Evaluating the Possible Association between PD-1 (Rs11568821, Rs2227981, Rs2227982) and PD-L1 (Rs4143815, Rs2890658) Polymorphisms and Susceptibility to Breast Cancer in a Sample of Southeast Iranian Women

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Abstract

Introduction: Programmed cell death-1 (PD-1) and its ligands (PD-L1 and PD-L2) play a critical role as a regulator of immune-system cells, including T cell, natural killer T (NKT), monocytes, dendritic cells (DC), and B cells. Objective: This study aimed to find a possible association between PD-1 (rs11568821, rs2227981, rs2227982), and PD-L1 (rs4143815, rs2890658) variants and Breast Cancer (BC) risk in a sample of southeast Iranian women. Method: The case-control study consisted of 520 individuals, including 260 histologically confirmed BC patients and 260 non-cancer age-matching healthy women as the control group. The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and Tetra-Primer Amplification Refractory Mutation System-Polymerase Chain Reaction (T-ARMS-PCR) methods were used for genotyping of PD-1 (rs11568821, rs2227981, rs2227982), and PD-L1 (rs4143815, rs2890658) polymorphisms. Results and Conclusion: Our findings indicated that the PD-L1 rs4143815 (G/C) variant meaningfully reduced the risk of BC. However, the PD-L1 rs2890658 variant increased the BC risk in the AC genotype as well as the A allele. Furthermore, we could not find a meaningful association between PD-1 rs11568821, PD-L1 rs2227981, PD-L1 rs2227982, and BC. Our team examined the possible association between variants and clinicopathological characteristics, including age, size of tumour, lymph node, histology, grade of tumour, estrogen and progesterone receptors status as well as human growth factor receptor 2 (HER2). Our findings demonstrated that PD-L1 rs4143815, PD-L1 rs2890658, PD-L1 rs2227982 had a significant association with age. Additionally, we found a significant relation between PD-1 rs2227982 variant and tumour size. Statistical analyzes of PD-1 rs2227981 and PD-L1 rs11568821 variants showed a meaningful relation between tumour grade and tumour stage (p=0.006), respectively.

Keywords: Apoptosis- PD-L1- PD-1- cancer- polymorphism

Introduction

Breast Cancer (BC), the second most frequently diagnosed carcinoma and the primary cause of cancer mortality among women all around the world, is becoming the main concern for public health in all communities (Ferlay et al., 2015). Development of BC begins with the abnormal and rapid division of breast cells, resulting in tumour formation that could invade other breast tissues and spread to other organs (Yu, 2019; Umami et al., 2020) such as brain, lungs, and bones (Eckhardt et al., 2012; Vickers, 2017).

The findings of numerous studies have shown a strong correlation between the susceptibility to BC and factors such as genetic mutation, diet, physical activity, breastfeeding, level of estrogen and progesterone hormones (Jayasekara et al., 2016, Pizot et al., 2016; Anstey et al., 2017; Dall and Britt, 2017; Shikanbola et al., 2017; Bertoni et al., 2019; McTiernan et al., 2019). However, the leading cause of BC has yet to be identified.
Genetic mutation, critical factor to cancer susceptibility, are responsible for permanent alternations in DNA and RNA sequences. Single nucleotide polymorphisms (SNPs) are responsible for phenotype variations in populations (Kitts and Sherry 2002). SNPs’ could be located in various regions of the human genome, such as in promoters, exons, introns, as well as 5’- and 3’ UTRs. Consequently, small changes in these parts might affect the expression of genes and also increase or decrease the vulnerability to certain diseases (Taylor et al., 2001; Bond et al., 2005; Fan et al., 2010; Wu et al., 2014; Schirmer et al., 2016; Hashemi et al., 2020).

