdata.fda.gov.2019[WWWdocument].URL https://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/016608s098,020712s029,021710_ClinRev.pdf [accessed 6 March 2019]
7. Lonjou C, Thomas L, Borot N, *et al.* A marker for Stevens-Johnson syndrome ...: ethnicity matters. *The Pharmacogenomics J.* 2006; **6**(4): 265-8.
8. Gunathilake K, Wettasinghe K, Dissanayake V. A study of HLA-B*1502 in a Sri Lankan population: Implications for pharmacogenomic testing. *Hum Immunol.* 2016; **77**(5): 429-31.
9. Roujeau J. The Spectrum of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis: A Clinical Classification. *J Invest Dermatol.* 1994; **102**(6): 28S-30S.
10. Man C, Kwan P, Baum L, *et al.* Association between HLA-B*1502 Allele and Antiepileptic Drug-Induced Cutaneous Reactions in Han Chinese. *Epilepsia.* 2007; **48**(5): 1015-18.
11. Pagano M, Gauvreau, K. Principles of biostatistics. 2nd ed. Belmont CA: Brooks/Cole. (2000)
12. Mockenhaupt M, Viboud C, Dunant A, *et al.* Stevens-Johnson syndrome and Toxic Epidermal Necrolysis: Assessment of Medication Risks with Emphasis on Recently Marketed Drugs. The EuroSCAR-Study. *J Invest Dermatol.* 2008; **128**(1): 35-44.
13. Kinoshita Y, Saeki H. A Review of the Pathogenesis of Toxic Epidermal Necrolysis. *J Nippon Med Sch.* 2016; **83**(6): 216-22.
14. Downey A, Jackson C, Harun N, Cooper A. Toxic epidermal necrolysis: Review of pathogenesis and management. *J Am Acad Dermatol.* 2012; **66**(6): 995-1003.
15. Accessdata.fda.gov.2019[WWWdocument].URL https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/016608s101,018281s048lbl.pdf [accessed 6 March 2019].
16. Shankarkumar U, HLA-A, -B and -Cw allele frequencies in a Bhil population from Western India. *Hum Immunol.* 2004; **65**(9-10): 960-61.
17. Rajalingam R, Parham P, Mehra N. HLA-A, -B, -Cw, -DQA1, -DQB1 and -DRB1 alleles and KIR alleles in a Hindu population from Punjab, India. *Hum Immunol.* 2004; **65**(9-10): 958-60.
18. Shankarkumar U. HLA-A, -B and -Cw allele frequencies in a Nadar population from Tamil Nadu, India. *Hum Immunol.* 2004; **65**(9-10): 958.