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Is human papillomavirus associated with breast cancer or papilloma presenting with pathologic nipple discharge?

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ABSTRACT

Purpose: There are little data on the presence or interaction of human papillomavirus (HPV) in intraductal papilloma or Breast cancer (BC) presenting with pathologic nipple discharge (PND). The study aimed to determine whether the HPV-genotypes are identifiable in papilloma or carcinoma of the breast by real-time PCR with broad-spectrum genotyping.

Methods: Formalin-fixed-paraffin-blocks obtained from the patients who were suffering from PND and underwent ductoscopic papilloma extraction (n = 27) or segmental/total mastectomy for cancer diagnosis (n = 18). HPV-DNAs were identified by PCR with broad-spectrum genotyping. Mc Nemar test was used to compare cancer-involved cases to normal-adjacent tissue concerning HPV positivity. Chi-Square test was used to analyze the association for receptor status in HPV positive cancer-involved cases.

Results: The mean age (± SD) was 49 ± 16 in papilloma and 52 ± 14 in BC patients, respectively. We found high prevalence of HPV in papilloma and carcinoma: 29.6% (n = 8) and 44.4% (n = 8), respectively. The most common type identified in breast lesions was HPV-11, and the others were HPV- 6, -11, -39, and -82. Cancer-involved samples were more contaminated by HPV in comparison to normal-adjacent tissues (p = 0.016). In HPV positive cancer-involved cases, hormone receptors were found to be more positive than HER2-Neu (p = 0.035).

Conclusions: Our data suggest that HPV might be a causative agent for the development of papilloma and carcinoma of the breast in some cases presenting with PND. HPV positive breast cancers are more likely to be hormone positive. Further studies needed for validation regarding the integration of HPV-DNAs into the human genome that causes BC.

Introduction

Although the association between HPV infection and the development of genital tumors is widely accepted, little is known about the influence of these viruses on breast lesions. Discordant results have been obtained from several studies; however, the overall prevalence of HPV in breast cancer (BC) was reported to be 23% (range of 13–42%) [1]. Some studies speculated that the variation depends on several reasons such as the use of methodology, which is less sensitive with low spectrum HPV primers, paraffin-embedded tissues that have less DNA integrity, and the geographic variety of HPV.

The biological mechanisms by which HPVs are tropic and oncogenic to epithelial cells are reasonably well known from studies of cervical oncogenesis. The interaction of HPV genes E6 and E7 with the host cell causes degradation of p53 and a loss of p16 transcription inhibition, respectively [2]. Lower expressions of p53 and p21 proteins in HPV-positive breast cancers have been observed versus those that are HPV-negative [3]. Little is known about the correlation between the hormone receptors as well as HER2-neu status of BC and HPV. Previous studies have shown that HPV-harboring tumors were less likely to express ER receptors [4].

Papilloma or papillomatosis are benign intraluminal growths that can be located in a variety of sites such as the esophagus, larynx, sinonasal sinus, cervix and breast. HPV 6 and 11 were predominantly identified and found to be an inductor of tumorigenesis from oropharyngeal cavity and cervix [5]. However, there is little data regarding the identification of HPV in the intraductal papilloma [6,7].

Considering the controversial reports on the association of HPV and breast lesions [8], the aim of this study was to determine whether the HPV-DNA can be identifiable in intraductal papilloma or carcinoma of

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the breast among a cohort of Turkish population presenting pathologic nipple discharge (PND).

Materials and methods

Formalin-fixed paraffin blocks (FFPB)obtained from the patients who underwent ductoscopy-aided papillomectomy ($n = 27$) or segmental/total mastectomy ($n = 18$). Patients who underwent segmental/total mastectomy were diagnosed with invasive ductal and lobular cancer ($n = 15$, $n = 3$, respectively). HPV-DNA presence was analyzed by PCR with broad-spectrum genotyping in 87 histopathological slides achieved from a total of 45 FFPBs including benign and malignant cases. Comparison analyses were done between cancer-involved cases and normal-adjacent tissues concerning HPV positivity. Association analyses including estrogen, progesterone or HER2/neu receptor were done for HPV positive cancer-involved cases.

