INTRODUCTION

Pemphigus vulgaris (PV) is a potentially lethal autoimmune blistering disease mediated by autoantibodies directed against Desmogleins (Dsg) located on the surface of keratinocytes which leads to an intraepithelial loss of adhesion called acantholysis with clinical presentation of vesicles and blisters.1

The worldwide prevalence of PV is reported to be 0.5 - 3.2/100,000.2 In Iran, it is reported as 0.98/100,000.3 Chumurova et al. reported the prevalence of PV in Jerusalem and UK to be 1.6/100,000 and 0.68/100,000 respectively with more prevalence in Jewish population of Jerusalem. Male to female ratio is 1:1.4 and mean age of onset is 40 - 60 years.4

Various environmental factors have been reported as triggering factors for PV and genetic association has been suggested to be the most important predisposing factor.5 The Human Leukocyte Antigen (HLA) locus is a genomic region in the chromosomal position 6p21. It encodes the six classical transplantation HLA genes and at least 132 protein coding genes which have important roles in the regulation of the immune system and some other important molecular and cellular processes. This genomic region has been associated with more than 100 different diseases, including various autoimmune disorders. HLA molecules perform a fundamental function in the regulation of the immune response.6

In different populations and ethnic groups, HLA class II alleles associated with PV are different. In North-East China, the gene frequencies of HLA DRB1*14, DRB1*12 and DQB1*0503 alleles in PV patients were reported to be significantly higher than a control group in Han subjects.7 In a Japanese study, all the patients carried one or two alleles of DRB1*04 and DRB1*14.8 In a Turkish study, HLA DRB1*04⁄DQB1*03 was the most common haplotype (32.0%) and the frequency of this haplotype was significantly higher than that of control subjects (32.0% vs. 6.2%, \( \chi^2 = 28.142, p < 0.001 \)).9 Delgado et al. reported that PV patients from Pakistan had significantly increased frequencies of DRB1*1404 (\( p = 0.01 \)), DQA1*0101 (\( p = 0.02 \)), and DQB1*0503 (\( p = 0.01 \)).10 PV has still remained a relatively less explored entity in our part of the world and there are not much reports on the molecular genotyping of HLA class-II alleles in Pakistani patients. This study will highlight the association of HLA-DR alleles in Pakistani patients of PV.

The objective of this study was to determine frequency of HLA-DR alleles in Pakistani patients of pemphigus vulgaris in comparison with local healthy controls.

ABSTRACT

Objective: To determine frequency of HLA-DR alleles in Pakistani patients of pemphigus vulgaris in comparison with local healthy controls.

Study Design: Cross-sectional, comparative study.

Place and Duration of Study: Department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from January 2011 to January 2014.

Methodology: Twenty eight patients with biopsy proven diagnosis of pemphigus vulgaris referred from Department of Dermatology, Military Hospital, Rawalpindi were included. Patients were compared with a group of 150 unrelated local healthy subjects. DNA was extracted from peripheral blood collected in Tri-potassium EDTA. HLA-DRB1 typing was carried out on allele level (DRB1*01 - DRB1*16) using SSP (sequence specific primers). HLA type was determined by agarose gel electrophoresis and results recorded. Phenotype frequency of various alleles among patient group and control group was calculated by direct counting and significance of their association was determined by Fisher's exact test/ Chi square test.

Results: A total of 12 male and 16 female patients, with age ranging from 21 to 34 (mean 23.4 years) were genotyped for HLA-DRB1 loci. A statistically significant association of the disease with HLA-DRB1*04 was observed (50% versus 20.7% in controls, \( p < 0.05 \)).

Conclusion: There is a strong association of HLA-DRB1*04 with pemphigus vulgaris in Pakistani population.

Key Words: Pemphigus vulgaris. Human leukocyte antigen. Pakistan. Haplotype.
METHODOLOGY

Twenty eight unrelated adult patients of PV attending the Dermatology Clinic at Military Hospital, Rawalpindi, from January 2012 to January 2014, with a biopsy proven diagnosis on histopathology and confirmed by the immunofluorescence examination of skin biopsy sections, were included in the study. HLA data obtained from these patients was compared with a panel of 150 healthy unrelated local individuals from Rawalpindi. Peripheral blood was collected into EDTA containing tubes, and chromosomal DNA was extracted according to the manufacturer's instructions (Puregene DNA Purification Kit; Gentra Systems, Inc.). DNA was amplified using sequence specific primers for HLA-DRB1*01 - *16. Amplified DNA was subjected to electrophoresis on 2% agarose followed by ethidium bromide staining for 30 minutes. HLA-DR alleles were determined by recording specific band patterns observed on the gel under UV illumination.

The phenotype frequency of various alleles among patient group and control group was calculated by direct counting and significance of their association was determined by Fisher’s exact test or Chi-square test using Open Epi Info software. Level of significance was set to 0.05. Odds ratio (OR) was calculated at 95% confidence limits.

