Distinct clinico-immunological profile of patients infected with human papilloma virus genotypes 6 and 11

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Abstract Anogenital warts are primarily caused by Human Papillomavirus (HPV) type 6 and 11, which belong to the taxonomic family Papillomaviridae, genus alpha-papillomavirus and species 10. The presentation of the warts is varied and most of the patients have high recurrence rate of wart lesions. Studies had shown that an effective cellular immune response is required for the control of HPV infection. Here, we report distinct clinico-immunological profile of two patients presenting with venereal warts caused by HPV genotypes 6 and 11. The Case 1 manifested greater number of verrucous warts and case 2 had fewer subtle lesions. Further, evaluation of HPV antigen-specific cellular immune response revealed a robust T cell response against HPV6 peptide and a weak response against HPV11 in case 1. Interestingly, HPV genotyping revealed type 6 in case 1 with greater severity of infection and robust immune response against HPV6

peptide. In contrast, case 2 presented with milder infection and weak immune response and was positive for genotype 11. More extensive study with larger cohorts will strengthen our observation and could be relevant for designing immunotherapeutic adjunct strategies along with the standard treatment for rapid clearance of HPV infections in these patients. This communication reports immune status of two patients with venereal warts and their correlation with clinical presentation and the genotyping.

Keywords Human Papillomavirus · HPV Genotype 6 · HPV Genotype 11 · IFN- γ · IL-4

Human Papilloma virus (HPV) infection caused by genotypes 6 and 11 [1–4] is the most common infection of the anogenital region in humans with varied presentations of the warts [5]. Approximately 90–95% of these warts are caused by HPV genotypes 6 and 11 [3, 4, 6, 7], which belong to the taxonomic family Papillomaviridae, genus alpha-papillomavirus and species 10 [8]. HPV infection has been detected in more than 99% of the cervical cancer patients [9] of which 70% had high-grade genotype 16 or 18 [10]. Studies had demonstrated that most of the HPV infections regress within 8 months to 2 years [11, 12] and the infection with subtypes 16 and 18 are likely to persist longer [13]. Few studies had shown that an effective cellular immune response is required for the control of HPV infection [14]. However, to the best of our knowledge, no data is available on the association between HPV genotype, clinical presentations and its immunological profile. In this communication, we demonstrated the clinico-immunological correlation with distinct genotypes in two patients with ano-genital warts, which might have therapeutic implications.

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A 26 year-old male presented with multiple (>30) verrucous perianal and gluteal warts (Fig. 1a) in OPD of Department of Dermatology and Venereology at the Dr. Ram Manohar Lohia (RML) Hospital, New Delhi, India. The duration of the lesions was 6 months. The patient was HIV negative. The warts were verrucous, keratinized and were of varying size located in the perianal and gluteal region. The size of the largest lesion was 8×10 mm and of smallest lesion was 2×3 mm. The total number of

lesions was around 30. For HPV genotyping, genomic DNA was isolated from genital wart tissues as described earlier with minor modification [15]. HPV DNA was detected by PGMY-PCR using PGMY09/11 primers followed by reverse line blot (RLB) assay [16].

The genotyping of the lesion revealed infection with HPV6. The peripheral blood cells were stimulated with HPV peptide 6 or 11, co-stained for cell surface marker (CD3) and intracellular cytokines (IFN- γ and IL-4) and

Fig. 1 Clinico-immunological profile of patient 1 infected with HPV genotype 6. **a** Anogenital region showing gluteal and perianal warts. **b** *In vitro* T cell response against HPV6 and 11 peptides after stimulation of PBMCs from patient 1. *Dot plots* showing percent

frequency of T cells positive for IFN- γ (*top panel*) and IL-4 (*bottom panel*). Relative increase in percent frequency of cytokine producing T cells in peptide stimulated PBMCs were compared to unstimulated control

Fig. 2 Clinico-immunological profile of patient 2 infected with HPV genotype 11. Anogenital region showing (a) penile warts and (b) perianal warts (c) *In vitro* T cell response against HPV6 and 11 peptides after stimulation of PBMCs from patient 2. Dot plots

showing percent frequency of T cells positive for IFN- γ (top panel) and IL-4 (bottom panel). Relative increase in percent frequency of cytokine producing T cells in peptide stimulated PBMCs were compared to unstimulated control

acquired by Flow cytometer (FC500, Beckman Coulter) and analysed by CXP analysis software. The HPV peptide specific T cell response of the PBMCs revealed a robust and dominant response to HPV6 peptide and a poor or absent response to HPV11 peptide in comparison with unstimulated control (Fig. 1a). The percent frequency of T cells expressing IFN- γ was low as compared to the IL-4⁺T cells indicating higher Th2 response as compared to Th1 response (Fig. 1b).

The second patient was a 23-year-old male who presented with relatively fewer warts of the mucosal region of penile shaft (Fig. 2a), Left panel) and perianal region (Fig. 2b), Right Panel) as compared to case 1. The patient was HIV negative. The clinical appearance of the warts present at mucosal region of penile shaft showed small papular, subtle warts of 2 × 3 mm in size. The total number of the warts was about 10. The clinical appearance of the perianal warts showed non-verrucous, non-

keratinized and subtle warts. The size of the largest lesion in the perianal area was 7×8 mm in size and smallest of 2×3 mm in size. The total number of the warts was around 13–14 in the perianal area. The genotyping of the lesion (by PGMY RLB method) revealed HPV11 infection. The HPV peptide specific T-cell response revealed a weak response to HPV11 peptide and a poor or absent response to HPV6 peptide. The percent frequency of both IFN- γ^+ and IL-4 $^+$ T cells was low in this patient suggesting poor immunogenicity of HPV11 genotype (Fig. 2c).

The clinico-immunological comparison in both the wart patients with different HPV genotypes revealed a significant correlation between the clinical presentation, type of the lesion and immune response. Interestingly, we observed that although the percent frequency of peptide specific IFN- γ^+ T cells was decreased and IL-4 $^+$ T cells was higher, there was a robust response to HPV 6 peptide as compared to HPV11 peptide. In the patient with HPV6 positive warts the Th2 response was more robust as compared to Th1 response to HPV6 specific peptide and no response was observed to HPV11 specific peptide; suggesting that there was functional skewing of Th1 response in favor of Th2 response. However, in patient with HPV11 positive warts, there was a weak Th2 response to HPV6 and HPV11 specific peptides.

This indicated that persistence of HPV induced warts could be due to suppression of immune profile. Besides, the HPV6 genotype seems to be responsible for causing multiple verrucous clinical lesions as compared to HPV11 genotype, which causes milder and fewer lesions. Our findings seem to be interesting and warrant a larger study correlating the clinico-immunological profile with different HPV genotypes. This could have potential future implications in designing immunomodulation strategies to boost immune response along with the standard treatment for early cure and prevention of recurrence, especially in patients infected with genotype HPV6.

Several researchers have been evaluating newer therapies for improvement of efficacy of treatment for warts, either by modulating host immunity for eradication of HPV infection or by enhancing drug delivery to HPV lesions but with limited success. Intra-lesional as well as systemic interferons were used in the past as an adjuvant to other modalities of treatment, as an immunomodulatory drug [17–19]. But the therapy is costly, and associated with high incidence of side effects [19]. Thus, the current treatment options may not be well suited for developing nations. Understanding the role of immunity and its response to specific genotype and the clinical presentation might help the clinicians to give an adjuvant therapy to the patients infected with HPV6 for more rapid and complete remission and prevention of recurrence.

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