ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
In press.

Association between HLA-DRB1.2 Genotypic Diversity and Cervical Cancer in Women Infected with the Human Papillomavirus

Somaye Sedaghat[1], Gholamreza Nikbakht Brujeni[2], Seyyed Ali Akbar Shamsian[3], and Nakisa Sohrabi Haghdoost[1]

[1] Department of Pathobiology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3] Department of Parasitology and Mycology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Received: 18 December 2024; Received in revised form: 3 February 2025; Accepted: 20 February 2025

ABSTRACT

Cervical cancer is a significant public health concern, particularly in women infected with the human papillomavirus (HPV). Recent evidence suggests that host genetic factors, specifically those related to the human leukocyte antigen (HLA) system, may also play a crucial role in determining susceptibility to cervical cancer in HPV-infected individuals.

In this study, 86 patients with HPV and 27 healthy donors were selected from May 2023 to February 2024. *HLA-DRB1* genotypes were determined using polymerase chain reaction followed by high-resolution melting curve analysis (HRM). Genotype frequencies in patients were compared with those in the control group from donors.

Based on the HRM analysis, 10 genotypes were found in both patients and controls (profiles A-J). In the analysis of *HLA-DRB1* genotypes, C, F, and I showed significant associations with HPV infection, indicating a possible protective effect against infection. Notably, genotype B was strongly linked to high-risk HPV, while genotype A was associated with low-risk HPV and is relevant to infection history. However, the remaining genotypes examined in the study did not exhibit significant associations with the analyzed parameters.

This study contributes valuable evidence regarding the role of *HLA-DRB1* genotypes in cervical cancer susceptibility and highlights the potential clinical implications for risk assessment and targeted immunotherapies. The use of HRM for *HLA* typing offers advantages that are efficient, accurate, and scalable, making it suitable for large-scale studies and clinical applications.

Keywords: Cervical cancer; HLA-DRB1.2; HRM; MHC; Papillomavirus

INTRODUCTION

Cervical cancer, a significant public health concern, particularly in women infected with the human papillomavirus (HPV), is a leading cause of morbidity and mortality worldwide.[1] Extensive research has focused on elucidating the risk factors and origins of cervical cancer, with HPV infection recognized as a

Corresponding Author: Gholamreza Nikbakht Brujeni, PhD; Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Tel: (+98 21) 6111 7057, Fax: (+98 21) 6111 7001, Email: nikbakht@ut.ac.ir

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

major contributor.[2] However, recent evidence suggests that host genetic factors, specifically those related to the human leukocyte antigen (HLA) system, may also play a crucial role in determining susceptibility to cervical cancer in HPV-infected individuals.[3]

The HLA system, also known as the major histocompatibility complex (*MHC*), is a highly polymorphic region of the human genome that encodes cell surface antigens crucial for the immune system function.[4] HLA class II molecules, including HLA-DR, -DP, and -DQ, play a pivotal role in presenting antigens to CD4+ T cells and initiating adaptive immune responses.[5] Within the HLA class II region, the *HLA-DRB1* gene is highly polymorphic and has been associated with various diseases, including malignancies, due to genetics. Despite the importance of HLA genes in immune regulation, the association between specific *HLA-DRB1* genotypes and HPV infection remains inconclusive, with studies yielding conflicting results.[6] These inconsistencies may be attributed to genetic diversity among different populations, as the distribution of *HLA-DRB1* genotypes varies significantly.[7] Therefore, it is crucial to examine the diversity of *HLA-DRB1* in different populations and their relationship with cervical cancer in HPV-infected women. Understanding the diversity of *HLA-DRB1* and its impact on resistance or susceptibility to infection is not only vital for unraveling the mechanisms of disease development but also holds potential clinical implications.[6] Knowledge of *HLA-DRB1* diversities and their influence on disease progression can facilitate risk assessment, prognosis, and the development of targeted immunotherapies for cervical cancer.[8]

In recent years, high-resolution melting analysis (HRM) has emerged as a powerful technique for *HLA* typing.[9] HRM is a rapid, cost-effective, and high-throughput method that enables the detection of sequence variations in PCR amplicons based on their melting behavior.[10] This technique relies on the principle that different DNA sequences have distinct melting profiles when subjected to increasing temperatures.[11] By analyzing the melting curve of PCR products, HRM can accurately identify single-nucleotide polymorphisms (SNPs) and small indels in *HLA* genes, including *HLA-DRB1*.[12]

In the context of our study on the association between *HLA-DRB1* genotypes and cervical cancer in HPV-infected women, HRM will be employed for *HLA* typing. By leveraging the advantages of HRM, we aimed to accurately determine the diversity of *HLA-DRB1* genotypes in our study population and investigate their potential associations with HPV infection risk. The utilization of HRM will enhance the efficiency and accuracy of our *HLA* typing, contributing to the comprehensive analysis of HRM and melt curves.

