Case-control study measuring the association between HLA-B*13:01 and dapsone hypersensitivity syndrome in Indian patients

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Summary
Objectives: Dapsone hypersensitivity syndrome (DHS) is associated with HLA-B*13:01 in Chinese and south-east Asians. This association has not been studied among Indians. The objective was to study the association between HLA-B*13:01 and DHS in Indian patients.

Methodology: A case – control study was done in south India (January 2016 to March 2018). Prevalence of HLA-B*13:01 in 8 DHS cases was compared to its prevalence in 324 controls (113 patient controls, 211 population controls). HLA-B*13 typing was done in 2 steps: First, HLA-B*13 positivity was determined using intermediate resolution PCR (SSOP HLA typing kit, Luminex platform) in the 332 study participants followed by Sanger sequence-based typing on 17 HLA-B*13 positive patients (7 cases, 10 patient controls). 1 DHS case (HLA-B*13:01 negative) was also screened for HLA-DRB*15:01 and 16:02. Odds ratio, CI and population attributable risk were calculated.

Results: There were 8 cases of DHS and 324 controls including 113 patient controls and 211 population controls. 7/8 cases (87.5%) of DHS and 9/113 of patient controls (7.96%) were HLA-B*13:01 positive. Among those in whom high resolution typing was done, 1/17 (5.7%) were HLA-B*13:02 positive. Among population controls, 9.9% (21/211) were HLA-B*13 positive. HLA-DRB*15:01 and 16:02 were negative in the...
1 patient tested. Two HLA-B*13:01 positive patient controls were dapsone tolerant. HLA-B*13 and specifically HLA_B*13:01 was significantly associated with DHS. (Odds ratio of 66.16 for HLA-B*13 and 80.89 for HLA-B*13:01). Population attributable risk was 86.2%.

**Conclusion:** HLA-B*13:01 appears to be a significant risk factor for DHS in Indians. Implementation of a pre-treatment screening strategy may decrease the incidence of DHS.

**Keywords:** leprosy, dapsone hypersensitivity syndrome, HLA-B*13:01

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**Introduction**

Dapsone is a commonly used drug for dermatological and non-dermatological indications ranging from acne, leprosy, vasculitis, connective tissue disorders, immuno-bullous disorders, and immune thrombocytopenic purpura to name a few. The prevalence of DHS in India has been reported to be 0.25% by Sharma et al. and 1.3% by Rege et al.1,2 It is a morbid condition with a mortality rate of 9.6–11.1%.3,4

In 2013, a population wide genomic study in Chinese patients with leprosy found that HLA-B*13:01 was significantly associated with the development of DHS [OR: 20.53].5 More recent studies have shown an even higher association in Chinese and Thai populations.6,7 In 2018, a meta-analysis re-emphasized the need for HLA-B*13:01 testing prior to initiation of dapsone [OR: 43].8 In 2017, HLA-DRB*15:01 and 16*02 were also found to have an association with DHS.9 Based on the data from allele frequency net, Zhang et al. reported that the Indian population has a high incidence of HLA-B*13:01 ranging from 1 to 12%.5 Till date, to the best of our knowledge, there has been no study of HLA-B*13:01 positivity in patients with DHS from India. Given that pre-treatment HLA-B*13:01 testing has not yet become the standard of care in India, and that dapsone hypersensitivity has a high morbidity and mortality, we undertook this case-control study to assess the HLA-B*13:01 positivity and its association with DHS in our population.

**Materials and methods**

This was a case record-based case-control study done in a dermatology unit of a tertiary care hospital in south India. The duration of study was 27 months (January 2016 to March 2018). Institution review board clearance was obtained [Ref no: 11006(retro)]. Patients who fulfilled the Richardus and Smith criteria for DHS were included as cases.10 Patients who had adverse events following use of dapsone but did not fulfill the criteria for DHS were excluded from the study. Controls included patient controls and population controls. Patient controls were those patients who were screened for HLA-B*13 but did not develop DHS. The secondary data of population controls, consisting of HLA class 1 type of the 211 most recent stem cell and voluntary kidney donors tested was provided by the HLA laboratory of our institution.

