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Relation between HPV genotypes and BRCA mutation in familial breast cancer

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Abstract:

Breast cancer is a multistep disease, and infection with a DNA virus could play a role in one or more steps in this pathogenic process. High-risk human papillomaviruses (HPV) are the causative agent of several cancers. In this study, we investigated HPV genotypes associated with breast cancers and its relation with BRCA mutation for the detection of familial breast cancers. We analyzed 84 formalin-fixed, paraffin-embedded tissue blocks from 38 familial breast cancer and 46 non-familial breast cancer samples by multiplex PCR and clinical parameters. Overall prevalence of HPV infection was 27 out of 84: 10 (37.03%) HPV 16, 9 (29.62%) HPV 18, 4 (14.81%) HPV 11, 1 (3.7%) HPV 31, 1 (3.7%) HPV 33 and 2 (7.4%) HPV35. Furthermore, seventeen mtDNA4977 deletions and five 5382insC mutations were detected from 38 familial breast cancers. Our results demonstrate that infection with HPV was prevalent among Iranian women with familial breast cancer and the testing of mtDNA4977 deletion and 5382insC mutation in combination with clinical parameters can serve as major risk factors in the identification of familial breast cancers.

Key words: familial breast cancer, HPV genotyping, BRCA1 mutation and mtDNA4977 deletion

Introduction:

Breast cancer is the first most common cause of cancer-related death among Iranian women [11]. According to statistics of WHO/ICO Information Centre on HPV and Cervical Cancer 2010, an incidence of breast cancer in the Iran was 15/8 per 100,000 women [19, 2]. Germ-line BRCA1 and BRCA2 mutations account for most of familial breast-ovarian cancer, and BRCA1 expression is frequently decreased in sporadic cancers [12, 3]. A positive family history of the disease is one of the strongest risk factors for developing breast cancer in Iran, especially among young women and most families with detected BRCA1 or BRCA2 gene mutation are qualified for molecular testing on the basis of family history of breast or ovarian cancers [4].

However, The BRCA1 has 5592 nucleotides and 22 exons distributed in 89 kb of genomic DNA and encode a protein of 1863 amino acids. This protein is present in normal breast and ovarian epithelium and is altered, reduced, or absent in some breast and ovarian tumors. The BRCA2 gene was localized in 1994 in the 13q12-13 region. It was sequenced in 1995 and has 10443 nucleotides and 26 exons distributed in 70 kb of genomic DNA. The gene encodes a protein of 3418 amino acids [11].

Furthermore, infection with a DNA virus could play a role in one or more of the steps in breast cancer pathogenic process. Human papillomavirus (HPV) infection is now a well-established cause of cervical cancer and there is growing evidence of HPV being a relevant factor in several cancers (cervix, anus, vulva, vagina, penis and breast) [13]. There are approximately 100 types of HPV in that over 30 types can be passed from one person to another through sexual contact. Transmission can occur in the genitals, anal, mouth, or breast regions. On the basis of their epidemiological association with the development of cervical carcinoma, a group of so-called high-risk HPV genotypes has been defined. These include HPV genotype 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68[17]. Other genotypes, such as HPV-6, -11, -42, -43, and -44, are classified as low-risk types [18]. HPV-DNA testing is widely used throughout the world and today is an important part of cervical cancer screening programs. The accurate genotyping of HPV is clinically important because the oncogenic potential of HPV is dependent on specific genotypes [15, 6].

Data on HPV type distribution in women with invasive cervical cancer and its precursor lesions are essential to predict the potential worldwide impact of new prophylactic vaccines against HPV16/18, as well as to determine priorities for the inclusion of HPV types in future HPV vaccines and HPV-based screening tests [16]. In order to evaluate the role of HPV and major BRCA mutations in initiating breast carcinoma, it is necessary to use optimal techniques such as PCR for mutation detection and HPV typing [8]. Here, we evaluate three major BRCA mutations and the prevalence of HPV by Multiplex PCR in breast cancer samples.

