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Association of HPA Antigens with Immune Thrombocytopenia: A Case-Control Study by PCR-SSP Method

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ABSTRACT

Background: Human platelet antigens (HPAs) play a clinically significant role in alloimmunization and the development of immune-mediated disorders such as immune thrombocytopenia (ITP), fetal and neonatal alloimmune thrombocytopenia (FNAIT), and post-transfusion purpura (PTP). Understanding the genetic profiles of HPAs is critical for preventing and treating these conditions. Given the limitations of serological methods in determining HPA genotypes, this study aims to investigate the association between the genotypes of HPA1, HPA2, HPA3, HPA4, and HPA15 antigens and autoimmune thrombocytopenia in Lorestan Province, utilizing the PCR-SSP method.

Materials and Methods: This case-control study involved 80 individuals diagnosed with ITP and 120 healthy controls. DNA samples were extracted using a commercial DNA extraction kit, with concentrations quantified via a Nanodrop spectrophotometer. Genotyping was performed using the PCR-SSP method with specific primers for each HPA gene. The genotype data were verified using previously established sample sets. The frequencies of each HPA genotype were recorded, and a comparative analysis was conducted between the patient and control groups to evaluate the study hypothesis.

Results: The results revealed that individuals carrying the HPA2b allele had a 5.31-fold increased risk of developing ITP, a statistically significant finding ($P < 0.05$, OR = 5.31). Similarly, the presence of the HPA15b allele was associated with a 6.54-fold increased risk ($P < 0.05$, OR = 6.54).

Conclusion: These findings, in conjunction with previous studies, suggest the need for larger-scale investigations across different populations. Such research could aid in the early diagnosis and prediction of thrombocytopenia severity, inform treatment strategies, and facilitate the removal of pathogenic antibodies from circulation.

Keywords: Genotype; Autoimmune thrombocytopenia; Platelet-specific antigens (HPA)

INTRODUCTION

Platelets, or thrombocytes, are a type of blood cell with a diameter of 2-4 microns and a volume of 8-10 femtoliters[1]. These cells are formed in the bone marrow through the fragmentation of the cytoplasm of megakaryoblasts, a large precursor cell. In 1882, Bizzozero first identified these discoid cells. Structurally, platelets consist of a glycocalyx, cytoplasmic membrane, and submembrane regions.

They contain various hormones and growth factors, such as TGF-β, PDGF, FGF, and VEGF, which stimulate several cellular functions [2-4].

Platelets also house different types of granules, including alpha granules, delta granules, lysosomal granules, and microperoxisomes. Alpha granules, the largest type, contain key coagulation factors[5]. The primary function of platelets is hemostasis, or blood clotting, achieved through platelet aggregation, clot formation, and the prevention of bleeding[6]. Hemostasis involves a series of processes mediated by platelet membrane receptors, intracellular signals, and platelet-derived substances[7]. Beyond hemostasis, platelets also participate in inflammation, innate and adaptive immunity, fibroblast and endothelial cell proliferation, collagen secretion, and wound healing [8].

When observed under an electron microscope, platelets are seen to be encased in a cell membrane called the glycocalyx. This membrane layer contains numerous complex glycoprotein molecules [9]. These glycoproteins act as receptors for proteins such as collagen, fibronectin, ADP, and thrombin, facilitating platelet aggregation [10]. Numerous antigenic markers are present on the platelet membrane; some are shared with other cells, such as ABH and HLA antigens, while others, such as human platelet antigens (HPA), are specific to platelets [11, 12].

HPAs, or platelet-specific antigens, are located on the membrane surface and begin to be expressed in platelets during the 16th to 18th week of embryonic development [13]. To date, 34 HPA antigens have been identified, each associated with one of six platelet glycoproteins: GPIIb, GPIIIa, GPIa, GPIbα, GPIbβ, and CD109. These antigens can be expressed in both homozygous and heterozygous patterns [14, 15]. Due to their polymorphic nature, HPAs function as alloantigens. This polymorphism is typically caused by a single amino acid change, leading to the formation of single nucleotide polymorphisms (SNPs)[16]. These alterations in amino acid composition can modify platelet glycoproteins, resulting in the emergence of new antigens that antibodies can target [17].

Most known HPAs are expressed on glycoproteins IIb and IIIa. The first and most critical alloantigen, HPA1a, is found on glycoprotein IIIa and is responsible for 85% of fetal and neonatal alloimmune thrombocytopenia (FNAIT) cases. HPA4 antigens, also located on glycoprotein IIIa, are implicated in disorders such as post-transfusion purpura (PTP), FNAIT, and multi-platelet refractoriness (MPR). Similarly, HPA3 antigens are found on glycoprotein IIb, and antibodies targeting HPA1a, HPA1b, and HPA3b are linked to platelet transfusion refractoriness [11, 18-20].