Programmed cell death-1 (PD-1) is an immune checkpoint key receptor responsible for maintaining self-tolerance and inhibition of uncontrolled inflammation (Francisco et al., 2010; Hashemi et al., 2019; Hashemi et al., 2020). PD-1 is mainly expressed on the surface of several immune system cells (Inman et al., 2007; Keir et al., 2008; Ahmadzadeh et al., 2009; Hashemi et al., 2019). The human PD-1 gene is located on chromosome 2q27.3, which consists of two domains, extracellular immunoglobulin V domain and an intracellular domain containing an inhibitory motif (ITIM) and immune receptor tyrosine-based switch motif (ITSM). Programmed cell death ligand 1 and 2 (PD-L1 and PD-L2) are two main ligands for PD-1 receptor. ITIM is activated by binding PD-L1 or PD-L2 ligands to the PD-1 receptor, which could promote inhibitory signals to decrease the activation of T lymphocytes and cause proliferation (Hashemi et al., 2019).

PD-L1, located at 9p24 chromosome, is one of the transmembrane type 1 glycoproteins. Several studies showed that the upregulation of PD-L1 is a major cause of cancer immune evasion (Yamazaki et al., 2002; Nguyen and Ohashi, 2015; Salmaninejad et al., 2018; Hashemi et al., 2019). Binding the PD-L1 ligand with PD-1 receptor can dephosphorylate several kinases, including ZAP70, AKT, PI3K, and ERK-R, which can inhibit IL-2 production on T cells and cause T cell proliferation and apoptosis (Zheng and Zhou, 2015; Catakovic et al., 2017). The abnormal expression of PD-Ls on the cell lines or tissues of several tumours, including cervical cancer (Karim et al., 2009), gastric carcinoma (Ohigashi et al., 2005), and breast cancer (Ghebeh et al., 2006) have been comprehensively investigated.

Genetic polymorphism of PD-L1 and PD-1 were reported in several malignancies (Haghsenas et al., 2011; Hua et al., 2011; Qiu et al., 2014; Ma et al., 2015; Dougan, 2017; Haghsenas et al., 2017; Juchem et al., 2018; Kuol et al., 2018; Tan et al., 2018; Zhang et al., 2018). Therefore, in the current investigation, our team evaluated the correlation between PD-1 (rs11568821, rs2227981, rs2227982) and PD-L1 (rs4143915, rs2890658) and BC susceptibility in a sample of southeast Iranian women.

Materials and Methods

Patients

Our case-control study was performed on 520 individuals comprising 260 histologically confirmed BC patients, and 260 control healthy individuals within the same age group. The protocol of this study has been designed based on previous investigations (Danesh et al., 2018; Hashemi et al., 2018). The institutional review board approved the current research at Zahedan University of Medical Sciences (IR.ZAUMS.REC.1397.386). Proper consent was obtained from all participants. The genomic DNA samples were extracted using the salting-out method and were collected in separate special tubes containing EDTA (Hashemi et al., 2013).

Genotyping

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method was used for genotyping the PD-L1 rs2890658, PD-1 rs11568821, PD-1 rs2227981 and PD-1 rs2227982 polymorphisms. For identification of the PD-L1 rs4143915 variant, we used Tetra-Primer Amplification Refractory Mutation System-Polymerase Chain Reaction (T-ARMS-PCR) technique. The procedure used for this investigation, previously designed and testified by Hashemi’s lab, is as follow (Hashemi et al., 2012; Hassanzarei et al., 2017):