Materials and Methods:

Patients

Patient samples were drawn from three medical centers in Iran: Khatam Hospital, Baghiatollah Hospital and Iranian Center for Breast Cancer. We retrieved archive breast tissue samples from women aged 25- 80 years who were diagnosed with breast cancer. All the cases were reviewed using a special questionnaire which allowed taking into account the presence or absence of family history of breast cancer and other pathology information. The family history characteristics were associated with an increased likelihood of carrying a *BRCA1* or *BRCA2* mutation including multiple cases of breast cancer in the family. We analyzed 84 formalin-fixed, paraffin- embedded tissue blocks from 38 familial breast cancer

patients and 46 non-familial breast cancer patients. Verification of each cancer reported in a relative was sought through the pathology reports of the hospital records.

Optimization of DNA Extraction by PCR

Successful DNA extraction was assessed by PCR amplification of β-actin gene (99bp). DNA extraction was performed as previously described [13]. Briefly, tumor pathology in archive samples was reviewed and regions of tumor tissue were selected for dissection from the paraffin blocks. Paraffin was removed from the 20-mm sections by agitation in a 200 µl solution of Tris-HCL + 0.5% Tween-20 and then it was heated in a 650 W microwave oven for up to 45 s. After that, the tubes were spun whilst heated at 12,000 rpms for 15 min and then were placed on ice. Prior to digestion, the solid wax disc was removed using a sterile pipette tip. Then 5 µl of 10mg/ml Proteinase K was added to each tube and was digested for 3-5 h at 65 °C, with 10 s gentle vibration in every hour. Finally, we followed the kit (CinaGene,Garmdareh, Tehran) process for each sample.

HPV genotyping by multiplex PCR

A rapid method was used to detect the simultaneous amplification of the HPV consensus L1 region and HPV-16,-18,-31,-11,-33 and -35, along with the β-globin gene as an internal control. The MY09/MY11 primers amplify the HPV L1 consensus sequence and type-specific primers use for the HPV E6-E7 gene region in HPV-16, -18,-31,-11,-33 and -35[14]. The PCR primers are described in Table 1. PCR amplification was performed using 100 ng of DNA derived from archive samples with primers and PCR amplification conditions as published by Rassi H and et al.[14].

Detection of major BRCA mutations and mtDNA4977 deletion

A simple method was used to detect the simultaneous detection of four mutations: 185delAG , 5382insC, 6174delT and mtDNA4977 deletion. For each *BRCA* mutation, three primers (one common, one specific for the mutant, and one specific for the wild-type allele) were used [13]. The PCR-primers are described in Table 2. PCR amplification was performed using 100 ng of DNA derived from archive samples with primers and PCR amplification conditions.

Clinical Parameters

Clinical parameters of 38 familial and 46 non-familial breast cancers were retrieved from their hospital records. The tubules, mitotic activity, necrosis, polymorphism and grade of breast cancer were staged by Nottingham histological grading. Immunohistochemical staining of the sections from the paraffin wax embedded tissues for the expression of ER, PR and p53 was carried out using a standard method. Sections from the tissue were immersed in boiling 10 mM sodium citrate at pH 6.5 for 2 min in a pressure cooker. The percentage of stained nuclei, independent of the intensity, was scored for ER, PR and p53. For categorical analysis, a case was considered positive when >10, 10; and 25% of the cells were stained with ER, PR, and p53, respectively.

Statistical Methods

Chi-square test for trend was used to compare the familial and non-familial patients. Statistical analyses were performed using the 7 version of the Epi Info(TM) 2012 software.