HPA2a is expressed on GPIbα, while HPA12bw is found on GPIbβ. Antibodies against these antigens contribute to the risk of FNAIT [21, 22]. HPA15 antigens, expressed on CD109 glycoprotein, are more challenging to detect due to the low expression levels of this glycoprotein on platelets. Nevertheless, studies have identified anti-HPA15 antibodies in the maternal serum of FNAIT patients [23].

The frequency of HPA expression varies across populations, and understanding these variations can help minimize incompatibilities[24]. Incompatibilities in platelet antigens between mother and fetus during pregnancy or between donor and recipient during transfusion can induce antibody production, resulting in platelet destruction and conditions such as FNAIT, PTP, and MPR. Thrombocytopenia, a common and potentially fatal complication, highlights the importance of further HPA research[2,25].

Understanding the biochemical and molecular basis of platelet antigens will enhance our ability to develop new therapeutic approaches and diagnostic methods for managing platelet-related immune disorders. The primary aim of this study is to investigate the relationship between the genotypes of HPA1, HPA2, HPA3, HPA4, and HPA15 antigens and autoimmune thrombocytopenia in Lorestan Province, using the PCR-SSP method.

MATERIALS AND METHODS

Study Population

This case-control study includes 80 patients diagnosed with immune thrombocytopenia (ITP) as the patient group and 120 healthy individuals as the control group. A non-probability sampling method was employed for ease of access. The study was conducted among individuals residing in Lorestan Province, with patient samples collected from the

oncology department of Shahid Madani Hospital in Khorramabad. Patients were selected based on a platelet count of less than 50 × 10^9/L. Control samples, consisting of healthy individuals with normal platelet counts (150-450 × 10^9/L), were obtained from the Khorramabad Blood Transfusion Organization. Both groups were matched by gender and Lor ethnicity, with age considered as a covariate for further analysis.

Sample size

The sample size was calculated using a proportion comparison formula. Based on previous studies, the following parameters were used: $\alpha = 0.05$, $\beta = 0.20$, $P_0 = 0.12$, $P_1 = 0.29$, and a control-to-patient sample size ratio of 1.5 (K = 1.5). This resulted in a sample size of 80 participants in the patient group and 120 participants in the control group.

Study design and data collection

This case-control study involves 80 individuals diagnosed with ITP and 120 healthy individuals as controls. Patients were selected based on the following criteria: platelet counts below 50 × 10^9/L, the presence of bruising, petechiae, and a confirmed diagnosis by Shahid Madani Hospital[8]. Control subjects had normal platelet counts between 150 and 450 × 10^9/L. The study included both male and female participants, aged between 1 and 40, who were non-relatives and residents of Lorestan Province. Individuals with platelet disorders other than ITP were excluded.

Sampling was performed using a non-probability, convenient sampling approach in the oncology department of Shahid Madani Hospital and the Blood Transfusion Organization in Khorramabad. The two groups were homogenized by gender and Lor ethnicity, controlling for age as a covariate by incorporating it into the statistical model.

DNA extraction

DNA was extracted from the nuclei of white blood cells obtained from 3 milliliters of whole blood collected in EDTA tubes using a commercial DNA extraction kit. The concentration of the extracted DNA was measured using a spectrophotometer. The maximum optical absorption of nucleic acids occurs at a wavelength of 260 nm, while the maximum optical absorption of proteins occurs at 280 nm. The 260/280 ratio was used to assess the purity of the DNA; a high ratio indicates contamination with substances such as alcohol, while a low ratio suggests low DNA purity. The concentration of the extracted DNA was expressed in nanograms per microliter (ng/µL). All equipment was sterilized prior to use. After completing the quantitative and qualitative analyses, the DNA was stored at -70°C.

Primer design

Specific primers (manufactured by Takaposist) were used for the PCR-SSP method to identify the HPA genes in patients with ITP and control subjects. The sequences of these primers were obtained from previous studies. The primers were provided in lyophilized form. To prepare them for use, 150 µL of sterile distilled water was added to each vial. The primers were initially prepared at high concentration, followed by a 1/10 dilution. The diluted primers were aliquoted into small vials and stored at -20°C for future use. The primer sequences used are reported in the following Table 1 [26].