RESULTS

Most of them belonged to the provinces of Punjab and Khyber Pakhtunkhwa with only 2 patients from Baluchistan and no patient from Sindh. The clinical features of these 28 patients were consistent with PV (Table I). The median age of the patients at the time of diagnosis was 23.5 years (range 21 to 34 years). Most of the patients had severe mucocutaneous lesions with ulcers in the oral cavity (82.1%) and on the skin of back (60.71%) and flexures (60.71%) being the most common presenting features.

Table II illustrates the HLA-DRB1 alleles in PV patients and healthy controls. Fourteen out of 28 patients were positive for DRB1*04 (50%) when compared with its frequency of 20.7% (31 out of 150) in controls (p < 0.05). Of the 28 patients, 2 were DRB1*04 homozygous (7.1%), 2 were DRB1*13 homozygous (7.1%) and one each were DRB1*03, DRB1*15 and DRB1*14 homozygous (3.6%). Four each were DRB1*04/*12, DRB1*04/*13 and DRB1*04/*15 heterozygotes (14.3%); 2 each were DRB1*07/*12, DRB1*03/*08 and DRB1*07/*15 heterozygotes (7.1%). One each were DRB1*03/*15, DRB1*11/*14 and DRB1*03/*11 heterozygotes (3.6%).

DISCUSSION

Clinical presentation of PV in these patients indicate that ulcers of oral cavity and skin of back and flexures are the most common presenting complaints. The results obtained on HLA-DR association in our patients are in agreement with those reported in other ethnic groups, with high frequency of DR4 positive haplotypes being reported in studies from North America (50%), Turkey (68% vs. 30.97%) and in a meta-analysis of 18 studies (54.46% vs. 18.93%). Although HLA-DQ alleles were not determined directly, given the tight linkage of DQ3 to DR14 and DR4, it may be likely that all PV patients express the DQ3 or DQ5 heterodimers, suggesting high prevalence of DR4-DQ3 and DR4-DQ5 haplotypes in our PV patients. Similar findings were depicted by another study, where majority of PV patients were found to carry HLA-DQ3 and HLA-DR4 haplotypes. In an Indian study DQB1*0503 was reported to be primarily associated with PV. An analysis of haplotypes in a Turkish population revealed that HLA DRB1*04/DQB1*03 was the most common haplotype and the frequency of this haplotype was significantly higher than that of control subjects (32.0% vs. 6.2%). A study in 2009 by Shams et al. revealed that HLA-DRB1*04, DRB1*1401, DRB4, DQA1*0104, DQA1*03011, DQB1*0302 and DQB1*0502 alleles were significantly increased in Iranian patients with PV. In contrast, HLA-DRB1*15, DRB1*0301, DRB1*07, DRB1*11, DRB5, DQA1*0101, DQA1*0103, DQA1*201, DQA1*05, DQB1*0201,
It has been reported that only antigen presenting cells expressing HLA-DRB1*0402 and DQB1*0503 were capable of presenting Dsg 3 (the protein present on the surface of keratinocytes) to auto reactive Th1 and Th2 clones, stressing a direct involvement of particular HLA antigens in the pathophysiology of PV. HLA-B*44:02, -C*04:01, -C*15:02 alleles and HLA-A*03:01, -B*51:01, -C*16:02 haplotype are susceptibility factors for development of pemphigus vulgaris in the Iranian population, while HLA-C*06:02, -C*18:01 alleles and HLA-A*26:01, -B*38, -C*12:03 haplotype may be considered as protective alleles.

Such haplotype diversion suggests that genes lying outside DR4-DQ3/DR4-DQ5 region may exert an additional influence on PV susceptibility beyond HLA-DQ association in PV patients.

Despite the strong association of HLA-DR4-DQ3/DR4-DQ5 with PV, only a minor proportion of people possessing these alleles develop the disease. This raises the question of how healthy individuals bearing the typical DR4 heterodimer remain protected. It is possible that HLA genes are not the only factors to be involved in the determination of disease. In some studies, the role of adjacent MHC genes (major histocompatibility genes) such as TNF-α (tumor necrotic factor-alpha) -308, MIC (MHC class I chain-related genes), and others has also been highlighted.

Slomov et al. in 2005 identified two PV transporter associated with antigen processing (TAP2) risk alleles (TAP2*C and TAP2*D), the frequency of which was estimated to be 37.8% in the patients and 5.3% in the controls. This association was found to be independent of HLA-DR. It is, therefore, possible that TAP2 genes are involved in susceptibility to development of PV. In several studies, role of other environmental triggering factors like intake of drugs, stress, ionizing radiations, dietary factors and viral infections especially herpetic ones has also been highlighted.

**CONCLUSION**

This data reconfirms the observation that PV has one of the strongest HLA class II associations of any human illnesses. DR4 alleles are crucial in development of PV conferring an Odds Ratio (OR) of 3.84 when compared with other DR alleles in Pakistani patients.

**REFERENCES**


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