PCR reaction for β-globin was done to identify and exclude the false negative samples due to the presence of PCR inhibitors or the lower DNA integrity for each extracted DNA template. PC04 (5′- CAA CTT CAT CCA CGT TCA CC-3′) and GH20 5′-GAA GAGCCAAGGACAGG TAC-3′ primers were used to amplify β-globin gene [9]. For run validation and quality control, positive and contamination (H20) controls were included in each HPV PCR run. We followed the following steps to prevent false positive results: standard precautions such as working in distinct pre- and post-PCR sites, working in PCR workstation, using disposable blades to cut tissues from the paraffin blocks and changing it from one block to another, frequent use of UV-C lamp and surface decontaminants, recruiting filter tips, waste management, and safe handling of PCR products. No carryover or failure of the test system was observed according to in run control results.

Broad spectrum HPV genotyping

All HPV positive templates were subject to HPV genotyping by an HPV type 3.5 LCD Array kit (Chipron GmbH, Berlin) [10]. The prelabeled PCR primer mixes were provided with the kit-generated labeled fragments of the viral DNA. The non-fluorescent labeled PCR fragments were combined with the hybridization buffer and hybridized to the individual array fields of one chip. During hybridization, the labeled PCR fragments bound to the specific capture probes were immobilized as spots on the bottom of each field. Following a short washing procedure, each field incubated with a secondary solution (enzyme-conjugate). After a second washing step, those positions (spots) where PCR fragments and secondary labels were bound could be visualized by the enzyme substrate provided as “BLUE stain”. The data read-out could either be done by simple optical examination using the pattern matrix provided by the kit or by the scanner. The kit provided two primer sets for PCR amplification—one based on the published and commonly used MY09/MY11 system—the second produced a shorter PCR product 125 bp long. According to the manufacturer, two independent PCR reactions were carried out on each HPV positive template; then, 5 μL of each PCR product was mixed together to follow the post-PCR hybridization steps on polymer LCD-chip. The HPV kit 3.5 LCD Array is capable of detecting 32 HPV types including 6, 11, 16, 18, 31, 33, 35, 39, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 70, 72, 73, 81, 82, 83, 84, 90, and 91.

TNM classification and immunohistochemistry

The AJCC 7th edition based on TNM classification obtained from pathology records was used in the staging of patients diagnosed with cancer. Estrogen (ER) and progesterone receptors (PR), as well as overexpression of HER2-neu, were determined by immunohistochemistry. Nuclear staining >1% was considered positive for ER and PR.

Statistical analyses

Quantitative variables are presented as mean and standard deviation (SD) and/or median. For the associations and comparisons of proportions, chi-square and Mc Nemar tests were used. Statistical significance was set at 0.05 and analyses were conducted using NCSS (Number Cruncher Statistical System) 2007 Statistical Software (NCSS LLC, Kaysville, Utah, USA).

Results

Of 87 FFPB samples with a total of 45 patients, only 4 (4.5%) FFPBs were positive for inhibitory effects or insufficient DNA integrity at PCR for β-globin. Despite four FFPEs were excluded from the study, the recruited 83 FFPB samples were enough to analyze all enrolled patients. Thirty-eight FFPBs were used for the analysis of 19 HPV negative intraductal papilloma cases, and 14 FFPBs for 8 HPV positive intraductal papilloma cases. Nineteen FFPBs were used to analyze 10 HPV negative cancer cases, 12 FFPBs for 8 HPV positive cancer cases. The FFPBs from the same HPV-positive case tested all positive for HPV, but they were not all positive for the same HPV types. The mean age ± SD was 49 ± 16 in papilloma, and 52 ± 14 in BC patients, respectively. We found that 29.6% (8 of 27) of intraductal papillomas and 44.4% (8 of 18) of BC paraffin blocks were positive for HPV-DNA. The most common type in both groups was HPV- 11 (Tables 1 and 2); 50% (4 of 8) of HPV-DNA positive papillomas had only HPV-11. The remaining papillomas were HPV-6; HPV-6, 11; HPV-11; HPV-11, 39, 82. Cancer samples with only HPV-11 were present in 62.5% (5 of 8) of cancer cases. Multiple HPV identification per patient was found in three patients as HPV-11, 39; HPV-11, 82; HPV-6, 11, 39. Only one normal specimen adjacent to associated cancer was found to be positive for HPV-16. Cancer-involved samples were more contaminated by HPV in comparison to normal-adjacent tissue ($p = 0.016$) (Table 3). In HPV positive cancer-involved cases, hormone receptors were found to be more positive than HER2-Neu ($p = 0.035$). Only one out of eight (12.5%) patient was stained as triple negative. Table 2 summarizes the variation of HPV genotypes, histopathology and receptor status.