The PCR-SSP (sequence-specific primer) method was used to determine HPA genotypes. The required master mix was prepared using Sinacloon reagents (Lot No: 9920604 and Lot No: 9920603). The total reaction volume was 25 µL, composed of the following components: 12.5 µL of master mix, 6.5 µL of water, 1 µL of forward primer, 1 µL of reverse primer, 1 µL of forward HGH primer, 1 µL of reverse HGH primer, and 2 µL of DNA. These components were mixed in a 0.2 mL microtube, centrifuged briefly, and placed in a thermocycler for amplification.

The reagents for determining the genotype of each of the 10 alleles were prepared in separate microtubes following the same procedure. The study analyzed the genotypes of HPA1a/b, HPA2a/b, HPA3a/b, HPA4a/b, and HPA15a/b in patients with ITP and healthy controls. The allele frequencies were calculated, and a comparative analysis between the patient and control groups was conducted. Based on the results, conclusions were drawn regarding the study's hypothesis.

Table 1: HPA antigen primers with their sequence and product size

Primer	Sequence	Product Size
HPA1a	5' TCACAGCGAGGTGAGGCCA 3'	90 Bp
HPA2b	5' TCACAGCGAGGTGAGGCCG 3'	90 Bp
Reverse	5' GGAGGTAGAGAGTCGCCATAG 3'	90 Bp
HPA2a	5' GCCCCCAGGGCTCCTGAC 3'	258 Bp
HPA2b	5' GCCCCCAGGGCTCCTGAT 3'	258 Bp
Reverse	5' TCAGCATTGTCCTGCAGCCA 3'	258 Bp
HPA3a	5' TGGACTGGGGCTGCCCAT 3'	267 Bp
HPA3b	5' TGGACTGGGGCTGCCCAG 3'	267 Bp
Reverse	5' TCCATGTTCACTTGAAGTGCT 3'	267 Bp
HPA4a	5' GCTGGCCACCCAGTGCG 3'	120 Bp
HPA4b	5' GCTGGCCACCCAGTGCA 3'	120 Bp
Reverse	5' CAGGGGTTTTCGAGGGCCT 3'	120 Bp
HPA15a	5' TTCAAATTCTTGGTAAATCCTCG 3'	225 Bp
HPA15b	5' TTCAAATTCTTGGTAAATCCTCT 3'	225 Bp
Reverse	5' ATGAACCTTATGATGACCTATTC 3'	225 Bp
Forward HGH	5' GCCTTCCCAACCATTCCCTTA 3'	429 Bp
Reverse HGH	5' TCACGGATTTCTGTTGTGTTTC 3'	429 Bp

Statistical analysis

Data were collected and entered into SPSS statistical software for analysis. Qualitative variables were reported as numbers and percentages, while quantitative variables were presented as mean ± standard deviation (SD). The data were analyzed using an independent chi-square test and multivariable logistic regression. Odds ratios (ORs) were calculated to assess the strength of associations, with results reported at a 5% significance level ($P < 0.05$).

RESULTS

HPA1 Gene Genotyping by PCR-SSP Method

The HPA1 gene, which consists of the HPA1a and HPA1b alleles, was genotyped using the PCR-SSP method. Separate forward-specific primers were used for each allele, while a common reverse primer was employed. Initial temperature cycles were based on previous studies; however, these settings yielded unsatisfactory results, with the formation of numerous non-specific bands. The initial PCR protocol was as follows:

- **Initial denaturation:** 95°C for 10 minutes (1 cycle)
- **Amplification:** 95°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds (35 cycles)
- **Final extension:** 72°C for 10 minutes (1 cycle)

To improve specificity and strengthen the bands, the temperature cycles were modified, particularly the annealing temperature. The revised PCR protocol was as follows:

- **Initial denaturation:** 95°C for 5 minutes (1 cycle)
- **Amplification:** 95°C for 15 seconds, 59°C for 30 seconds, 72°C for 30 seconds (40 cycles)
- **Final extension:** 72°C for 10 minutes (1 cycle)

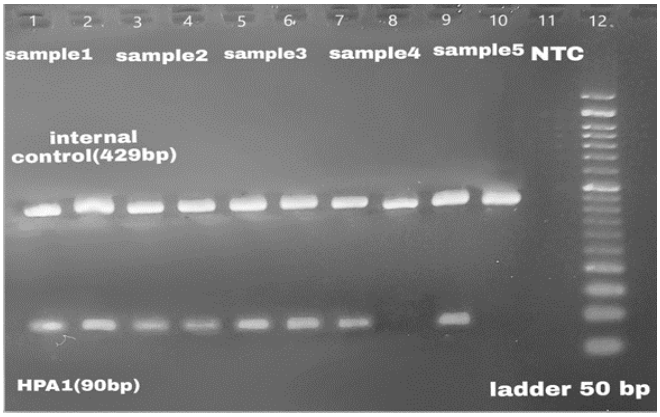

Figure 1. HPA1 Gene Genotyping Results after PCR-SSP Setup

The Figure depicts the genotyping results for the HPA1 gene using the PCR-SSP method. From left to right:

- **Wells 1 and 2**: Sample 1, HPA1a/1b genotype
- **Wells 3 and 4**: Sample 2, HPA1a/1b genotype
- **Wells 5 and 6**: Sample 3, HPA1a/1b genotype
- **Wells 7 and 8**: Sample 4, HPA1a/1a genotype
- **Wells 9 and 10**: Sample 5, HPA1a/1a genotype
- **Well 11**: No template control (NTC)
- **Well 12**: 50 bp DNA ladder (band at 429 bp)

HPA2 gene genotyping by PCR-SSP method

The genotyping of the HPA2 gene was conducted using the PCR-SSP method, with the following temperature cycling conditions:

- **Initial denaturation:** 95°C for 5 minutes (1 cycle)
- **Amplification:** 95°C for 15 seconds, 62°C for 30 seconds, 72°C for 30 seconds (40 cycles)
- **Final extension:** 72°C for 10 minutes (1 cycle)

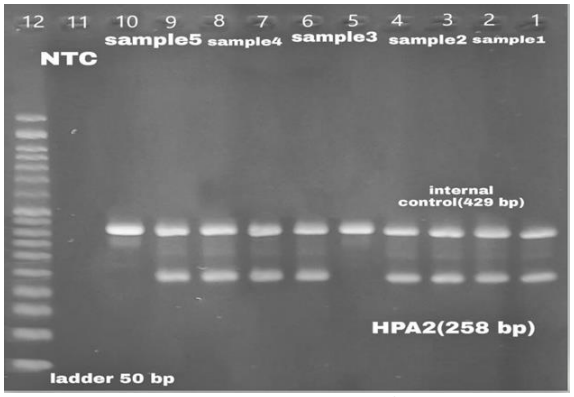

Figure 2. HPA2 Gene Genotyping Results after PCR-SSP Setup

The Figure shows the genotyping results for the HPA2 gene using the PCR-SSP method. From right to left

- **Wells 1 and 2**: Sample 1, HPA2a/2b genotype
- **Wells 3 and 4**: Sample 2, HPA2a/2b genotype
- **Wells 5 and 6**: Sample 3, HPA2b/2b genotype
- **Wells 7 and 8**: Sample 4, HPA2a/2b genotype
- **Wells 9 and 10**: Sample 5, HPA2a/2a genotype
- **Well 11**: No template control (NTC)
- **Well 12**: 50 bp DNA ladder (band at 429 bp)

HPA3 gene genotyping by PCR-SSP method

The HPA3 gene was genotyped using the PCR-SSP method, following these temperature cycling conditions:

- **Initial denaturation**: 95°C for 5 minutes (1 cycle)
- **Amplification**: 95°C for 15 seconds, 62°C for 30 seconds, 72°C for 30 seconds (40 cycles)
- **Final extension**: 72°C for 10 minutes (1 cycle)

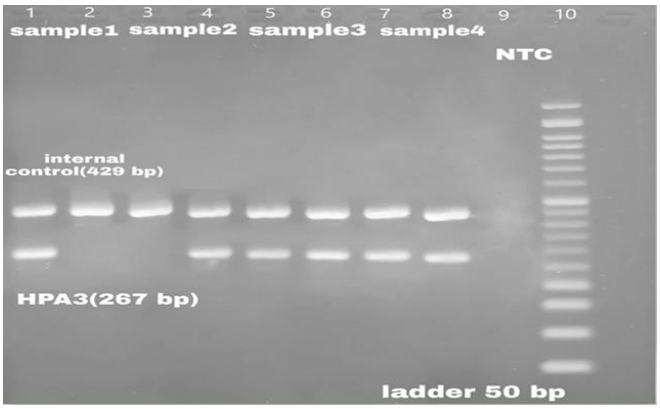

Figure 3. HPA3 Gene Genotyping Results after PCR-SSP Setup

The Figure illustrates the genotyping results for the HPA3 gene using the PCR-SSP method. From left to right:

- **Wells 1 and 2**: Sample 1, HPA3a/3a genotype
- **Wells 3 and 4**: Sample 2, HPA3b/3b genotype
- **Wells 5 and 6**: Sample 3, HPA3a/3b genotype
- **Wells 7 and 8**: Sample 4, HPA3a/3b genotype
- **Well 9**: No template control (NTC)
- **Well 10**: 50 bp DNA ladder (band at 429 bp)