Results

HPV genotyping in archival breast samples by multiplex PCR

When the quality of isolated DNA was checked using agarose gel electrophoresis prior to PCR amplifications, DNA was then successfully extracted from every block. The success of DNA amplification after microwave treatment and purification using simple boiling occurred in 84 samples. High degradation of target DNA after electrophoresis may occur due to long time lapses between surgical tissue removal and fixation, the type of fixative used, and the duration of the fixation. The fraction of DNA suitable for amplification of HPV genotypes was also relatively low. Prevalence of HPV infection was 32.1 % in the archival breast cancer samples. HPV DNA typing identified 27 infections: 10 (37.03%) HPV 16, 9 (29.62%) HPV 18, 4 (14.81%) HPV 11, 1 (3.7%) HPV 31, 1 (3.7%) HPV 33 and 2 (7.4%) HPV35. In this study HPV types 16, 18, 31, 33 and 35 were considered high risk. The presence of HPV11, a low risk type, was very remarkable since mostly high risk HPV 16 and 18 types were reported in breast cancer. However, the DNA extracted may not be long enough for mutation detection; thus, the amplification of smaller gene fragments may be successful but, the amplification of longer gene fragments may be slightly limited.

Detection of BRCA mutations and mtDNA4977 deletions

Mutation analysis of mtDNA and BRCA are helpful in the determination of potential developmental, early diagnosis and gene therapy for breast cancer. In this study, five

5382insC mutations were detected by multiplex PCR from 38 familial samples and one mutation in non-familial breast cancer. Also, seventeen mtDNA4977 deletions were detected from familial breast cancers but eleven deletions in non-familial samples. The mtDNA4977 deletion and 5382insC mutation was highly prevalent in familial breast cancer but it was low in the breast tissue of the cancer cases.

Comparison of Results of Multiplex PCR with Clinical Parameters

In total, DNA and breast tissues of 38 familial breast cancer patients and 46 non-familial breasts cancer patients were investigated. The ovarian cancer was observed in 8 familial breast cancer patients. Table 3 demonstrates the distributions of clinical parameters of tumors in the familial and non-familial cases. Tumors in familial breast cancer exhibited higher mitotic activity (OR= 5.484, P<0.00001), higher polymorphism (OR=1.375, P<0.004), lower necrosis (OR= 0.372, P<0.02) and lower tubules (OR= 0.540, P<0.05), compared with the non-familial cancers. Estrogen receptor ER (OR= 4.953, P <0.00001) and progesterone receptor PR (OR= 4.314, P <0.00001) were observed less frequently in the familial cancer cases than the non-familial ones. A significantly higher level of TP53 (OR=0.228, P<0.00001) expression was observed in tumor tissues in the familial breast cancer patients compared with the non-familial cases. The frequency of BRCA mutation, mtDNA4977 deletions and HPV positive was higher in the familial breast cancer cases (P<0.05).

Discussion:

Today, many risk factors have been associated with the pathogenesis of breast cancer; including family history, BRCA mutation, hormones, cigarette smoking, alcohol consumption and infection with HPV, but the molecular mechanisms related to role of this virus in breast carcinogenesis remain poorly understood [20]. Furthermore, mutation analysis of mitochondrial genome and BRCA genes are helpful in the early diagnosis of familial breast cancers [1-19]. Breast Cancer Information Core (BIC) database indicates that the mutations with the highest number of registrations associated with breast cancer are 185delAG, exon 2 and 5382insC, exon 20 in the BRCA1 gene; and 6174delT in *BRCA2* gene [1]. The BRCA1 protein is found primarily in cell nuclei and plays an important role in the DNA damage response and transcriptional regulation [12-13]. Deficiencies in DNA repair capabilities have been associated with higher histopathological grade and worse prognosis in breast cancer. In women carrying mutations in BRCA1 gene are more frequently estrogen receptor negative than the nonhereditary breast cancer [7].