Discussion

We found a high prevalence of HPV in intraductal papilloma (29.6%) and breast cancer (44.4%) in a cohort of Turkish patients. Discordant results have been obtained from previously published studies (Tables 4 and 5). Simoes et al. reported an overall prevalence of 23% HPV in breast cancer (range 13–42%) [1].

Intraductal papillomas are benign tumors that originate from the epithelium of the lactiferous ducts. The incidence of these tumors is 2–3%; they develop in women between the ages of 30 and 77 years

Table 1
HPV genotypes detected in intraductal papillomas.

Patient	HPV genotypes	Histopathology
A1	11, 39, 82	Intraductal papilloma
A2	11, 39	Intraductal papilloma
B	11	Intraductal papilloma
C1	6, 11	Intraductal Papilloma, Ductal ectasia, fibrosis, fibrocystic changes
C2	6, 11	Intraductal Papilloma, Ductal ectasia, fibrosis, fibrocystic changes
D1	11, 39	Intraductal papilloma, intraductal hyperplasia
D2	39	Intraductal papilloma, intraductal hyperplasia
E	11	Intraductal papilloma
F1	11	Intraductal papilloma
F2	11	Intraductal papilloma
F3	11	Intraductal papilloma
G 1	11	Intraductal papilloma
G2	11	Intraductal papilloma
H	6	Intraductal papilloma

Table 2
Demographic and tumor characteristics of patients with HPV-DNA positivity.

Patient, sample	Age	HPV types detected by PCR	Histopathology	ER	PR	HER2-neu	pTNM
1,a	56	11	Invasive Ductal Carcinoma	+ + +	+	negative	T3N1M0
1,b	56	11	Invasive Ductal Carcinoma	+ + +	+	negative	T3N1M0
2,a	60	11	Invasive Ductal Carcinoma	negative	negative	negative	T2N1M0
2,b	60	11	Invasive Ductal Carcinoma	negative	negative	negative	T2N1M0
3	51	6,11,39	Invasive Ductal Carcinoma	negative	negative	+ + +	T1N2M0
4,a	46	11	Invasive Ductal Carcinoma	+ +	+ +	negative	T2N0M0
4,b	46	11,39	Invasive Ductal Carcinoma	+ +	+ +	negative	T2N0M0
5	59	11	Invasive Ductal Carcinoma	+ + +	+ + +	+ + +	T1N1M0
6	44	11	Invasive Ductal Carcinoma	+	+ +	negative	T1N0M0
7	73	11	Invasive Lobular Carcinoma	+ +	+	negative	T2N2M0
8,a	80	11, 82	Invasive Ductal Carcinoma	+ + +	+ +	negative	T2N1M0
8,b	80	11	Invasive Ductal Carcinoma	+ + +	+ + +	negative	T2N1M0

Table 3
Comparison of cancer-involved specimens to adjacent normal breast tissues concerning HPV-DNA positivity.

	Cancer-involved specimen	Adjacent normal tissue	*p*
HPV DNA (+)	8 (44,4%)	1 (5,6%)	**0,016***
HPV DNA (-)	10 (55,6%)	17 (94,4%)	
Total	18 (100%)	18 (100%)	

Mc Nemar test.
* $p < 0,05$.

[11]. Clinically, 72% of all cases present with bloody discharge from the nipple caused by the fragility of the proliferating and disorganized epithelium, which tends to decompose and bleed [12,13]. Although these tumors are benign, there is a great deal of controversy surrounding their diagnosis [14]. Our results suggested HPV as a causative agent for the breast papilloma or papillomatosis because we demonstrated a 29.6% prevalence of HPV in intraductal papillomas in this study.

While the intraductal papilloma has been a clinical and pathological challenge, the etiology or pathophysiology of these lesions remains unclear. It resembles the pathogenesis of papilloma or papillomatosis in the larynx and sinonasal sinus, and we hypothesize that the development of intraductal breast papilloma or papillomatosis may be triggered by HPV. In the literature, only two reports have been published regarding the HPV occurrence in the papilloma specimen presented with or without pathologic nipple discharge. Similar to our study, multiple types as 16, 33, 58 and 71 were identified from the papillary lesion using a broad-spectrum linear array genotyping test [6]. Choi et al. studied HPV presence in nine intraductal papillomas by real-time PCR and found 22.2% prevalence of HPV types 33 and 53 [7]. These studies—including our findings—suggest that a comprehensive genotype analysis of HPV is needed for the etiology of benign papillary lesions in future studies.