This study can contribute valuable evidence regarding the association of *HLA-DRB1* genotypes with susceptibility or resistance to infection. The findings may aid in identifying individuals at higher risk of developing cervical cancer and inform personalized interventions, including immunotherapies targeted toward specific *HLA-DRB1* genotypes.

MATERIALS AND METHODS

The research is classified as a case-control study, with a specific focus on retrospective analysis. The suspected subjects were referred by gynecologists, urologists, and dermatologists from teaching hospitals of Mashhad University of Medical Sciences, Mashhad, Iran. Samples were collected and examined by a molecular medical diagnostic laboratory, ACECR-Central Medical Lab (Academic Center for Education, Culture and Research, Razavi Khorasan, Mashhad, Iran; https://jdmlabs.ir).

To investigate the association between HPV profiles (high risk and low risk), previous infection, and abortion history, this study design allows for the comparison of the patient group, consisting of individuals with confirmed HPV infection and cervical cancer, with a control group, consisting of individuals without HPV infection or any history of autoimmune disease, malignancy, or HIV. The patients had a mean age of 33.2±9.3 years, whereas the controls without HPV infections were 32.8±7.2 years old.

The ethical approval to conduct this study was provided by the research cluster at Azad University of Research Sciences (Ethical Approval No: IR.MUMS.REC.1402.161). Written informed consent was obtained from each patient.

Acceptance and Exclusion Criteria

To be included in the patient group, individuals had to meet the following criteria: confirmation of HPV infection, referral to the Laboratory, and absence of autoimmune disease, malignancy, and risk of HIV infection. For the control group, individuals had to meet the following criteria: absence of any infectious disease, autoimmune disease, malignancy, and HIV infection.

Individuals with a history of autoimmune disease, malignancy, and a risk of HIV infection were excluded from both the patient and control groups. According to previous studies, there is a direct relationship between some types of HPV and cervical cancer. So far, about 80 subtypes of the virus have been known, of which 20 types cause cervical cancer. They are involved, and are subgroups 16, 18, and 25 in order of importance.

Sample Collection

Sampling for this study was done randomly. The diagnosis of HPV infection in the patient group was based on clinical symptoms and the Pap smear test. In the patient group, a total of 86 individuals with confirmed HPV infection and cervical cancer, who were referred to the laboratory, were selected as participants. In the control group, a total of 27 individuals without any infectious disease or any history of autoimmune disease, malignancy, or HIV were selected. The participants were matched in age and sex to ensure comparability between the two groups.

For individuals who were pregnant or virgins, patient consent was obtained before taking the samples. It was ensured that there was no contact in the last 2 days, at least 3 days had passed since the end of menstruation, and the patient had not used cream or gel in the genital area in the last 3 days.

The sampling tools used included a speculum, an LBC brush, a swab, an LBC vial, and distilled water or sterile physiological serum. The LBC pap smear solution, containing a sample stabilizing and lysing solution, was used to preserve the samples. For patients without visible lesions, a speculum was used to open the vagina, and a sample was taken from the cervix using a brush. The brush was then placed into the LBC vial.

Personal protective equipment, including latex gloves, masks, sleeves, and protective glasses, was used during the sampling procedure. After completing the procedure, gloves were disposed of in a designated yellow plastic bin. Sampling tools, such as the speculum and brush handle, were placed on a paper-lined sampling bed, and any contaminated consumables were discarded in an infection bucket. The sampling room was equipped with a UV lamp that automatically turned on for 20 minutes every night to ensure proper disinfection.

DNA Extraction

DNA extraction from the collected samples was performed using the i-genomic Blood DNA Extraction Mini Kit from Intron, Korea. The kit's protocol was followed, with slight modifications depending on the sample type. For blood samples, the procedure involved mixing the sample with lysing buffer, incubating it at room temperature, and centrifuging it to collect the DNA. The paraffin-embedded tissue samples were first separated from the tissue, and then DNA extraction was performed using the same kit.

The first step of DNA extraction involved dissolving proteinase K powder in toilet-free water and carrier reagent in the release buffer. The samples were then mixed with the carrier reagent and binding buffer, vortexed, and incubated at 56°C. After incubation, the samples were centrifuged, and the supernatant was discarded. The columns were washed with wash buffer 1 and wash buffer 2, and the DNA was eluted using release buffer. The extracted DNA was stored at −20°C until further analysis.