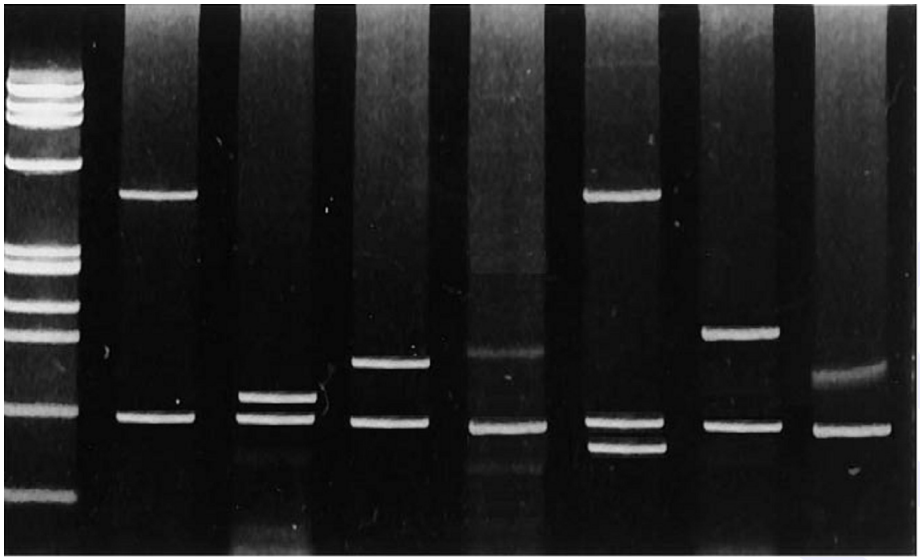
In other hand, more than 100 HPV genotypes have been isolated to date; types 16, 18, 31, 33, 35, 39, 45, 52, and 58 are considered to belong to the group that puts patients infected with these types at high risk for cervical carcinogenesis. Several HPV has been detected in the oral cavity of infants and breast cancer tissue, suggesting its vertical transmission through maternal milk, but result shows HPV infection through maternal milk may occur, but its likelihood is low[4,3]. Furthermore, the important roles of mitochondria in cellular energy production suggest that mitochondria may serve as a key switch in the breast cancer process [13, 5]. However, the most common somatic mutation is deletion (mtDNA4977) which occurs between nucleotides 8,470 to 13,477 and has been reported in a wide range of tumors, stressed tissues, and even in normal appearing tissues [5]. Here, seventeen mtDNA4977 deletions and five 5382insC mutations were detected from familial breast cancers. These mutations were highly prevalent in familial breast cancer but they were low in the breast tissue of the non-familial cancer cases. In our results prevalence of HPV infection was 32.1 % in the archival breast cancer samples. HPV DNA typing identified 27 infections: 10 (37.03%) HPV 16, 9 (29.62%) HPV 18, 4 (14.81%) HPV 11, 1 (3.7%) HPV 31, 1 (3.7%) HPV 33 and 2 (7.4%) HPV35. In this study HPV types 16, 18, 31, 33 and 35 were considered high risk. In other study, Li et al conducted a meta-analysis and revealed that 24.5% of the breast carcinoma cases were associated with HPV, of which 32.4% occurred in Asia and 12.9% in Europe [8].

In vitro studies have shown that the main oncoproteins E6 and E7 from HPV16 are able to immortalize primary mammary epithelial cells and provided additional evidence for a possible role of this virus in breast carcinogenesis [9]. The E7 gene product of human papillomavirus type 16 (HPV16) binds to the retinoblastoma gene product (pRb) and dissociates pRb-E2F complexes. Point mutations within the zinc finger domains of E7 and E6 inactivated the binding to the N terminus of BRCA1 and reduced their ability to rescue BRCA1 inhibition of ER-α. E6 and E7 also antagonized the ability of BRCA1 to inhibit c-Myc E-box-mediated transactivation and human telomerase reverse transcriptase promoter activity, in a manner dependent upon the zinc finger domains. Also, the ability of E6 and E7 to antagonize BRCA1 did not involve proteolytic degradation of BRCA1. These findings suggest functional interactions of BRCA1 with E7 and E6 [21]. It appeared likely that the mechanism(s) by which E6 and E7 inactivate BRCA1 is not identical to those pertaining to p53 or RB1 because both E6 and E7 can inactivate BRCA1, although E6 selectively targets p53 and E7 selectively targets RB1.