HPV might also be a causative agent for BC. Only 5–10% of BCs arise in women with family history; 90–95% are sporadic [15]. Thus, environmental factors are prominent in the etiology of this malignancy [16]. Authors from different countries have analyzed HPV in BC specimens suggesting a causal association. Various types of HPV have been identified from different geographic locations. HPV-16 is the most common HPV identified in BC specimens followed by the genotypes 18, 6, 11, and 33 [17].

Nevertheless, we found that HPV-11 is the most common type in this cohort of the Turkish population. As a result of a systematic review and meta-analysis of 29 studies including 2011 samples obtained from all around the world, the overall prevalence was found to be 23% [1]. The prevalence of HPV infection in breast cancer showed great variation ranging from 13% to 42% suggesting that demographic features may contribute to geographic differences of HPV infection [1]. This considerable worldwide variation depends on the sensitivity and low primer spectrum of the selected methods. We found a variety of HPV types in paraffin-embedded blocks of BC patients as types 6, 11, 39 and 82, which confirm the need for broad-spectrum primers.

The meta-analysis of case-control studies showed that HPV-positive women are 5.9-times more likely to have BC compared to HPV-negative patients [1]. The question raised from the meta-analysis is whether the identification without integration analyses of HPV in a BC specimen is adequate to consider the HPV as a causative agent. Thus, Aguayo et al. investigated the presence of E6 and E7 gene transcripts as well as the physical status of HPV to confirm whether the virus is active, episomal, or integrated into the host genome [16]. Even though the prevalence of HPV in BC specimens was low (8.7%), all cases were infected by the integrated HPV-16. Nevertheless, none of them had E6 or E7 gene transcript expressions in the specimen. The absence of detectable levels of E6/E7 transcripts is a hallmark of functional activity in HPV-positive BC specimens. Importantly, the viral load in three of four HPV positive cases was compatible with an eventual direct carcinogenic role of HPV although no E6/E7 expression was detected. We Thus speculated a possible “hit and run” mechanism of HPV's initial action. In this “hit-and-run” hypothesis, the virus initiates cancer or plays a role in cancer development, but then disappears from tumor cells (probably by immune surveillance) by the time the cancer is clinically detected [18]. HPVs also appear to influence the cell cycle control enzyme APOBEC that leads to genomic instability and ultimately to breast cancer [19,20]. Ngan et al. found high HPV E7 oncoprotein expression in benign breast tissues but low HPV E7 expression in subsequent breast cancer that develops in the same patients [18]. These observations are consistent with the viral “hit and run” hypothesis. This early influence of HPV may be the reason why there is no increase in the prevalence of HPV-associated breast cancer in immunocompromised patients as compared to HPV-associated cervical cancer. On the contrary, Tsuboi et al. identified HPV16 in a rate of%27.3 (24/88) but found no correlation between HPV 16 infection (known as APOBEC-3B inducer) and APOBEC-3B expression [21].

Most reports studied viral DNA rather than the expression of viral transcripts. Gannon et al. screened a large cohort of fresh-frozen cancer and healthy breast specimens of patients in Australia for the presence of HPV DNA. They found an overall prevalence of HPV of 16% and 10% in malignant and non-malignant tissue, respectively [22]. Samples that were positive for HPV DNA were further screened using RNA-sequencing for the presence of viral transcripts. Interestingly, they were unable to show any evidence for HPV or other viral transcripts in those HPV-DNA positive samples suggesting that transcription of viral genomes is unlikely to be a significant factor in breast cancer pathogenesis.

In the literature, most studies used FFPB tissues to identify the HPV [3,23–29,33–35,37,38,40–43,45–47,49–53,55,57,58,60,66]. Studies in which fresh tissues were analyzed mostly showed a higher prevalence of HPV in comparison to studies used FFPBs [4,17,30–32,36,39,44,54] (Table 4). On the contrary, others found no HPV DNA in the fresh

Table 4
Studies reporting positive HPV-DNA identification in breast cancer tissues.