HPA4 gene genotyping by PCR-SSP method

The HPA4 gene was genotyped using the PCR-SSP method. The following temperature cycling conditions were used:

- **Initial denaturation**: 95°C for 5 minutes (1 cycle)
- **Amplification**: 95°C for 15 seconds, 61°C for 30 seconds, 72°C for 30 seconds (40 cycles)
- **Final extension**: 72°C for 10 minutes (1 cycle)

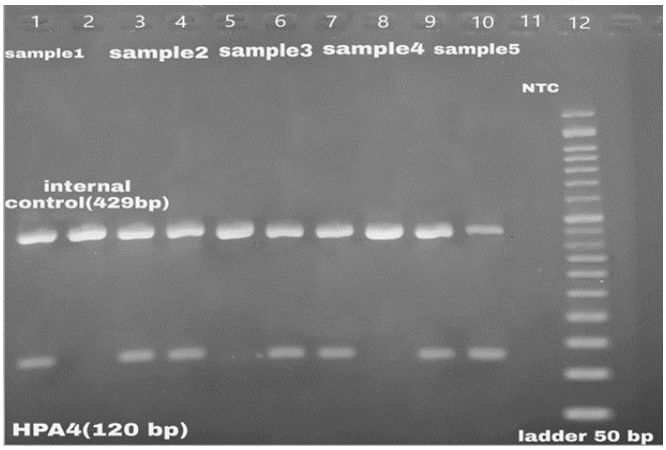

Figure 4. HPA4 Gene Genotyping Results after PCR-SSP Setup

The Figure displays the genotyping results for the HPA4 gene using the PCR-SSP method. From left to right:

- **Wells 1 and 2**: Sample 1, HPA4a/4a genotype
- **Wells 3 and 4**: Sample 2, HPA4a/4b genotype
- **Wells 5 and 6**: Sample 3, HPA4b/4b genotype
- **Wells 7 and 8**: Sample 4, HPA4a/4a genotype
- **Wells 9 and 10**: Sample 5, HPA4a/4b genotype
- **Well 11**: No template control (NTC)
- **Well 12**: 50 bp DNA ladder (band at 429 bp)

HPA15 gene genotyping by PCR-SSP method

For HPA15 gene genotyping, the PCR-SSP method was employed using the following thermal cycling conditions:

- **Initial denaturation**: 95°C for 5 minutes (1 cycle)
- **Amplification**: 95°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds (40 cycles)
- **Final extension**: 72°C for 10 minutes (1 cycle)

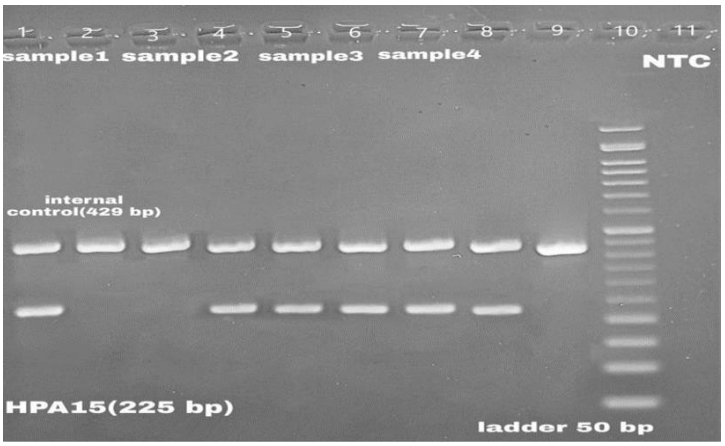

Figure 5. HPA15 Gene Genotyping Results after PCR-SSP Setup

The Figure shows the genotyping results for the HPA15 gene using the PCR-SSP method. From left to right:

- **Wells 1 and 2**: Sample 1, HPA15a/15a genotype
- **Wells 3 and 4**: Sample 2, HPA15b/15b genotype
- **Wells 5 and 6**: Sample 3, HPA15a/15b genotype
- **Wells 7 and 8**: Sample 4, HPA15a/15b genotype
- **Well 10**: 50 bp DNA ladder (band at 429 bp)
- **Well 11**: No template control (NTC)

Findings

In this study, 200 participants were included: 80 individuals in the patient group and 120 in the control group. The patient group consisted of 42 women (52.5%) and 38 men (47.5%), while the control group included 67 women (55.8%) and 53 men (44.2%). Gender distribution was statistically similar between the two groups (P = 0.67). In terms of viral history, 47 individuals in the patient group (58.8%) and 47 in the control group (39.2%) reported a recent history of viral disease, with a significant difference between the groups (P = 0.009). The average age in the control group was 19.24 ± 0.837 years (range: 1-40 years), while in the patient group, it was 12.06 ± 0.898 years (range: 2-43 years), showing a statistically significant difference in age between the two groups (P < 0.001) (Table 2, Figure 6).