Alternative individual criteria that can be used to identity BRCA1 gene carriers would, therefore, be of great value. It was recently predicted that individual morphological and immunohistochemical parameters might prove to be useful tools of establishing the BRCA1 status [7]. In this study, we established that familial breast cancer tumors exhibited higher mitotic activity, higher polymorphism, lower necrosis, lower tubules, higher ER- and PR-negatives and lower TP53-positives than the non-familial cancers. Although most BRCA1 mutant breast cancers are ER-α negative, the ability of BRCA1 to inhibit ER-α activity may contribute significantly to the development of BRCA1-mutant breast cancers based on a series of findings indicating that hormonal factors contribute to breast cancer risk in BRCA1 mutation carriers and that hormonal prophylaxis (bilateral oophorectomy) significantly reduces the incidence of BRCA1 mutant breast cancers. In summary, the primary goal of this study was to use multiplex PCR with morphological and immunehistochemical methods for the detection of BRCA mutation, mtDNA4977 deletion and HPV genotyping in familial and non-familial breast cancers. Our analysis indicates that infection with HPV was prevalent among Iranian women with familial breast cancer and the testing of mtDNA4977 deletion and 5382insC mutation in combination with clinical parameters such as mitotic activity, necrosis and ER negative can serve as major risk factors in the identification of familial breast cancers.

Table 1 PCR primers used for HPV genotyping

Type	Primer position	Primer sequence	Length (bp)
L1 (MY9/11)	Pr1. 7015–7034 Pr2. 6583–6602	5'-GCTCC AA GG AAC TGATC- 5'-GCCAGGG CAT AAAATGG-3’	450
HPV-16	Pr. 421–440 Pr. 521–540	5’-TCA AAA GCC ACT GTG TCC TGA-3’ 5’-CGT GTT CTT GAT GAT CTG CAA-3’	119 bp
HPV18	Pr. 533-553 Pr. 705-682	5’-CCG AGC ACG ACA GGA GAG GCT- 3’ 5’- TCG TTT TCT TCC TCT GAG TCG CTT-3’	172 bp
HPV-31	Pr. 3835–3875 Pr. 3963–3988	5’-CTA CAG TAA GCA TTG TGC TAT GC-3’ 5’-ACG TAA TGG AGA GGT TGC AAT AAC CC-3’	153 bp
HPV-11	Pr. 221–240 Pr. 291–301	5’-CGC AGA GAT ATA TGC ATA TGC-3’ 5’-AGT TCT AAG CAA CAG GCA CAC-3’	80 bp
HPV-33	Pr. 567–587 Pr. 758–778	5’-AAC GCC ATG AGA GGA CAC AAG-3’ 5’-ACA CAT AAA CGA ACT GTG GTG-3’	211 bp
HPV-35	Pr. 610–629 Pr. 821–840	5’-CCC GAG GCA ACT GAC CTA TA-3’ 5’-GGG GCA CAC TAT TCC AAA TG-3’	230 bp
b-actin	Pr. 6999-7018 Pr. 7097-7072	5’CCACACTGTGCCCATCTACG3’ 5’AGGATCTTCATGAGGTAGTCAGTCAG3’	99 bp



1 2 3 4 5 6 7 8

Figure 1: Electrophoresis of multiplex PCR products for HPV genotyping: Lane 1, 100-bp ladder ;lane 2, HPV + with MY; lane 3, HPV16; lane 4, HPV18, lane 5, HPV33; lane 6, HPV11; lane 7, HPV35 and lane 8, HPV31. The B-actin (length 99bp) in all samples used as internal control.