PCR Hybridization

PCR hybridization was used in this study to detect the presence of HPV DNA in the samples. The OpeGen High + Low PapillomaStrip PCR kit (Operon S.A., Zaragoza, Spain) was used for this purpose. The first stage of hybridization involved using strips with low-risk and high-risk genotypes, as well as a DNA solution for single-stranded DNA. The hybridization buffer dissolves any sediments and deposits in the solution. The wash buffers were used to wash the strips. The Cani HRP solution was also used in the hybridization process. The TMB solution was used to visualize the bands. A ticker-like sheet was used to identify the genotypes.

High-Resolution Melting Analysis for HLA-DRB1 Genotyping

The *HLA-DRB1* exon 2 sequence was amplified using a Qiagen real-time PCR machine (Rotor-Gen Q, Qiagen, Germany). The PCR amplification was carried out in a final volume of 25 µL. The reaction mixture consisted of 20 ng of genomic DNA, 1.5 mM MgCl2, 200 µM of each dNTP, PCR buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), 1 U/µL of Taq DNA polymerase (CinaClon, Iran), and 20 pmol of both primers Forward (DRBamp-A): CCCCACAGCACGTTTCTTG and Reverse (*DRB amp-B): CCGCTGCACTGTGAAGCTCT. This PCR step amplified a 260 bp DNA fragment specific to the *HLA-DRB1.2* locus. For HRM analysis, 1 µL (2 µM) Syto 9 dye (Life Technologies Corp., Carlsbad, CA) was added

to the PCR master mix. The thermal cycling profile for the second round of PCR was: 1 cycle of 95°C for 2 min, 30 cycles of 95°C for 30 s, 64°C for 30 s, and 72°C for 30 s, Repeat steps 2 to 4, 30 more times, 72ºC for 5 min and final extension of 74°C for 5 min. 5 µL of the last PCR stage was electrophoresed on 2% agarose gels in order to check the quality and specificity of DNA fragment amplification.

The optimal melting conditions for distinguishing between *HLA-DRB1* alleles were found when the PCR products were subjected to a ramping rate of 0.2 °C/s, ranging from 84 to 93°C. The resulting melting profiles were subsequently analyzed using Rotor-Gene 1.7 software and the HRM algorithm. Graphs exhibiting identical patterns were assigned to the same groups with a confidence level of 90%.

Statistical Analysis

The association between the *HLA-DRB1* genotype and HPV infection was determined using the odds ratio (OR). The frequency distribution of the genotype in each profile was compared using a general chi-square test to assess the contribution of each genotype to the total Chi-squared value. In cases where the genotype had a low or zero frequency, the denominator would be zero. In these instances, the odds ratio would be undefined, and Haldane's modified Woolf formula was utilized. Fisher's exact test was performed to determine whether the odds ratio was statistically significant or not. An odds ratio value less than 1.0 suggests that individuals with the genotype have a reduced risk of developing HPV, indicating a potential resistance to the infection. In contrast, an odds ratio greater than 1.0 indicates that individuals with that genotype face an increased risk of developing HPV, making them more susceptible to the infection. Association of HLA-DRB1.2 genotypes with HPV profiles (high risk and low risk), previous infection, and abortion history was determined using a general linear model and multivariate analyses. Association of HLA-DRB1.2 genotypes with HPV profiles, previous infection, and abortion history. The most frequent allele was designated as the reference, and the association study was evaluated using multivariate regression analysis and GLM procedures. The SPSS software version 21 was employed for data analysis, and a probability of $p<0.05$ was deemed statistically significant according to SPSS Institute guidelines in Chicago, IL, USA.

RESULTS

HLA-DRB1 profiles were analyzed using both melting curve analysis and high-resolution melting (HRM) analysis for cases positive and negative for human papillomavirus (HPV). The profiles were divided into 10 genotypes (A–J), as depicted. (Figures 1 and 2).

The control chart exhibited the highest frequency of genotype F (11 cases), while the positive HPV chart showed the highest frequency of genotype D (21 cases). Genotypes A, B, E, G, and H were observed in HPV-positive samples but were absent in the control group. The remaining genotypes were present in both HPV-positive and negative groups (Figure 3).