1. The volume for assembling the reaction solution in each PCR tube for RFLP-PCR and T-ARMS-PCR methods are accessible in Table 1.
2. Primers sequences used for the detection of PD-1 and PD-L1 are presented in Table 2.
3. PCR thermal cycling conditions used for amplification of PD-1 and PD-L1 polymorphisms, are listed in Table 3.
4. Corresponding restriction endonucleases used for the digestion of PCR products are mentioned in Table 2.
5. UV transilluminator was used to visualize the fragments digested and separated by agarose gel electrophoresis. Briefly, for PD-1 rs11568821, the PstI enzyme digested the A allele and produced a 93 base pair (bp) and 197 bp pattern. However, the G allele was undigested (290 bp fragment) Figure 1A. Regarding PD-1 rs2227981, we used the PvuII restriction enzyme to digest the T allele to a 74 bp and 133 bp pattern. Nevertheless, the C allele remained undigested (207 bp fragment) Figure 1B. For PD-1 rs2227982, we used the BceAI restriction enzyme to digest the C allele to 29 bp and 145 bp pattern. However, the T allele remained undigested (174 bp fragment) Figure 2A. The PD-L1 rs2890658 C allele was digested by HaeIII and made a 25 bp and 226 bp pattern, while the A allele stayed undigested (251 bp fragment) Figure 2B.

We used the T-ARMS-PCR method for genotyping the PD-L1 rs4143915 (Ye, Dhillon et al. 2001, Hashemi, Moazeni-roodi et al. 2012). In this technique, two external primers (forward outer and reverse outer) and two allele-specific internal primers (forward inner and reverse inner) were designed. Three bands were produced; 322 bp for control band, and two 203 bp and 176 bp bands related to C and G allele, respectively Figure 3. Furthermore, we sequenced the PD-L1 rs4143915 to validate our genotyping and results in Figure 4.

Statistical analysis

All of the statistical data was carried out by using computer software Statistical Package for Social
Our results showed that PD-L1 rs4143815 (G/C) variant significantly reduced the risk of BC in homozygous (OR=0.52, 95%CI=0.28-0.96, P=0.049, GG vs CC), recessive (OR=0.44, 95%CI=0.26-0.77, P=0.006, GG vs CG+CC) genetic models. Regarding the PD-L1 rs2890658 C/A polymorphism, our results show that PD-L1 rs2890658 significantly increased the risk of BC in heterozygous (OR=2.44, 95%CI=1.71-3.46, p<0.0001, CA vs CC), dominant (OR=2.48, 95%CI=1.74-3.51, p<0.0001, CA+AA vs CC), and A allele (OR=1.87, 95%CI=1.41-2.48, p<0.0001, A vs C) genetic models. We could not find a significant correlation between PD-1 rs11568821, PD-1 rs2227981, PD-1 rs2227982 and variants and the risk of BC.

Furthermore, our team examined the possible association between variants and clinicopathological characteristics, including age, size of the tumour, lymph node, histology, the grade of tumour, estrogen and progesterone receptors status as well as human growth factor receptor 2 (HER2) Table 5. Our findings demonstrated that PD-L1 rs4143815, PD-L1 rs2890658, PD-1 rs11568821, PD-1 rs2227981, PD-1 rs2227982 and variants and the risk of BC.

Table 1. The Volumes for Assembling the Reaction Solution in Each PCR tube for the Detection of PD-1 and PD-L1 Polymorphisms

| Polymorphisms | Reverse Inner Primer | Forward Inner Primer | Forward Outer Primer | Reverse Outer Primer | 2X Taq master mix | H₂O | DNA |
|---------------|----------------------|----------------------|----------------------|----------------------|------------------|-----|-----|
| PD-L1 Rs4143815 | 1 µl | 1 µl | 1 µl | 1 µl | 8 µl | 6 µl | 1 µl |
| PD-L1 Rs2890658 Tetra-ARMS | 1 µl | 1 µl | - | - | 8 µl | 6 µl | 1 µl |
| PD-L1 Rs11568821 RFLP | 1 µl | 1 µl | - | - | 8 µl | 6 µl | 1 µl |
| PD-L1 Rs2227981 RFLP | 1 µl | 1 µl | - | - | 8 µl | 6 µl | 1 µl |
| PD-L1 Rs2227982 RFLP | 1 µl | 1 µl | - | - | 8 µl | 6 µl | 1 µl |