Author, year	Country	Sample size (Cancer cases)	Prevalence of HPV in Cancer cases (%)	Prevalence of HPV in benign or normal controls	Genotype detected (Cancer cases)	Sample Source
Di Lonardo et al., 1992 [23]	Italy	47	29.4 %	0%	16	FFPB
Hennig et al.,1999 [3]	Norway	41	46.3 %	5%	11,16,18,33	FFPB
Yu et al., 1999 [24]	China/Japan	44	34 %	5%	16,18, 33	FFPB
Li et al., 2002 [25]	China	28	68 %	0%	16,18	FFPB
Damin et al., 2004 [26]	Brazil	101	25%	0%	16,18	FFPB
Widschwendter et al., 2004 [27]	Austria	11	63.6 %	NA	16	FFPB
De Villiers et al., 2005 [28]	USA	29	86%	NA	3,15,24,87, DL473	FFPB
Tsai et al., 2005 [29]	Taiwan	62	13 %	5%	NA	FFPB
Kan et al., 2005 [30]	Australia	50	48%	NA	16, 18, 33	Fresh
Kroupis et al., 2006 [4]	Greece	107	16 %	NA	6, 11, 16, 18, 31, 33, 52, 58	Fresh
Gumus et al., 2006 [31]	Turkey	50	74 %	32%	11, 16, 18, 33	Fresh
Choi et al., 2007 [32]	Korea	123	7%	0%	16,18,31,56,58,59,70	Fresh
Akil et al., 2008 [33]	Syria	113	61%	NA	16, 18, 31, 33, 35	FFPB
Khan et al., 2008 [34]	Japan	124	21%	NA	16, 18, 33	FFPB
De Leon et al., 2009 [35]	Mexico	51	29%	0%	16, 18	FFPB
He et al., 2009 [36]	China	40	60%	5%	16	Fresh
Heng et al., 2009 [37]	Australia	26	30.7%	17.6%	16, 18	FFPB
Mendizabal-Ruiz et al., 2009 [38]	Mexico	67	4%	0%	16, 18, 33	FFPB
Mou et al., 2011 [39]	China	62	6.5%	0%	16, 18	Fresh
Antonsson et al., 2011 [17]	Australia	54	50 %	NA	18	Fresh
Baltzell et al., 2012 [40]	USA	70	6%(with ISH) − 3%(with in situ-PCR)	NA	16,18,31,33,35,39,45,51,52,56,58,66	FFPB
Frega et al., 2012 [41]	Italy	31	9%	0%	NA	FFPB
Glenn et al., 2012 [42]	Australia	50 (withPCR	50%	8%		FFPB
		27 (with in situ-PCR)	37	3%	16,18,33	
Sigaroodi et al., 2012 [43]	Iran	58	25.9%	2.4%	6,11,15,16,18,23,124	FFPB
Liang et al., 2013 [44]	China	224	21.4%	16.2%	16,18,31,33,35,39,45,51,52,56,58,59,68	Fresh
Ali et al., 2014 [45]	Iraq	129	46.5%	3%(benign) 0%(normal)	16,18,31,33	FFPB
AhangarOskouee et al., 2014 [46]	Iran	65	33.8%	0%	6,11,16,35,52	FFPB
Manzouri et al., 2014 [47]	Iran	55	18.2%	13.7%	11,16,18,33,35,45,55	FFPB
Peng et al., 2014 [48]	China	100	2%	0%	18	Diverse samples (blood, cancer tissue, axillary lymph nodes, normal tissue)
Fu et al., 2015 [49]	China	169	14.8%	1%	58	FFPB
Li et al., 2015 [50]	China	187	1.6%	0%	NA	FFPB
Fernandes et al., 2015 [51]	Venezuela	24	41.6%	NA	18,33,51	FFPB
Wang et al., 2016 [52]	China	146	36%	4%	16,18,58	FFPB
Delgado-Garcia et al., 2017 [53]	Spain	251	52.8%	26.3%	16,31,39,51,59	FFPB
Salman et al., 2017 [54]	United Kingdom	110	42%	2%	16,31,33,39	Fresh
Naushad et al., 2017 [55]	Pakistan	250	18%	0%	NA	FFPB
Balci et al., 2019 (present study)	Turkey	18	44.4%	5.5%	6, 11, 39, 82	FFPB

Table 5
Studies reporting negative HPV-DNA identification in breast cancer tissues.