**HLA typing**

A 2-step process was followed for detection of HLA-B*13:01 among those with DHS and patient controls. Initial screening was done by using intermediate resolution polymerase chain reaction (PCR) with sequence-specific oligonucleotide probes (PCR-SSOP) on the luminex platform using the lifecodes HLA – B typing kits from Immucor GTI diagnostics Inc.
Among positive cases and patient controls, high resolution typing to define HLA-B*13 subtypes was done using Invitrogen B kits from One Lambda (SeCorew Sequencing Kits) performing bidirectional Sanger sequencing for exons 2,3,4,5. Sequencing fragments processing by capillary electrophoresis was done using the 3130XL genetic analyser (Applied Biosystems) for exon 2,3,4,5. Output files generated by the genetic analyser were imported into uTYPEw Dx HLA sequence analysis software and were analysed against IMGT/HLA database to determine molecular typing. The population controls underwent only the intermediate resolution PCR. In the DHS case who was HLA-B*13 negative, screening for HLA-DRB*15:01 and 16:02 was performed.

Figures 1 and 2 shows the algorithm of testing that we followed for HLA-B*13 screening and further evaluation of those who were found to be carrying the allele.

Odds ratio and population attributable risk was calculated to find out the statistical and epidemiological significance of our results.

Results

A total of 8 DHS cases and 324 controls (113 patient controls and 211 population controls) were recruited (Figure 2).
HLA typing

Results of HLA-B*13/-B*13:01 typing in patients with DHS, patient controls and population controls is shown in Figure 2.

7/8 (5 males and 2 females) patients with DHS were HLA-B*13:01 positive. The 1 DHS case who was HLA-B*13:01 negative, was also negative for HLA-DRB*15:01 and HLA-DRB*16:02.

Among the 113 patient controls, 10 were HLA-B*13 positive; 9/10 (90%) were HLA-B*13:01 positive (males: 5, females: 4) and 1/10 (10%) was HLA-B*13:02 positive (male). In total, of the 17 HLA-B*13 positive patients, 94·1% (16/17) were HLA-B*13:01 positive and 5·8% (1/17) were HLA-B*13:02 positive. Only 2/10 patient controls (both males) who were HLA-B*13:01 positive received dapsone and both (100%) were tolerant to dapsone.

Among the population controls (healthy kidney and stem cell donors), the prevalence of HLA-B*13 was 9·9% (21/211). High resolution testing was not done in population controls due to cost constraints.

The HLA-B*13 positive patients (DHS cases and patient controls) belonged to different regions in India and there was no clustering of cases in any particular region.

ASSOCIATION BETWEEN HLA-B*13:01 AND DHS

HLA-B*13 was significantly associated with DHS (OR: 66·16, 95% CI: 7·9 to 555·5, p < 0·001) (Table 1). This association was found to be stronger for HLA-B*13:01 (OR: 80·89, 95% CI: 8·93 to 732·38, P = 0·0001).

The population attributable risk was found to be 86·2%.

CLINICAL FEATURES OF CASES AND PATIENT CONTROLS

There were 8 cases of DHS. (M:6, F:2). The mean age of presentation was 31·75 years (range: 20 – 42 years). The indications included leprosy (3 patients), acne vulgaris (1), lichen planus (1), oral ulcers (1), urticaria (1) and immune thrombocytopenic purpura (ITP, 1). The dose varied from 50 to 100 mg daily. 7 patients were started on dapsone elsewhere and 1 was started at the Hematology department of the same institute for ITP. The latency of DHS ranged from 3·5 – 8 weeks (mean: 4·93 weeks). The type of rash was maculopapular in 5, erythroderma in 2 and TEN in 1 patient. Other features included fever (100%), transaminitis (ALT > 2x normal range), (87·5%), lymphadenopathy (62·5%), eosinophilia (25%), elevated creatinine (12·5%), myocarditis (12·5%). One patient expired due to dapsone induced myocarditis (mortality, 12·5%).

There were 113 patient controls. (M: 72, F: 41). Dapsone was administered in 80 patients. The indications for which dapsone was administered included leprosy (59 patients, 73·75%),

Table 1. Contingency ratio for odds ratio calculation: The odds ratio was found to be 66·16

<table>
<thead>
<tr>
<th>HLA-B*13</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7</td>
<td>31</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>293</td>
</tr>
</tbody>
</table>
bullous pemphigoid (4 patients, 5%), lupus erythematosus, pemphigus vulgaris, oral aphthae (2 patients each) and 1 patient each of 11 other diagnoses. In a few patients, dapsone had to be stopped for reasons such as hemolytic anemia in 7 patients, and the lack of response to dapsone in 1 patient who had a leg ulcer.