Figure 1. (1A) electrophoresis pattern of the PCR-RFLP method for the detection of PD-1 rs11568821(G/A) polymorphism. M: DNA marker; Lanes 1 and 4: GA; Lanes 2 and 3: GG (1B): Photograph of electrophoresis pattern of the PCR-RFLP method for detection of PD-1 rs2227981(C/T) polymorphism. M, DNA marker; Lanes 1 and 6, CT; Lanes 2 and 5, CC; lanes 3 and 4, TT
and PD-1 rs2227982 had a significant correlation with age (p=0.005, p=0.046, p<0.001). Additionally, we found a significant correlation between PD-1 rs2227982 variant and tumour size (p=0.049). Statistical analyzes of PD-1 rs2227981 and PD-1 rs11568821 variants showed the meaningful relation between tumor grade (p=0.049) and

Table 2. The Primers Used for Detection of PD-1 and PD-L1 Polymorphism

| Polymorphisms | Primer sequence (5′→3′) | Restriction Enzyme | Fragment (bp) |
|---------------|--------------------------|-------------------|--------------|
| PD-1 rs4143815 | FO: CTGTGACAGGGAGAAGGATCTTCTG | -- | Allele G=176 bp |
|               | RO: AGCAAGTGTGGTGGCGACAAAAATTG | | Allele C=203 bp |
|               | FI: TTTGCCCTCAACTCAGCTCATAAT | | Control= 322 bp |
|               | RI: AACACTGAGACTCTGATCAGTCAGAAATAC | | |
| PD-1 rs2890658 | FO: GCAAGAGGAGGTAGAATAATCAAG | HaeIII | Allele A=251 bp |
|               | RO: GATACCTGTGTITAAAATGAGACAG | | Allele C=226+25 bp |
| PD-1 rs11568821 | FO: CTCATATTCTAATAGCCAGGACC | PstI | Allele G=290 bp |
|               | RO: TAAGATAAAGAATGCACAGCCAC | | Allele A=197+93 bp |
| PD-1 rs2227981 | FO: TGACGCCAGGAGTATGACC | PvuII | Allele C=207 bp |
|               | RO: CTGAGGAAAATGCGCTGACC | | Allele T= 133+74 bp |
| PD-1 rs2227982 | FO: TGACTCCCTCTCTCTCTCTTC | BceAI | Allele T=174 bp |
|               | RO: GCCCATCCCTAGGAAAGA | | Allele C=145+29 bp |

Figure 2. (2A). Electrophoresis pattern of the PCR-RFLP method for the detection of PD-1rs2227982(C/T) polymorphism. M: DNA marker; Lanes 1 and 6: CT; Lanes 2 and 5: CC; lanes 3 and 4: TT. (2B) electrophoresis pattern of the PCR-RFLP method for the detection of PD-L1rs2890658(C/A) polymorphism. M: DNA marker; Lanes 1 and 6: CA; Lanes 2 and 5: AA; lanes 3 and 4: CC

Figure 3. Electrophoresis Pattern of the T-ARMS Method for the Detection of PD-L1rs4143815(G/C) Polymorphism. M, DNA marker; Lanes 1 and 6, GC; Lanes 2 and 5, CC; lanes 3 and 4, GG

Figure 4. Sequencing of PD-L1 rs4143815
Discussion

Our current study aimed to examine the possible association between PD-1 (rs11568821, rs2227981, rs2227982), PD-L1 (rs4143815, rs2890658) polymorphisms and susceptibility to BC in a sample of southeast Iranian women. Recent investigations showed that PD-1 and its ligand (PD-L1) play a critical role in regulation of immune system cells’ function (Inman et al., 2007; Keir et al., 2008). Several studies showed that there is a meaningful association between the abnormal expression of PD-1/PD-Ls and susceptibility to BC (Haghshenas et al., 2011, Hua et al., 2011; Ren et al., 2016). Thus, we considered the PD-1/PD-L1 pathway as a strong potential candidate for susceptibility to BC in a sample of southeast Iranian women.