Author, year	Country	Sample size	Prevalence (%)	Sample source
Wrede et al., 1992 [56]	UK	80	0	Fresh
Bratthauer et al., 1992 [57]	USA	28	0	FFPB
Czerwenka et al., 1996 [58]	Austria	20	0	FFPB
Gopalkrishna et al., 1996 [59]	India	30	0	Fresh
Lindel et al., 2007 [60]	Switzerland	81	0	FFPB
De Cremoux et al., 2008 [61]	France	50	0	Fresh
Hachana et al., 2010 [62]	Tunisia	123	0	Fresh /FFPE
Hedau et al., 2011 [63]	India	228	0	Fresh
Silva et al., 2011 [64]	Brazil	79	0	Fresh
Chang et al., 2012 [65]	China	48	0	Fresh /FFPE
Vernet-Tomas et al., 2015 [66]	Spain	76	0	FFPB
Koulora et al., 2018 [67]	Greece/Italy	201	0	Fresh

tissues as well as in FFPBs [56–67] (Table 5). The lack of HPV DNA identification might be due to methodological failures such as a low sample size using only specific primers and achieving low amounts of cells due to inappropriate sampling techniques. Furthermore, the high-risk genotype stratification of HPV is based on the well-defined interaction of HPV with the tumor in most popular sites such as cervix and oropharynx. Therefore, studies are needed to confirm whether the high-risk stratification is appropriate for the HPV interaction with the breast by the identification of rare types, subtypes or variations. Although this work used paraffin blocks, broad-spectrum primers were selected for the HPV genotyping. While untyped HPV-DNA-positive cases were not found in our study, subtypes or variations could not be studied. Future studies may involve different methods such as pyrosequencing that includes detecting untyped HPVs as subtypes or variations.

The expression of hormone receptors, estrogen receptors (ER), and progesterone receptors (PR), as well as overexpression or amplification of HER2, has been identified as important predictors for patients with breast cancer. Currently, these markers are commonly used to define treatment and establish disease prognosis associated with clinical and pathological variables. Most recently, several breast cancer prevention trials targeting HER2 have demonstrated the preventive efficacy of HER2-targeting drugs [68]. However, the identification and development of effective and safe targeted therapies for the prevention of TNBC remain challenging. Since the triple negative and HER2 positive cancers are associated with poor prognoses, the proposed virus effect on those molecular profiles has been studied. However, there is no robust evidence of correlation has been found. Fernandes et al. found no correlation between HPV presence and HER-2 overexpression [51]. Hennig et al. showed no significant difference in immunostaining for HER2-neu protein in HPV-positive versus HPV-negative breast cancer groups ($p = 0.15$) or for the co-expression of p53/c- erbB-2 ($p = 0.19$) [3]. In a PCR analysis of 67 BC frozen samples, HPV was found to be more prevalent among patients who were negative for estrogen receptor ($p = 0.04$) [69]. On the contrary, we found a high prevalence of hormone receptor-positive/HER-2 negative molecular profiling in 62.5% of HPV-positive cancer cases. Considering the limited number of cancer patients studied, further studies in larger cohorts would be crucial to determining whether the HPV is associated with a specific molecular subtype.

In conclusion, HPV might be causative for the development of some cases with intraductal papilloma or BC presenting with PND. Further studies are warranted not only for the identification but also the interaction of HPV on the tumorigenesis of the breast. The study is the first study to identify HPV types in an appropriate sample size of papillomas presenting with PND. Further studies are needed to identify the HPV-DNA in the nipple aspiration fluids. Identification studies of HPV in nipple aspiration fluids would be a pre-surgical method to manage the treatment of patients with intraductal papillary lesions. As a future direction, patients with a solitary intraductal papilloma could be treated by surgical or ductoscopic excision as well as the installation of specific intraductal agents against HPV.

Additional association studies with molecular profiling of BC are needed to understand whether a specific molecular subgroup has been affected by the HPV infection. These studies may help to categorize BC patients for vaccine trials who have been infected by HPV.

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Conflict of interest

Authors Fatih Levent Balci, MD and Sheldon Feldman, MD received a research grant from Susan Love Research Foundation. Author Fatih Levent Balci received a poster honorarium for the study from Avon Foundation as AACR International Scholar-in-Training Award in San Antonio Breast Cancer Symposium, Texas, USA, Dec. 6–10, 2011. Author Cihan Uras, MD declares that he has no conflict of interest.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the IRB (#079–2010) of Ankara Numune Teaching and Research Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

References

[1] P.W. Simoes, L.R. Medeiros, P.D. Simoes Pires, et al., Prevalence of human papillomavirus in breast cancer; a systematic review, Int. J. Gynecol. Cancer 22 (3) (2012) 343–347.
[2] H. Zur Hausen, Papillomaviruses and cancer; From basic studies to clinical application, Nat. Rev. Cancer 2 (2002) 342–350.
[3] E.M. Hennig, Z. Suo, S. Thoresen, et al., Human papillomavirus 16 in breast cancer of women treated for high-grade cervical intraepithelial neoplasia (CIN III), Breast Cancer Res. Treat. 53 (2) (1999) 121–135.
[4] C. Kroupis, A. Markou, N. Vourlidis, et al., Presence of high-risk human papillomavirus sequences in breast cancer tissues and association with histopathological characteristics, Clin. Biochem. 39 (2006) 727–731.
[5] J.D. Khoury, N.M. Tannir, M.D. Williams, et al., Landscape of DNA virus associations across human malignant cancers: analysis of 3775 cases using RNA-Seq, J.