Table 2. PCR primers used for BRCA mutations

Mutation	Primer sequence	Length (bp)
BRCA1 185delAG	Common forward (P1) 5′-ggttggcagcaatatgtgaa Wild-type reverse (P2) 5′-gctgacttaccagatgggactctc Mutant reverse (P3) 5′-cccaaattaatacactcttgtcgtgacttaccagatgggacagta	335 bp 354 bp
BRCA1 5382insC	Common reverse (P4) 5′-gacgggaatccaaattacacag Wild-type forward (P5) 5′-aaagcgagcaagagaatcgca Mutant forward (P6) 5′-Aatcgaagaaaccaccaaagtccttagcgagcaagagaatcacc	271 bp 295 bp
BRCA2 6174delT	Common reverse (P7) 5′-agctggtctgaatgttcgttact Wild-type forward (P8) 5′-gtgggatttttagcacagctagt	151 bp

	Mutant forward (P9) 5′- cagtctcatctgcaaatacttcagggatttttagcacagcatgg	 171 bp

Table 3. Clinical characteristics of Familial Breast Cancer (FBC) and Non-Familial Breast Cancer (NFBC)

Parameters	FBC (n=38) Number (%)	NFBC (n=46) Number (%)	Odds Ratio (OR)
Cancer type Ductal carcinoma Lobular carcinoma	 31(82%) 7(18%)	 39(85%) 7(15%)	 1.00 1.244 χ^2 =0.14(P~0.7)
Ductal carcinoma in situ Present Absent	 28(74%) 10(26%)	 31(67%) 15(33%)	 1.00 0.713 χ^2 =1.53 (P~0.21)
Necrosis Present Absent	 8(21%) 30(79%)	 4(9%) 42(91%)	 1.00 0.372 χ^2=6.59(P<0.02)
Tumor size < 2 cm 2–5 cm ≥ 5 cm	 10(26%) 25(66%) 3(8%)	 12(26%) 30(65%) 4(9%)	 1.00 1.015 0.889 χ^2 =0.6(P~0.8)
Grade I II III	 8(21%) 18(47%) 12(32%)	 7(15%) 30(65%) 9(20%)	 1.00 0.516 1.143 χ^2 =0.28(P~0.6)
Tubules 3(< 10%) 2(10-75) 1(>75%)	 30(79%) 8(21%) 0(0%)	 31(67%) 15(33%) 0(0%)	 1.00 0.540 0.0 χ^2 =4.2 (P<0.05)
Mitotic activity Low Moderate High	 14(37%) 12(32%) 12(31%)	 33(72%) 8(17%) 5(11%)	 1.00 3.663 5.484 χ^2=22.5(P<0.00001)
Polymorphisms 1 2 3	 29(76%) 2(5%) 7(19%)	 15(33%) 28(61%) 3(6%)	 1.00 0.033 1.375 χ^2 =10.92(P<0.004)
ER			

+ -	9 (24%) 29(76%)	28(61%) 18 (39%)	1.00 4.953 χ^2=26.38 (P<0.00001)
PR + -	12 (32%) 26(68%)	31(67%) 15 (33%)	1.00 4.314 χ^2=23.00(P<0.00001)
TP53 + -	24(63%) 14 (37%)	13 (28%) 33(72%)	1.00 0.228 χ^2=26.00(P<0,00001)
BRCA1/2 Mutation + -	5(13%) 33(87%)	1(2%) 45(98%)	1.00 0.137 χ^2=10.32(P<0,002)
mtDNA4977 deletion + -	17(45%) 21(55%)	11(24%) 35(76%)	1.00 0.386 χ^2=10.65(P<0,002)
HPV + -	15(39%) 22(61%)	12(26%) 36(74%)	1.00 0.550 χ^2=4.44(P<0,04)

χ^2 = Chi-square test for Trend

Reference:

1. An Open Access Online Breast Cancer Mutation Database. Bethesda (MD): National Human Genome Research Institute; 2002. Available from: http://www.nhgri.nih.gov.

2. Bosch, F. X., M. M. Manos, N. Munoz, M. Sherman, A. M. Jansen, J. Peto, M. H. Schiffman, V. Moreno, R. Kurman, K. V. Shah, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J. Natl. Cancer Inst. 1995; 87:796–802.

3. Burchell AN, Winer RL, Sanjos´e SD, Franco EL. Epidemiology and transmission dynamics of genital HPV infection. Vaccine.2006; 24: 52-61

4. Farshid G, Balleine RL, Cummings M, Waring P; Cuningham K. Consortium for Research into Familial Breast Cancer (kConFab). Morphology of breast cancer as a means of triage of patients for BRCA1 genetic testing. Am J Surg Pathol. 2006; 30: 1357-66.