Table 3. PCR Thermal Cycling Conditions for Amplification of PD-1 and PD-L1 Polymorphisms

| Polymorphism | Denaturation | Annealing | Extension | Cycles |
|--------------|--------------|-----------|-----------|--------|
|              | Time | Temp | Time | Temp | Time | Temp | |
| PD-L1 rs4143815 | 30s | 95°C | 30s | 58°C | 30s | 72°C | 30 |
| PD-L1 rs2890658 | 30s | 95°C | 30s | 59°C | 30s | 72°C | 30 |
| PD-1 rs11568821 | 30s | 95°C | 30s | 64°C | 30s | 72°C | 30 |
| PD-1 rs2227981 | 30s | 95°C | 30s | 61°C | 30s | 72°C | 30 |
| PD-1 rs2227982 | 30s | 95°C | 30s | 62°C | 30s | 72°C | 30 |

Table 4. The Association of PD-1 and PD-L1 Polymorphisms and Breast Cancer Risk

| PD-L1 Polymorphisms | Case n (%) | Control n (%) | OR (95%CI) |
|---------------------|------------|---------------|------------|
| PD-L1 rs4143815 Codominant |
| CC                  | 79 (30.4)  | 84 (32.3)     | 1          |
| CG                  | 161 (61.9) | 135 (51.9)    | 1.27 (0.87-1.86) |
| GG                  | 20 (7.7)   | 41 (15.8)     | 0.52 (0.28-0.96) |

| PD-L1 rs2890658 Codominant |
|---------------------------|-------------------|-----------------|
| CC                       | 101 (38.9)        | 159 (61.1)      | 1             |
| CA                       | 155 (59.6)        | 100 (38.5)      | 2.44 (1.71-3.46) |
| AA                       | 4 (1.5)           | 1 (0.4)         | 6.30 (0.69-57.14) |

| PD-L1 rs2227981 Codominant |
|-----------------------------|-------------------|-----------------|
| GG                          | 234 (90.0)        | 245 (94.2)      | 1             |
| GA                          | 26 (10.0)         | 15 (5.8)        | 1.81 (0.95-3.39) |
| AA                          | 0                 | 0               |               |

| PD-1 rs2227982 Codominant |
|---------------------------|-------------------|-----------------|
| GG                        | 494 (95.0)        | 505 (97.1)      | 1             |
| GA                        | 26 (5.0)          | 15 (2.9)        | 1.77 (0.95-3.35) |
| AA                        | 0                 | 0               |               |

| PD-1 rs2890658 Dominant |
|-------------------------|-------------------|-----------------|
| CC                      | 147 (56.5)        | 130 (50.0)      | 1.30 (0.93-1.83) |
| CT+TT                   | 356 (62.1)        | 385 (74.0)      | 1.62 (0.51-4.44) |

| PD-1 rs2227982 Recessive |
|--------------------------|-------------------|-----------------|
| CC                      | 155 (59.6)        | 153 (55.2)      | 1.19 (0.78-1.84) |
| CT+TT                   | 20 (7.7)          | 41 (15.8)       | 0.97 (0.52-1.82) |

| Allele | Case | Control | OR (95%CI) |
|--------|------|---------|------------|
| C      | 319  | 303     | 1          |
| A      | 201  | 217     | 0.88 (0.68-1.13) |

Table 4. Continued

| PD-L1 Polymorphisms | Case n (%) | Control n (%) | OR (95%CI) |
|---------------------|------------|---------------|------------|
| PD-L1 rs4143815 Recessive |
| CC                  | 113 (43.5)| 130 (50.0)    | 1          |
| CT+TT               | 20 (7.7)  | 41 (15.8)     | 0.97 (0.52-1.82) |

| PD-1 rs2227982 Allele |
|-----------------------|-----------------|-----------------|
| C                     | 469 (90.2)      | 456 (87.7)      | 1          |
| T                     | 51 (9.8)        | 64 (12.3)       | 0.77 (0.53-1.15) |