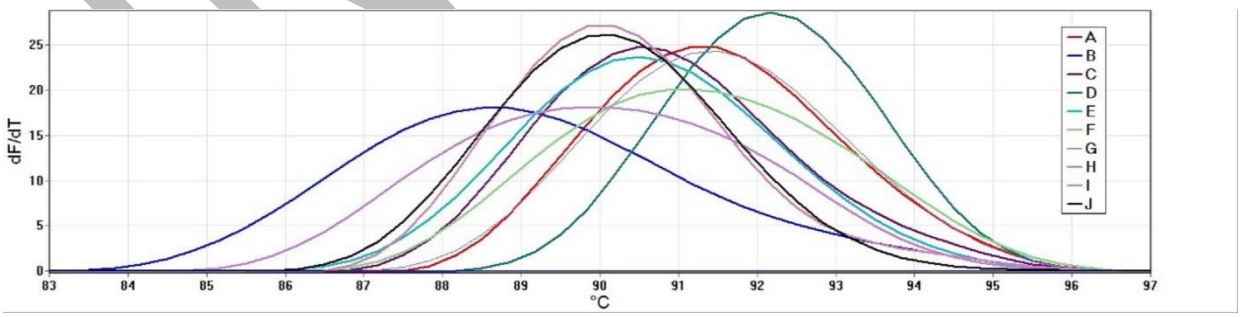

Figure 1. *HLA-DRB1* profiles detected by Melt curve analysis for HPV-positive and HPV-negative cases

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

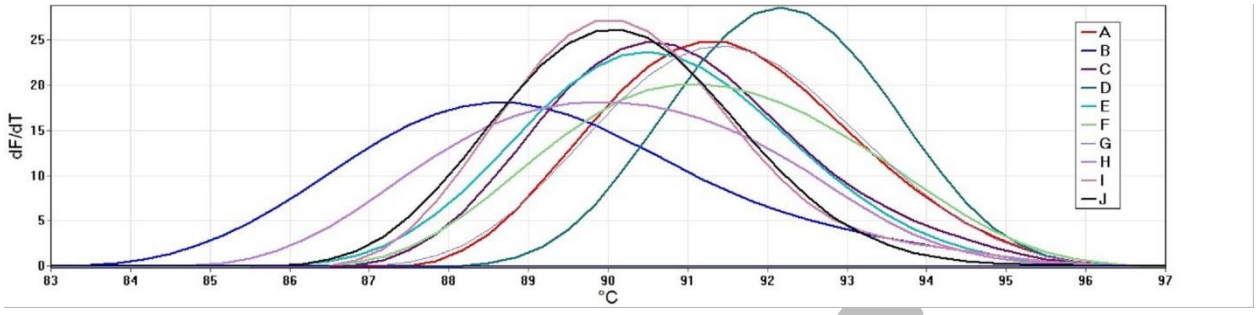

Figure 2. ***HLA-DRB1*** **profiles detected by HRM (high resolution melting curve) analysis for HPVpositive and HPV-negative cases**

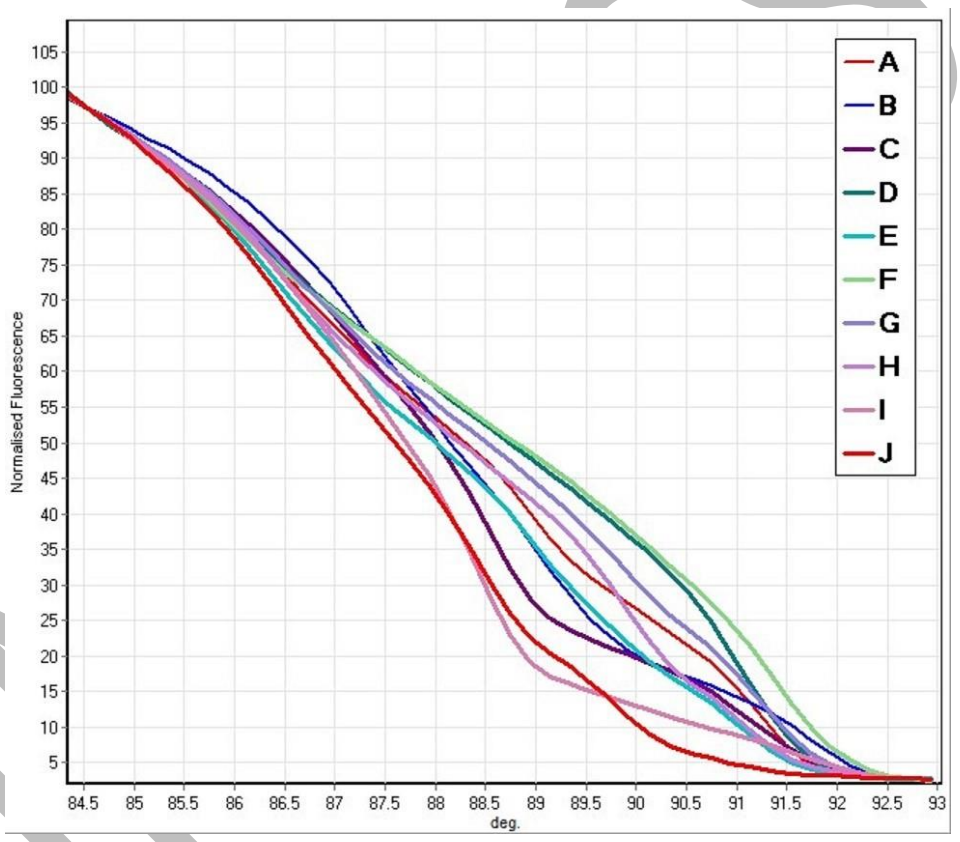

Figure 3. Frequency of HLA-DRB1 genotypes amongst human papillomavirus (HPV) positive cases and controls