5. Kang D, Hamasaki N. Mitochondrial disease: maintenance of mitochondrial genome and molecular diagnostics. Adv Clin Chem. 2006; 42: 217-54.

6. Karlsen F, Kalantari K, Jenkins A, Pettersen E, Kristensen G, Holm R, Johansson B, and B Hagmar. Use of multiple PCR primer sets for optimal detection of human papillomavirus. J Clin Microbiol. 1996; 34(9): 2095–2100.

7. Lakhani SR, Gusterson BA, Jacquemier J, Sloane JP, Anderson TJ, van de Vijver MJ, Venter D, Freeman A, Antoniou A, McGuffog L, Smyth E, Steel CM, Haites N, Scott RJ, Goldgar D, Neuhausen S, Daly PA, Ormiston W, McManus R, Scherneck S, Ponder BA, Futreal PA, Peto J, Stoppa-Lyonnet D, Bignon YJ, Stratton MR. The pathology of familial breast cancer: histological features of cancers in families not attributable to mutations in BRCA1 or BRCA2. Clin. Cancer Res 2000; 6: 782-9.

8. Li N, Bi X, Zhang Y, Zhao P, Zheng T, Dai M. Human papillomavirus infection and sporadic breast carcinoma risk: a meta -analysis. Breast Cancer Res Treat. 2011; 126: 515–520.

9. Liu Y, Chen JJ, Gao Q, Dalal S, Hong Y, Mansur CP, Band V, Androphy EJ. Multiple functions of human papillomavirus type 16 E6 contribute to the immortalization of mammary epithelial cells. Journal of Virology. 1999;73(9):7297–7307.

10. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Lui Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Skolnick MH A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994; 266: 66-71.

11. Parkin DM, Bray F. Chapter 2: the burden of HPV-related cancers. Vaccine. 2006;24(Suppl 3):S11–S25.

12. Pharoah PD, Day NE, Duffy S, Easton DF, Ponder BA. Family history and the risk of breast cancer: a systematic review and meta-analysis. Int J Cancer 1997; 71: 800-9.

13. Rassi H, Houshmand M, Hashemi M, Majidzadeh K, Hosseini Akbari MH. Investigation of Mitochondrial Common Deletion and BRCA Mutations for Detection of Familial Breast Cancers in Archival Breast Cancer Materials. Iranian Journal of Cancer Prevention. 2009;2: 77-83.

14. Rassi h, Salehi B, Mohammadian T, Nahavandi Araghi A. Prevalence of human papillomavirus genotypes associated with cervical and breast cancers in Iran. Monoclonal antibodies in immunodiagnosis and immunotherapy. 2013; 32(6): 399-403.

15. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. J Natl Cancer Inst.2011; 103: 368-83.

16. Sigaroodi A, Nadji SA, Naghshvar F, Nategh R, Emami H, Velayati AA. Human papillomavirus is associated with breast cancer in the north part of Iran. Scientific World Journal. 2012; 837191.

17. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer.2007; 12: 621-32.

18. Sotlar K, Diemer D, Dethleffs A, Hack Y, Stubner A, Vollmer N, Menton S Menton M, Dietz K, Wallwiener D, Kandolf R, Bültmann B. J Clinical Microbiol. 2004 ; 42: 3176-84.

19. WHO/ICO Information Centre on HPV and Cervical Cancer, 2010 http://apps.who.int/hpvcentre/statistics/dynamic/ico/country_pdf/IRN.pdf.

20. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995; 378: 789–92.

21. Zhang Y, Fan S, Meng Q, Ma Y, Katiyar P, Schlegel R, Rosen EM. BRCA1 interaction with human papillomavirus oncoproteins. J Biol Chem. 2005 Sep 30;280(39):33165-77.