Table 4. Continued

| PD-1 Polymorphisms | Case n (%) | Control n (%) | OR (95%CI) |
|---------------------|------------|---------------|------------|
| PD-L1 rs2227982 Dominant |
| CC                  | 147 (56.5)| 130 (50.0)    | 1.30 (0.93-1.83) |
| CT+TT               | 356 (62.1)| 385 (74.0)    | 1.62 (0.51-4.44) |

| Allele | Case | Control | OR (95%CI) |
|--------|------|---------|------------|
| C      | 319  | 303     | 1          |
| A      | 201  | 217     | 0.88 (0.68-1.13) |

| PD-1 rs2227982 Recessive |
|--------------------------|-----------------|-----------------|
| CC                      | 155 (59.6)      | 153 (55.2)      | 1.19 (0.78-1.84) |
| CT+TT                   | 20 (7.7)        | 41 (15.8)       | 0.97 (0.52-1.82) |

| Allele | Case | Control | OR (95%CI) |
|--------|------|---------|------------|
| C      | 469  | 456     | 1          |
| T      | 51   | 64      | 0.77 (0.53-1.15) |

tumor stage (p=0.006).
|                | Negative | Positive | p value |
|----------------|----------|----------|---------|
| HER2 status    | 32       | 68       | 0.194   |
| ER status      | 62       | 38       | 0.002   |
| PR status      | 19       | 84       | 0.008   |
| Stage          | 35       | 79       | 0.001   |
| Lymph node     | 54       | 22       | 0.001   |

**Table 5: Association of PD-1 and PD-L1 Polymorphisms with Clinicopathological Characteristics of Breast Cancer (BC) Patients**
Our result proposed that the GG genotype of the PD-L1 rs4143815 variant significantly decreased the risk of BC in our study. We also found that PD-L1 rs4143815 had a significant correlation with age (Table 5). Currently, there is still controversy about the direct function of PD-L1 rs4143815 in cancers. Several studies suggested that abnormal expression of PD-L1 rs4143815 increased the susceptibility risk to gastric cancer (Wang et al., 2013), ovarian cancer (Tan et al., 2018), and hepatocellular (HCC) carcinoma (Xie et al., 2018) in the Chinese population. However, there was no relation between PD-L1 rs4143815 and susceptibility or protection to esophageal squamous cell carcinoma (Zhou et al., 2016) and colorectal cancer (Catalano et al., 2018).

Regarding the PD-L1 rs2890658 C/A polymorphism, our results showed that the AC genotype and A allele of PD-L1 rs2890658 significantly increased the risk of BC. Similarly, Chen et al., (2014) showed that AC genotype and A allele increased the risk of non-small cell lung cancer (NSCLC) susceptibility in a Chinese population. Furthermore, PD-L1 rs2890658 was also recognized as a risk factor in NSCLC in two other studies of the Chinese population (Cheng et al., 2015; Ma et al., 2015). However, there is still controversy about the primary role of PD-L1 rs2890658 in cancer susceptibility. Xie et al., (2018) found that PD-L1 rs2890658 was not associated with HCC in a Chinese population. We also found that PD-L1 rs2890658 had a significant relationship with age (Table 5).

PD-I rs11568821 (PD-I-3) is located on intron 4 of the PD-I human gene. A “G” to “A” alteration might affect the runt-related transcription factor 1 (RUNX1) binding site (Prokunina et al., 2002). Our findings showed no meaningful differences in genotype and allele frequencies of PD-I rs11568821 variant and BC risk. Therefore, we concluded that RUNX1 activity was not correlated with BC risk in our study. Similar to our result, Haghshenas (2011) reported that the PD-I rs11568821 variant was not associated with BC and thyroid cancer in a sample of south Iranian females (Haghshenas et al., 2011). Furthermore, numerous investigations showed that PD-I rs11568821 was not associated with NSCLC (Ma et al., 2015), Leukemia (Ramzi et al., 2018), colorectal cancer (Yousefi et al., 2013), Benign Brain Tumors (Namavar et al., 2017) and hepatocellular carcinoma (Bayram et al., 2012). We also found that there is a significant association between PD-I rs11568821 and the stage of tumour in BC patients (Table 5).