Among the 10 genotypes, genotypes C, F, and I demonstrated a significant association with HPV between positive cases and healthy individuals. Specifically, genotype I displayed a highly significant relationship. Both genotypes A and B were found in 7 (8.24%) of HPV cases, while no instances were detected in control subjects. The odds ratios for both A and B (0.743) suggest a reduced odds of HPV presence among these genotypes, though the *p* values (0.248) indicate that these findings are not statistically significant (Table 1). Genotype J, like A, B, D, E, G, and H, presented an OR of 0.750 without statistical significance (p=0.248), suggesting no meaningful association with HPV infection.

Genotype C demonstrated a significant association with an OR of 0.284 and a *p* value of 0.046, indicating a lower likelihood of HPV infection in those with this genotype compared to controls. The presence of this

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

genotype in both groups (4.71% of individuals in HPV cases and 14.81% in controls) suggests that it may serve as a protective factor against HPV.

Genotype F exhibited an OR of 0.312 and a significant *P*-value of 0.023, suggesting that individuals with this genotype have a significantly lower odds of HPV infection compared to controls. The higher prevalence of genotype F (17.65% cases in HPV-positive individuals compared to 40.74% in controls) suggests its potential protective mechanisms against HPV.

Genotype I showed an OR of 0.217 with a highly significant *p* value of 0.014, indicating a strong association with HPV infection. The results suggest that genotype I may be a protective factor, especially since it is more prevalent among controls (18.52 % cases) compared to HPV-positive individuals (4.71% cases).

This research demonstrates a significant association between HLA-DRB1 genotypes, HPV profiles (High-risk or Low-risk, underlying disease history, and abortion history (Table 2). The analysis of HPV profiles across various factors, including high-risk and low-risk categories, infection history, and abortion history, was conducted to evaluate the significance of associations. The results for the HPV profiles A, B, C, F, G, H, and I are presented in terms of their genotype effect or coefficients (B) and significance levels (Sig.) for each category. Virus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 69 were detected in the high-risk group, and virus types 6, 11, 42, 54, 61, 67, 84 and 91 detected in low-risk group. In general, the types of genital warts that may lead to cancer are called high-risk.

Table 1. *HLA-DRB1* genotype frequencies and significant association of *HLA* genotypes and HPV between positive cases and healthy controls

Genotypes	HPV (n=85)	Controls (n=27)	Odds ratio (95% CI)	*p*
A	7 (8.24%)	0 (0%)	0.743	0.248
B	7 (8.24%)	0 (0%)	0.743	0.248
C	4 (4.71%)	4 (14.81%)	0.284	0.046*
D	21 (24.71%)	7 (25.93%)	0.938	0.542
E	4 (4.71%)	0 (0%)	0.750	0.326
F	15 (17.65%)	11 (40.74%)	0.312	0.023*
G	9 (10.59%)	0 (0%)	0.738	0.201
H	9 (10.59%)	0 (0%)	0.738	0.201
I	4 (4.71%)	5 (18.52%)	0.217	0.014**
J	4 (4.71%)	0 (0%)	0.750	0.248

* Significant, ** highly significant

Table 2. Association of *HLA-DRB1.2* genotypes with HPV profiles, previous infection and abortion history.

	High Risk		Low Risk		Infect. History		Abor. History	
HPV Profiles	B*	Sig.	B	Sig.	B	Sig.	B	Sig.
A	−0.066	0.882	0.991	0.015**	0.442	0.001**	0.525	0.096
B	1.562	0.001**	0.545	0.184	−0.12	0.347	−0.255	0.425
C	−0.604	0.183	−0.409	0.318	−0.12	0.347	0.019	0.952
F	−0.243	0.388	−0.099	0.698	−0.052	0.515	0.125	0.528
G	−0.095	0.826	−0.283	0.468	0.005	0.966	−0.192	0.527
H	−0.302	0.452	0.563	0.124	0.12	0.293	0.182	0.522
I	−0.337	0.406	−0.245	0.504	−0.12	0.295	0.303	0.291

*B. Genotype effect: Estimates of effects are relative to the D reference Genotype. Genotypes E, J, and K were excluded as rare genotypes. ** Highly significant

The analysis of high-risk HPV profiles revealed varying associations. For Profile A, the coefficient was −0.066 with a significance of 0.882, indicating no significant association with high-risk HPV. In contrast, Profile B exhibited a significant positive association (B=1.562, $p<0.001$), suggesting that individuals with this profile are significantly more likely to be associated with high-risk HPV. Profiles C, F, G, H, and I showed no significant associations, with coefficients of −0.604 ($p=0.183$), −0.243 ($p=0.388$), −0.095 ($p=0.826$), −0.302 ($p=0.452$), and −0.337 ($p=0.406$), respectively.