Discussion

The clinical features of DHS including mean age of presentation, latency of DHS and the cutaneous and extracutaneous manifestations were similar to that found in the literature. We had a male: female ratio of 3:1. This male preponderance seen in our study has been reported earlier from a study done in Nepal where the M:F ratio was 2-2: 1. However, since the number of cases in our study is small, this may not reflect a true male preponderance. A large meta-analysis by Wang et al. did not find any gender predominance in the Chinese population. (M:F = 1:26).

The results of our study show a significant association between HLA-B*13:01 and DHS in the Indian population making a strong case for pre-treatment screening to identify those at risk for DHS, and concur with previously published data from China and Thailand. The reported prevalence of HLA-B*13:01 in the Indian population is 1 – 12% and compares favourably with our population prevalence of HLA-B*13 in 9.9% of population controls.

The first reports of HLA-B*13:01 association with DHS were published in 2013. A study from Thailand showed that HLA-B*13:01 can also be associated with DHS manifesting with DRESS and SJS/TEN phenotypes [OR: 54-00 in comparison with dapsone tolerant patients and 26-11 with general population]. Further, a meta-analysis of the published studies has reaffirmed that there is a strong association between HLA-B*13:01 and DHS. Till date, there have been no similar studies from India regarding the association of HLA-B*13:01 and DHS. Our study findings (OR: 66-16 for HLA-B*13 and 80.89 for HLA-B*13:01) are in concurrence with previous studies, and has shown the association of HLA-B*13:01 in DHS patients of Indian origin. The results of a case-control study done in China that included 21 DHS patients, 105 patient controls and 100 population controls found a similar odds ratio of 69.5 with respect to population controls.

More recently, Yue Z et al. found that 5 amino acid variants of HLA-DR B1 (amino acid positions 133, 142, 17, 11 and 32) which are in high linkage disequilibrium are linked to DHS with -DRB*15:01 and 16*02 showing nominal significance. This was looked for in 1 of our patients with DHS who was HLA-B*13:01 negative and found to be absent.

The mechanism of HLA association of DHS was first described in 2017. It was found that dapsone fits within the structure of the antigen-recognition site of HLA-B*13:01 and thus changes the self-peptides that bind to HLA-B*13:01 causing an increased risk of allergic reaction to dapsone. Chen et al., further studied the role of HLA-B*13:01 in DRESS to dapsone and found that dapsone-specific cytotoxic T cells were 3-9 fold activated when co-cultured with HLA-B*13:01 – expressing antigen presenting cells in the presence of dapsone (p < 0.01) compared to HLA-B*13:01 negative cells.

Exposure to dapsone and the presence of HLA-B*13:01 positivity leading to a high incidence of DHS is a case of gene-environment interaction. These two factors almost provide a sufficient causal constellation apart from being necessary causes. The population attributable risk of 86.2% suggests that 86.2% of DHS can be eliminated if dapsone is not administered in patients who are HLA-B*13 positive.
Based on the initial reports, we started doing HLA-B*13 screening in our center from January 2015. As a cost saving measure, we advocated a 2-step process for detection of HLA-B*13:01 among those with DHS and dermatology patients. 94.1% of HLA-B*13 positive patients had the HLA-B*13:01 allele. This suggests that intermediate resolution HLA testing may be adequate for pre-treatment screening reducing the cost of testing considerably (unit cost of screening for HLA-B*13 is 21.50 USD, while for HLA-B*13:01 it is 63.00 USD).

In summary, our study has shown a strong association between DHS and HLA-B*13:01 suggesting that pre-treatment screening for the allele may result in reduction in the numbers developing DHS. As a cost saving measure, it may be adequate to use intermediate resolution HLA-B*13 typing over HLA-B*13:01 typing in our population. The results of a recently published large prospective cohort trial in Chinese patients with leprosy has shown that prospective screening identifies those with the HLA-B*13:01 allele and administering MDT without dapsone for positive patients significantly reduces the incidence of DHS.14

One of the limitations of this study is the small sample size. Larger multicenter studies are required to verify these results and to validate the risk reduction that can be obtained by making pre-dapsone HLA-B*13 testing a standard of care.

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Any conflict of interest disclosures

All authors declare that the answer to the question on competing interest form are all ‘No’, and therefore have nothing to declare.

Details of ethics approval

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Statement of independence of research from funders

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References