In the current research, we could not find any correlation between PD-I rs2227981 and BC. Similarly, numerous studies all around the world could not confirm a significant association between PD-I rs2227981 and susceptibility or protection to BC (Haghshenas et al., 2011), colorectal cancer (Savabkar et al., 2013), NSCLC (Ma et al., 2015) and epithelial ovarian cancer (Li et al., 2016). However, PD-I rs2227981 variant was recognized as a risk factor in several cancers including, cervical cancer (Li et al., 2016), BC (Hua et al., 2011), gastric cancer (Savabkar et al., 2013) and thyroid cancer (Haghshenas et al., 2017). Our findings also showed that PD-I rs11568821 is significantly associated with tumour grade (Table 5).

In the current study, we also confirmed that there was not any significant correlation between PD-I rs2227982 and BC. Similarly, several investigations were not able to find any association between PD-I rs2227982 variant and susceptibility or protection to BC (Hua et al., 2011), esophageal cancer (ESCC) (Qiu et al., 2014) and non-small cell lung cancer (NSCLC) (Ma et al., 2015). Despite our results, the PD-I rs2227982 variant was recognized as a protective factor in esophageogastric junction adenocarcinoma (Tang et al., 2017). However, the result of many studies revealed that PD-I rs2227982 variant was a risk factor in several cancers including, leukemia (Ramzi et al., 2018), gastric cardia adenocarcinoma (Tang et al., 2017), esophageal squamous cell carcinoma (Zhou et al., 2016) and ovarian cancer (Tan et al., 2018). Our findings also demonstrated that PD-I rs2227982 was significantly associated with age in the patient group (Table 5).

In conclusion, our current study suggested that two functional polymorphisms PD-L1 rs4143815 and PD-L1 rs2890658 were associated with BC protection and risk in a sample of southeast Iranian women. The previous investigations have shown that PD-I and PD-L1/PD-L2 belong to the family of immune checkpoint proteins which could induce proliferation and apoptosis in T Cells of cancer patients and causes cancer development (Li et al., 2016; Sacher and Gandhi, 2016, Hashemi et al., 2019). Thus, genetic variation in PD-L1 rs4143815 and PD-L1 rs2890658 could be a possible prognostic marker for the prediction of BC susceptibility and development. Additionally, we could not find a meaningful association between PD-I rs11568821, PD-I rs2227981, PD-I rs2227982 variants and BC risk or protection. Inconsistency in the results of several investigations might be associated with different genetic backgrounds, environmental factors, and the sample size of the study. Further investigations and larger sample sizes are needed to clarify the primary function of PD-I (rs11568821, rs2227981, rs2227982), PD-L1 (rs4143815, rs2890658) polymorphisms and BC susceptibility.

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Authors Contributions

M. Hashemi, H. Sattarifard, S. Sarabandi, S. Karami, M. Kiumarsi, involved in conceptualization, data collection, validation, statistical analysis and manuscript writing (first draft); S. Ghavami and R. Bahari, and M. Taheri formally analyzed and finalized the manuscript. S. Karami and S. Sarabandi collected the patient’s samples, did the benchwork. S. Ghavami led the team to finalize the project.

“The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher. Requests to access the datasets should be directed to “saeid.ghavami@umanitoba.ca”.

We would like to dedicate this article to the late
Professor Mohammad Hashemi, who passed away during the preparation and submission of this work. He was a pioneer in genetic studies.

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