Regarding low-risk HPV profiles, Profile A showed a significant positive association (B=0.991, $p=0.015$), indicating a potential relationship with lower-risk HPV types. However, Profiles B, C, F, G, H, and I did not show significant associations, with coefficients of 0.545 ($p=0.184$), −0.409 ($p=0.318$), −0.099 ($p=0.698$), −0.283 ($p=0.468$), 0.563 ($p=0.124$), and −0.245 ($p=0.504$), respectively. Overall, these findings suggest that while Profile B is significantly associated with high-risk HPV, Profile A emerges as a relevant factor in low-risk contexts.

In terms of infection history, profile A showed a significant positive association with a coefficient of 0.442 ($p<0.001$). Profiles B, C, F, G, H, and I exhibited no significant correlation with infection history. In the case of an abortion history, profile A had a coefficient of 0.525 ($p=0.096$), which did not reach significance but indicated a potential association. Profiles B, C, F, G, H, and I showed no significant associations for abortion history.

DISCUSSION

In this study, the association between *HLA-DRB1* genotypes and HPV infection was investigated in individuals both positive and negative for the virus. The results revealed distinct patterns in genotype distribution, odds ratios (OR), and the significance of the associations. The application of HRM for *HLA* typing offers several advantages over traditional methods, such as sequence-specific oligonucleotide probes (SSOP) or sequence-based typing (SBT). HRM eliminates the need for post-PCR processing steps, such as gel electrophoresis or hybridization, streamlining the workflow and reducing the turnaround time.[12] Moreover, HRM is highly sensitive and can detect both known and novel *HLA* alleles, enabling comprehensive and accurate *HLA* genotyping.[13] Furthermore, HRM is amenable to automation and can be easily integrated into high-throughput genotyping platforms, making it suitable for large-scale studies and clinical applications. Its robustness, cost-effectiveness, and scalability have positioned HRM as a promising tool for *HLA* typing in research, diagnostic laboratories, and population studies.[14]

Our results revealed that among the 10 genotypes analyzed, genotypes C, F, and I demonstrated a significant association with HPV infection. Specifically, genotype I showed a highly significant relationship. Previous studies have also reported an association between *HLA-DRB1* genotypes and HPV infection. For example, in Brazilian women, specific *HLA-DRB1* alleles were associated with an increased risk of persistent HPV infection.[15] Similarly, Wang et al observed that certain *HLA-DRB1* genotypes were associated with an increased risk of HPV-related cervical neoplasia.[16]

Our findings are in line with the previous studies, providing further evidence for the role of *HLA-DRB1* genotypes in resistance to HPV. Specifically, we found that individuals with genotypes C, F, and I had a significantly higher protection against HPV infection. These genotypes were found to be present in both HPV-positive cases and healthy controls, indicating that they may confer resistance to HPV rather than being a consequence of infection. These finding contrasts with previous studies that have reported an increased risk of cervical cancer associated with genotype.[16-17] Further investigations are needed to clarify the role of these genotypes in HPV infection and their potential protective effects. The presence of genotype I was found to be strongly associated with HPV infection, with individuals carrying this genotype exhibiting a significantly lower risk of cervical cancer. These results highlight the importance of considering genotype I as a potential marker for HPV-related cancer risk assessment.[17] Our study focused solely on *HLA-DRB1* genotypes and their association with HPV infection. The contribution of other *HLA* genes and genetic factors to HPV susceptibility should be explored in future research. These findings contribute to our understanding of the genetic basis of HPV susceptibility and may have implications for the development of targeted prevention and treatment strategies for HPV-related diseases.[18]

The MHC gene locus contains the most information for proper antigen delivery. HLA, which in humans is

encoded by the major histocompatibility complex. There is a significant relationship between HLA-DRB and *HLA-DRQ* alleles and HPV positive type. Both were significantly associated with cancer and HPV positivity. Cervical cancer is the fourth most common cancer in the world. It accounts for 6.6% of all female cancers. *HLA-DRB1* is a suitable genetic marker in which certain alleles help reduce the HPV infection rate.[19] Women who had cervical HPV infections were more likely to have *DRB1* alleles compared to women without cervical HPV infection. In this study, the relationship between the *HLA-DRB1* genotype and HPV was observed. HLA-DRB1 is a gene within the HLA system that plays a crucial role in the immune response, particularly in presenting antigens to T cells. Its importance in immunological reactions makes it a prime candidate for understanding the specific interactions between host genetics and HPV. Additionally, *HLA-DRB1* has a higher level of allelic diversity compared to some other *HLA* genes, which allows for a broader range of immune responses to various pathogens, including HPV. This diversity is fundamental in studying disease susceptibility, as different alleles can influence susceptibility and disease progression in distinct ways.