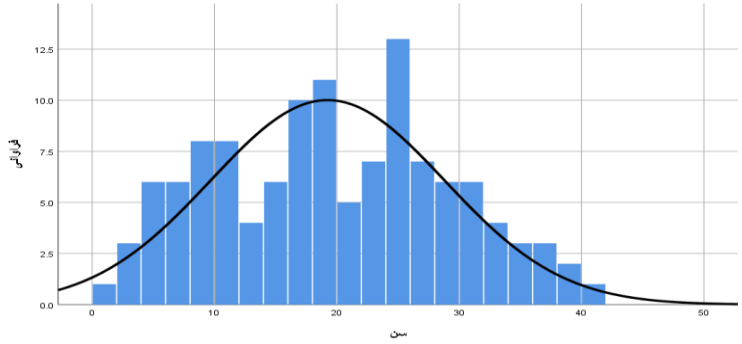

Figure 6. Age Distribution of the Control Group in the Study

Table 2: Comparison of Gender Distribution, Viral Disease History, and Age between Patients and Controls

		Patient (n=80)	Control (n=120)	Total	P
Gender	Woman	42 (52.5%)	67 (55.8%)	109	0.67
	Man	38 (47.5%)	53 (44.2%)	91	
History of viral disease	Yes	47 (58%)	47 (39%)	94	0.009
	No	33 (41%)	73 (60%)	106	
Age (standard deviation ± mean)		8.03 ± 12.6	9.56 ± 19.24		0.001>

Next, to compare the presence or absence of specific genes between the patient and control groups, the OR with a 95% confidence interval (CI) was calculated using univariate logistic regression. It is important to note that if the confidence interval does not include the value of 1, this indicates statistical significance for the OR.

Comparison of HPA1a gene between patient and control groups

In the patient group, 78 individuals (97.5%) carried the HPA1a gene, while two individuals (2.5%) did not. In the control group, 115 individuals (95.8%) had the HPA1a gene, and five individuals (4.2%) did not. The relative risk of developing the disease in those with the HPA1a gene was 69% higher compared to those without the gene; however, this difference was not statistically significant (P = 0.534) (Table 3).

Table 3: The presence and absence of HPA genes in the studied groups

Gene	Group	Yes	No	(95% confidence interval) odds ratio	P
HPA1a	Patient	78 (97.5%)	2 (2.5%)	1.69 (0.32, 8.96)	0.534
	Healthy	115 (95.8%)	5 (4.2%)		
HPA1b	Patient	25 (31%)	55 (67%)	1.19 (2.29,0.64)	0.560
	Healthy	33 (27%)	87 (72%)		
HPA2a	Patient	77 (96%)	3 (3.7%)	0.43 (0.07, 2.66)	0.368
	Healthy	118 (98%)	2 (1.6%)		
HPA2b	Patient	22 (27.5%)	58 (72.5%)	5.31 (2.22, 12.66)	0.000
	Healthy	8 (6.7%)	112 (93.3%)		
HPA3a	Patient	59 (73%)	21 (26%)	0.89 (0.46, 1.71)	0.739
	Healthy	91 (75%)	29 (36%)		
HPA3b	Patient	52 (65%)	28 (35%)	0.93 (0.51, 1.68)	0.807
	Healthy	80 (67%)	40 (33%)		
HPA4a	Patient	71 (88%)	9 (11%)	1.04 (0.42, 2.53)	0.928
	Healthy	106 (88%)	14 (11%)		
HPA4b	Patient	66 (82%)	14 (17%)	1.01 (0.47, 2.1)	0.990
	Healthy	99 (82%)	21 (17%)		
HPA15a	Patient	44 (55%)	36 (45%)	0.9 (0.51, 1.6)	0.727
	Healthy	69 (57%)	51 (42%)		
HPA15b	Patient	70 (87.5%)	10 (12.5%)	6.5 (3.08, 13.9)	0.000
	Healthy	62 (51.7%)	58 (48.3%)		

Comparison of HPA1b gene between patient and control groups

In the patient group, 25 individuals (31%) carried the HPA1b gene, while 55 individuals (67%) did not. In the control group, 33 individuals (27%) had the HPA1b gene, and 87 individuals (72%) did not. The relative risk of developing the disease in those with the HPA1b gene was 19% higher than in those without it; however, this increase was not statistically significant (P = 0.534) (Table 3).