22. Zhu W, Qin W, Bradley P, Wessel A, Puckett CL, Sauter ER. Mitochondrial DNA mutations in breast cancer tissue and in matched nipple aspirate fluid. Carcinogenesis 2005; 26: 145-52.

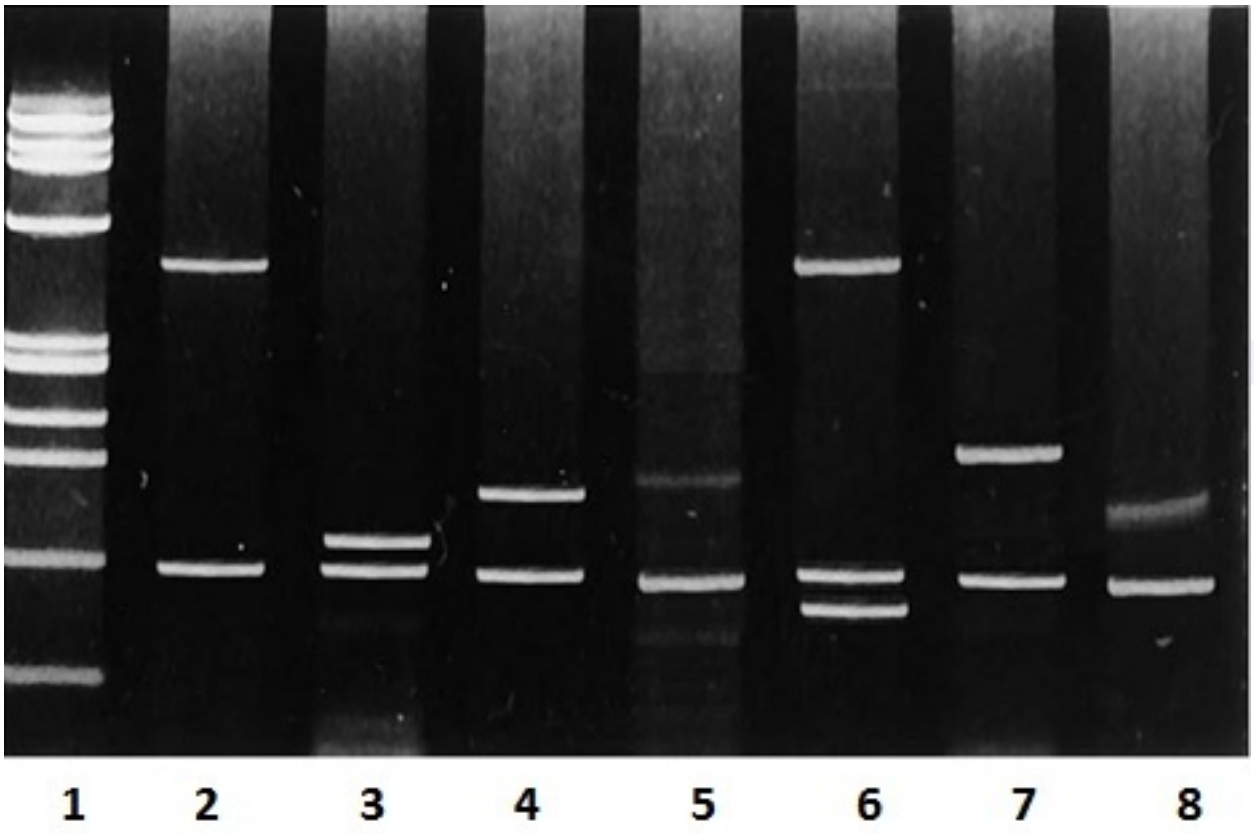


Fig. 1 Electrophoresis of multiplex PCR products for HPV genotyping: Lane 1, 100-bp ladder; lane 2, HPV + with MY; lane 3, HPV16; lane 4, HPV18, lane 5, HPV33; lane 6, HPV11; lane 7, HPV35 and lane 8, HPV31. The B-actin (length 99bp) in all samples used as internal control.