The studies showed that the presence of the *HLA-DRB1* genotype is related to the highest risk of HPV18, not HPV16, 52, or 58. HLA-DRB1 genes express HLA class II molecules in immune cells. Also, this study showed the relationship between the *HLA-DRB1* genotypes and HPV infection. More than 100 genotypes of these viruses are known, and some genotypes are classified as oncogenic or high-risk types (HR).[20] The results of the analysis provide valuable insights into the associations between various profiles and HPV risk categories, highlighting the complexity of these relationships. For high-risk HPV genotypes, genotype A demonstrated no significant association with high-risk HPV (coefficient=-0.066, p=0.882). This suggests that individuals with this genotype may not be at an increased risk for high-risk HPV types. However, the findings contrast sharply for genotype B, which exhibited a significant positive association with high-risk HPV (B=1.562, p<0.001). This substantial association indicates that individuals with Genotype B are significantly more likely to be affected by high-risk HPV, underscoring its potential role as a marker for susceptibility to more dangerous HPV strains. Conversely, the analysis of low-risk HPV genotypes reveals that genotype A shows a significant positive association (B=0.991, p=0.015), indicating that it may be relevant for lower-risk HPV types. This finding contrasts with Genotypes B, C, F, G, H, and I, which did not demonstrate significant associations with low-risk HPV. The coefficients for these genotypes ranged from 0.545 to −0.409, all with p values above 0.05, suggesting a lack of substantial influence on low-risk HPV susceptibility.

In examining the infection history, genotype A again indicates a significant positive association (coefficient=0.442, p<0.001), suggesting that individuals with this genotype may have a higher likelihood of past HPV infections. Meanwhile, the other genotypes—B, C, F, G, H, and I—did not show significant correlations with infection history, potentially indicating that they do not significantly influence an individual's infection pattern. Interestingly, when looking at abortion history, Genotype A had a coefficient of 0.525 (p=0.096), approaching significance and suggesting a potential relationship worth investigating further. The absence of significant associations for Genotypes B, C, F, G, H, and I in this context reinforces the idea that Genotype A may have unique relevance in these historical contexts.

Overall, these findings suggest that Genotype B is a significant predictor for high-risk HPV, while Genotype A may serve as an important factor, particularly in low-risk HPV scenarios and infection history. Further research is needed to explore the underlying mechanisms at play and to clarify the roles of these genotypes in HPV risk and infection dynamics. Future studies could also consider expanding the sample size or including a broader range of genotypes to provide a more comprehensive understanding of these associations.

The results of this study indicate that certain genotypes, particularly C, F, and I, may play protective roles against HPV infection, while others show no significant associations. The clinical and epidemiological significance of these findings suggests that further research is warranted, including larger sample sizes and more comprehensive genotyping. The limitations of this study, including the small size of the control group and the potential for confounding variables, could influence the outcomes. Future studies should consider multi-center approaches, larger sample sizes, and additional factors such as viral load or co-infections to clarify the relationships between these genotypes and HPV infection.

In conclusion, our study provides evidence for a significant association between specific HLA-DRB1 genotypes (C, F, and I) and HPV infection, and their impact on the risk of uterine cancer. Genotype I, in particular, displayed a highly significant relationship with uterine cancer development. The identification of individuals with these genotypes may aid in early detection and intervention strategies, ultimately leading to improved outcomes for patients at risk of uterine cancer. This knowledge can contribute to the development of personalized approaches to the prevention, diagnosis, and treatment of uterine cancer. Future studies can explore how these genotypes could inform personalized screening intervals and methods. For example, women with protective genotypes might be candidates for less frequent screening, while those with genotypes linked to increased risk could benefit from more rigorous surveillance. Utilizing HLA-DRB1 genotyping in conjunction with current screening methods could enhance the stratification of women at higher risk for HPV-related cervical cancer. This could allow for more targeted follow-up and intervention for those with genotypes associated with increased susceptibility. In addition, identifying high-risk *HLA-DRB1* alleles could inform the development of targeted immunotherapies. These strategies may help boost the immune response in individuals who are genetically predisposed to higher HPV-related cervical cancer risk.