Virol. 87 (16) (2013) 8916–8926.
[6] T. Khammapirad, J. Prueksadee, C. Diaz-Arrastia, et al., Intraductal papilloma of the breast in association with preoncogenic gene of breast cancer, Asian Pac. J. Trop. Biomed. 1 (2) (2011) 161–163.
[7] J. Choi, C. Kim, H.S. Lee, et al., Detection of human papillomavirus in korean breast cancer patients by real-time polymerase chain reaction and meta-analysis of human papillomavirus and breast cancer, J. Pathol. Transl. Med. 50 (6) (2016) 442–450.
[8] S. Hedau, U. Kumar, S. Hussain, et al., Breast cancer and human papillomavirus infection: no evidence of HPV etiology of breast cancer in Indian women, BMC Cancer 11 (2011) 27.
[9] S.D. Vernon, E.R. Unger, Detection of human papillomaviruses by polymerase chain reaction and in situ hybridization, Eds, in: JE Roulston, J Bartlett (Eds.), Molecular Diagnosis of Cancer: Methods and Protocols (Methods in Molecular Medicine), second edition, Humana Press, New Jersey, 2004, pp. 159–180.
[10] S. Fischer, M. Bettstetter, A. Becher, et al., Shift in prevalence of HPV types in cervical cytology specimens in the era of HPV vaccination, Oncol. Lett. 12 (1) (2016) 601–610.
[11] S. Ganesan, G. Karthick, M. Joshi, V. Damoradan, Ultrasound spectrum in intraductal papillary neoplasms of breast, Br. J. Radiol. 79 (2006) 843.
[12] S. Poma, A. Longo, The clinician's role in the diagnosis of breast disease, J. Ultrasound 14 (2) (2011) 47.
[13] J. Tominaga, H. Hama, N. Kimura, S. Takahashi, Magnetic resonance imaging of intraductal papillomas of the breast, J. Comput. Assist. Tomogr. 35 (1) (2011) 153.
[14] C. Richter-Ehrenstein, F. Tombokan, E.M. Fallenberg, et al., Intraductal papillomas of the breast: diagnosis and management of 151 patients, Breast 20 (2011) 501.
[15] J. Palacios, M.J. Robles-Frias, M.A. Castilla, et al., The molecular pathology of hereditary breast cancer, Pathobiology 75 (2008) 85–94.
[16] F. Aguayo, N. Khan, C. Koriyama, et al., Human papillomavirus and Epstein-Barr virus infections in breast cancer from Chile, Infect. Agent Cancer 6 (1) (2011) 7.
[17] A. Antonsson, T.P. Spurr, A.C. Chen, et al., High prevalence of human papillomaviruses in fresh frozen breast cancer samples, J. Med. Virol. 83 (12) (2011) 2157–2163.
[18] C. Ngan, J.S. Lawson, R. Clay, W. Delprado, N.J. Whitaker, W.K. Glenn, Early human papilloma virus (HPV) oncogenic influences in breast cancer, Breast Cancer 9 (2015) 93–97.
[19] K. Ohba, K. Ichiyama, M. Yajima, et al., In vivo and in vitro studies suggest a possible involvement of HPV infection in the early stage of breast carcinogenesis via APOBEC3B induction, PLoS One 9 (2014) e97787.
[20] V.C. Vieira, B. Leonard, E.A. White, et al., Human papillomavirus E6 triggers upregulation of the antiviral and cancer genomic DNA deaminase APOBEC3B, MBio 5 (2014) e02234–14.
[21] M. Tsuboi, A. Yamane, J. Horiguchi, et al., APOBEC3B high expression status is associated with aggressive phenotype in Japanese breast cancers, Breast Cancer 23 (2016) 780–788.
[22] O.M. Gannon, A. Antonsson, M. Milevsky, et al., No association between HPV positive breast cancer and expression of human papilloma viral transcripts, Sci. Rep. 14 (5) (2015) 18081.
[23] A. Di Lonardo, A. Venuti, M.L. Marcante, Human papillomavirus in breast cancer, Breast Cancer Res. Treat. 21 (1992) 95–100.