Comparison of HPA2a gene between patient and control groups

In the patient group, 77 individuals (96%) carried the HPA2a gene, while three individuals (3.7%) did not. In the control group, 118 individuals (98%) had the HPA2a gene, and two individuals (1.6%) did not. The likelihood of disease in those with the HPA2a gene was 2.32 times higher than in those without the gene, though this increase was not statistically significant (P = 0.368) (Table 3).

Comparison of HPA2b gene between patient and control groups

In the patient group, 22 individuals (27.5%) carried the HPA2b gene, while 58 individuals (72.5%) did not. In the control group, eight individuals (6.7%) had the HPA2b gene, while 112 individuals (93.3%) did not. The relative risk of developing the disease in those with the HPA2b gene was 5.31 times higher than in those without the gene, which was statistically significant (P = 0.000) (Table 3).

Comparison of HPA3a gene between patient and control groups

In the patient group, 59 individuals (73%) carried the HPA3a gene, while 21 individuals (26%) did not. In the control group, 91 individuals (75%) had the HPA3a gene, and 29 individuals (36%) did not. The relative risk of disease in those with the HPA3a gene was 12% higher than in those without the gene, though this increase was not statistically significant (P = 0.739) (Table 3).

Comparison of HPA3b gene between patient and control groups

In the patient group, 52 individuals (65%) carried the HPA3b gene, while 28 individuals (35%) did not. In the control group, 80 individuals (67%) had the HPA3b gene, while 40 individuals (33%) did not. The relative risk of disease in those with the HPA3b gene was 7% higher than in those without the gene, but this increase was not statistically significant (P = 0.807) (Table 3).

Comparison of HPA4a gene between patient and control groups

In the patient group, 71 individuals (88%) carried the HPA4a gene, while nine individuals (11%) did not. In the control group, 106 individuals (88%) had the HPA4a gene, and 14 individuals (11%) did not. The relative risk of disease in those with the HPA4a gene was 4% higher than in those without the gene, though this increase was not statistically significant (P = 0.928) (Table 3).

Comparison of HPA4b gene between patient and control groups

In the patient group, 66 individuals (82%) carried the HPA4b gene, while 14 individuals (17%) did not. In the control group, 99 individuals (82%) had the HPA4b gene, and 21 individuals (17%) did not. The relative risk of disease in those with the HPA4b gene was 1% higher than in those without the gene, but this increase was not statistically significant (P = 0.99) (Table 3).

Comparison of HPA15a gene between patient and control groups

In the patient group, 44 individuals (55%) carried the HPA15a gene, while 36 individuals (45%) did not. In the control group, 69 individuals (57%) had the HPA15a gene, and 51 individuals (42%) did not. The relative risk of disease in those with the HPA15a gene was 10% higher than in those without the gene, though this increase was not statistically significant (P = 0.727) (Table 3).

Comparison of HPA15b gene between patient and control groups

In the patient group, 70 individuals (87.5%) carried the HPA15b gene, while 10 individuals (12.5%) did not. In the control group, 62 individuals (51.7%) had the HPA15b gene, while 58 individuals (48.3%) did not. The relative risk of disease in those with the HPA15b gene was 6.5 times higher than in those without the gene, which was statistically significant (P = 0.000) (Table 3).

Logistic regression

To assess the effect of different genes on disease susceptibility, adjusting for sex, age, and recent viral disease history, a multiple logistic regression analysis was performed. The results indicated that assuming the influence of other genes remains constant, individuals with the HPA2b gene had a 5.31-fold higher risk of contracting the disease compared to those without this gene, which was statistically significant (P = 0.000). Additionally, individuals with the HPA15b gene had a 6.54-fold increased risk of contracting the disease, and this association was also statistically significant (P = 0.000). No significant associations were found between other genes and the disease (P > 0.05) (Table 4).

Table 4: Modeling the Effect of Different Genes on Disease Risk by Adjusting for Age and Viral History Using Multivariate Logistic Regression

Gene	B	S.E.	Wald	Sig.	OR	Upper 95% C.I. for OR	Lower 95% C.I. for OR
HPA1a	0.525	0.84	0.38	0.534	1.69	8.96	0.32
HPA1b	0.181	0.31	0.32	0.567	1.19	2.22	0.64
HPA2a	–0.832	0.92	0.81	0.368	0.435	2.66	0.071
HPA2b	1.67	0.44	14.1	0.000	5.31	12.66	2.22
HPA3a	–0.111	0.33	0.11	0.739	0.895	1.71	0.46
HPA3b	–0.074	0.30	0.059	0.807	0.929	1.68	0.51
HPA4a	0.041	0.45	0.008	0.928	1.04	2.53	0.42
HPA4b	0.00	0.38	0.00	1.00	0.99	2.1	0.47
HPA15a	0.102	0.29	0.12	0.727	0.903	1.59	0.511
HPA15b	1.87	0.38	23.9	0.001	6.54	13.9	3.084
History of recent viral disease	0.79	0.29	7.28	0.007	2.21	3.93	1.24
Age	–0.09	0.02	23.47	0.000	0.90	0.94	0.87
gender	0.134	0.29	0.215	.643	1.14	1.99	0.64