STATEMENT OF ETHICS

All of the patients had informed consent. The Ethics Committee of the Azad University of Research Sciences approved this study. (IR.MUMS.REC.1402.161).

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

This manuscript is based on the thesis of the First and corresponding author at Azad University of Research Sciences.

DATA AVAILABILITY

Upon reasonable request from the corresponding author.

AI ASSISTANCE DISCLOSURE

No artificial intelligence (AI) tools used in preparing this manuscript.

REFERENCES

1. Pimple S, Mishra G. Cancer cervix: Epidemiology and disease burden. CytoJournal. 2022;19:21.
2. Franco EL, Schlecht NF, Saslow D. The epidemiology of cervical cancer. Cancer J. 2003;9(5):348-59.
3. Paaso A, Jaakola A, Syrjänen S, Louvanto K. From HPV Infection to Lesion Progression: The Role of HLA Alleles and Host Immunity. Acta Cytol. 2019;63(2):148-58.
4. Mahdi BM. Introductory chapter: concept of human leukocyte antigen (HLA). human leukocyte antigen (HLA). 2019 Jan 18:1-8.5.
5. Suzuki K, Luo Y. Histone Acetylation and the Regulation of Major Histocompatibility Class II Gene Expression. Adv Protein Chem Struct Biol. 2017;106:71-111.
6. Kamiza AB, Kamiza S, Mathew CG. HLA-DRB1 alleles and cervical cancer: A meta-analysis of 36 case-control studies. Cancer Epidemiol. 2020;67:101748.
7. Latsoudis H, Stylianakis E, Mavroudi I, Kanterakis A, Pavlidis P, Georgopoulou A, Batsali A, et al. Significance of regional population HLA immunogenetic datasets in the efficacy of umbilical cord blood banks and marrow donor registries: a study of Cretan HLA genetic diversity. Cytotherapy. 2022;24(2):183-92.
8. Chambuso R, Ramesar R, Kaambo E, Denny L, Passmore JA, Williamson AL, et al. Human Leukocyte Antigen (HLA) Class II -DRB1 and -DQB1 Alleles and the Association with Cervical Cancer in HIV/HPV Co-Infected Women in South Africa. J Cancer. 2019;10(10):2145-52.
9. Kim N, Kwon JS, Kang WH, Yeom SI. High-Resolution Melting (HRM) Genotyping. Methods Mol Biol. 2023;2638:337-49.
10. Smith RA, Lam AK. Single Nucleotide Polymorphisms in Papillary Thyroid Carcinoma: Clinical Significance and Detection by High-Resolution Melting. Methods Mol Biol. 2022;2534: 149-59.
11. Wittwer CT, Hemmert AC, Kent JO, Rejali NA. DNA melting analysis. Mol Aspects Med. 2024;97.

12. Tucker EJ, Huynh BL. Genotyping by high-resolution melting analysis. Methods Mol Biol 2014;1145:59-66.
13. Zhang L, Ma X, You G, Zhang X, Fu Q. A novel multiplex HRM assay to detect clopidogrel resistance. Sci Rep. 2017;22 (1):16021.
14. Nathalang O, Intharanut K, Chidtrakoon S. High-resolution melting curve analysis to predict extended blood group phenotypes among Thai donors and patients. Transfus Med Hemother. 2022;22 (3):163-71.
15. Smith JM, S. N. a. J. T., HLA-DRB1*13 and its associated alleles are linked with persistent human papillomavirus (HPV) 16 infection. J Med Microbiol. 2015;64:530-6.
16. Wang SS TM, Schiffman M. Human leukocyte antigen (HLA) class I DRB1*04 alleles protect against persistent cervical neoplasia in women coinfected with oncogenic human papillomavirus (HPV) types. J Infect Dis. 2017;216(4):474-8.
17. Zhang QX, Zhong A. Association between human leukocyte antigen variants and human papillomavirus 16-positive cervical cancer. Biomed Res Int. 2018;16;570-81.
18. Bouza E, Martín Jiménez M, Alemany L, Arribas J, Bañares R, Barragán MB, et al. Overview of virus and cancer relationships. Position paper. Rev Esp Quimioter. 2021;34(6):525-55.
19. Chan PK, Cheung JL, Cheung TH, Lin CK, Siu SS, Yu MM, et al. HLA-DQB1 polymorphisms and risk for cervical cancer: a case-control study in a southern Chinese population. Gynecol Oncol. 2007;105(3):736-41.
20. Delahaye-Sourdeix M, Urayama KY, Gaborieau V, Veenstra R, Foll M, Chabrier A, Benavente Y, et al. A Novel Risk Locus at 6p21.3 for Epstein-Barr Virus-Positive Hodgkin Lymphoma. Cancer Epidemiol Biomarkers Prev. 2015;24(12):1838-43.