DISCUSSION

Glycoproteins on the platelet membrane surface express polymorphic antigenic markers that can trigger immune responses. When platelet antigens induce the production of antibodies, they can lead to complications such as thrombocytopenia and bleeding [27]. Immune thrombocytopenia (ITP) results from the generation of autoantibodies against platelets, leading to their destruction and inhibiting their production [28, 29].

Platelet alloantigens were historically detected using human serum containing alloantibodies. However, this approach faced challenges such as the limited availability of antisera in all healthcare facilities and the difficulty in obtaining a sufficient number of platelets from thrombocytopenic patients for analysis. As a result, molecular methods have become the preferred approach for detecting platelet antigens. Among these, the PCR-SSP method is the most widely used and the simplest [30, 31]. HPA antigens are inherited in a codominant or autosomal manner, and their frequency and distribution vary across different populations. Consequently, the pathological impact of these antigens can differ between individuals [2].

Numerous studies have demonstrated a relationship between HPA genotypes and disease susceptibility. For instance, HPA1 and HPA5 are key factors in neonatal alloimmune thrombocytopenia (NAIT), while antibodies against HPA1, HPA3, HPA4, HPA5, and HPA15 are implicated in the development of post-transfusion purpura (PTP)[32-34].

In this study, we explored the relationship between HPA1, HPA2, HPA3, HPA4, and HPA15 genotypes and the risk of ITP. In addition to environmental factors, genetic predisposition plays a role in the development of this disease. Given that the frequency of platelet antigens varies across populations, this variation influences the alloimmunization response to platelet antigens. Therefore, understanding the prevalence and distribution of these antigens in different populations is essential.

This study is the first to report the association between HPA1, HPA2, HPA3, HPA4, and HPA15 genotypes and ITP in individuals of Lor ethnicity in Iran. In this research, the genotypes of HPA antigens were determined using the PCR-SSP method, marking the first time this technique has been employed for such an investigation in Iran. Our findings indicate that the presence of the HPA2b allele significantly increases the risk of ITP ($P < 0.05$, OR = 5.31). Similarly, Thude et al. (1999) demonstrated a significant association between the HPA2b genotype and ITP (27). Pavkovic et al. (2012) also concluded that the HPA2b allele is more prevalent in ITP patients and may contribute to the formation of a specific epitope[35]. Additionally, in 2018, Tayssir Kamel et al. found that individuals with the HPA2b allele were 2.37 times more likely to develop ITP compared to healthy individuals[36].

In this study, the HPA15b allele was associated with a significantly increased risk of developing ITP ($P < 0.05$, OR = 6.54), a finding not observed in previous similar studies. In 2019, Muhiddin et al. conducted a study investigating the prevalence, genotype, and frequency of HPA in patients with immune and non-immune thrombocytopenia. They found that the HPA1b and HPA15a alleles were present in both immune thrombocytopenia groups but were absent in the normal population [37].

Given the limited available information on the mechanisms underlying ITP and the challenges in detecting antibodies against platelet antigens, further research is necessary to deepen our understanding of this condition.

CONCLUSION

This study aimed to investigate the relationship between the genotypes of HPA1, HPA2, HPA3, HPA4, and HPA15 antigens and autoimmune ITP in Lorestan Province using the PCR-SSP method. The findings revealed a significant association between certain HPA genotypes and ITP. Specifically, the presence of the HPA2b allele was identified as a potential factor that increases the risk of developing ITP, consistent with results from other studies. However, the association of the HPA15b allele with ITP has not been reported in previous studies, possibly due to racial differences across various populations. Therefore, it is essential to genotype ITP patients for

platelet antigens and screen for antibodies against these antigens in our population.

There was no significant relationship between age and sex and ITP susceptibility. One limitation of this study was the lack of precise data regarding viral infections in many patients prior to the onset of ITP, which prevented a clear understanding of the relationship between viral infections and the disease.

Future studies are recommended to be conducted on a larger scale and across different ethnic groups. This could help refine diagnostic methods, predict the severity of thrombocytopenia, improve treatment approaches, and assist in the removal of antibodies implicated in the disease from the bloodstream.

CONFLICT OF INTERESTS

The authors have no funding or conflicts of interest to disclose.

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