[24] Y. Yu, T. Morimoto, M. Sasa, et al., HPV33 DNA in premalignant and malignant breast lesions in Chinese and Japanese populations, Anticancer Res. 19 (1999) 5057–5061.
[25] T. Li, Z.M. Lu, M. Guo, et al., p53 codon 72 polymorphism (C/G) and the risk of human papillomavirus-associated carcinomas in China, Cancer 95 (2002) 2571–2576.
[26] A.P. Damin, R. Karam, C.G. Zettler, et al., Evidence for an association of human papillomavirus and breast carcinomas, Breast Cancer Res. Treat. 84 (2004) 131–137.
[27] A. Widschwendter, T. Brunhuber, A. Wiedemair, et al., Detection of human papillomavirus DNA in breast cancer of patients with cervical cancer history, J. Clin. Virol. 31 (2004) 292–297.
[28] E.M. De Villiers, R.E. Sandstrom, H. Zur Hausen, et al., Presence of papillomavirus sequences in condylomatous lesions of the mamillae and in invasive carcinoma of the breast, Breast Cancer Res. 7 (2005) R1–R11.
[29] J.H. Tsai, C.H. Tsai, M.H. Cheng, et al., Association of viral factors with non-familial breast cancer in Taiwan by comparison with non-cancerous, fibroadenoma, and thyroid tumor tissues, J. Med. Virol. 75 (2005) 276–281.
[30] C.Y. Kan, B.J. Iacopetta, J.S. Lawson, N.J. Whitaker, Identification of human papillomavirus DNA gene sequences in human breast cancer, Br. J. Cancer 93 (2005) 946–948.
[31] M. Gumus, P.F. Yumuk, T. Salepci, et al., HPV DNA frequency and subset analysis in human breast cancer patients' normal and tumoral tissue samples, J. Exp. Clin. Cancer Res. 25 (4) (2006) 515–521.
[32] Y.L. Choi, E.Y. Cho, J.H. Kim, et al., Detection of human papillomavirus DNA by DNA chip in breast carcinomas of Korean women, Tumour Biol. 28 (6) (2007) 327–332.
[33] N. Akil, A. Yasmeen, A. Kassab, et al., High-risk human papillomavirus infections in breast cancer in Syrian women and their association with Id-1 expression: a tissue microarray study, Br. J. Cancer 99 (2008) 404–407.
[34] N.A. Khan, A. Castillo, C. Koriyama, et al., Human papillomavirus detected in female breast carcinomas in Japan, Br. J. Cancer 99 (2008) 408–414.
[35] D.C. De Leon, D.P. Montiel, J. Nemcova, et al., Human papillomavirus (HPV) in breast tumors: prevalence in a group of Mexican patients, BMC Cancer 9 (2009) 26.
[36] Q. He, S.Q. Zhang, Y.L. Chu, et al., The correlations between HPV16 infection and expressions of c-erbB-2 and bcl-2 in breast carcinoma, Mol. Biol. Rep. 36 (2009) 807–812.
[37] B. Heng, W.K. Glenn, Y. Ye, et al., Human papilloma virus is associated with breast cancer, Br. J. Cancer 101 (2009) 1345–1350.
[38] A.P. Mendizabal-Ruiz, E.J.A. Morales, L.J. RamirezJirano, et al., Low frequency of human papillomavirus DNA in breast cancer tissue, Breast Cancer Res. 114 (2009) 189–194.
[39] X. Mou, L. Chen, F. Liu, et al., Low prevalence of human papillomavirus (HPV) in Chinese patients with breast cancer, J. Int. Med. Res. 39 (2011) 1636–1644.
[40] K. Baltzell, G.C. Buehring, S. Krishnamurthy, et al., Limited evidence of human papillomavirus in breast tissue using molecular in situ methods, Cancer 118 (2012) 1212–1220.
[41] A. Frega, L. Lorenzon, M. Bononi, et al., Evaluation of E6 and E7 mRNA expression in HPV DNA positive breast cancer, Eur. J. Gynaecol. Oncol. 33 (2012) 164–167.
[42] W.K. Glenn, B. Heng, W. Delprado, et al., Epstein-Barr virus, human papillomavirus and mouse mammary tumour virus as multiple viruses in breast cancer, PLoS One 7 (2012) e48788.
[43] A. Sigaroodi, S.A. Nadji, F. Naghshvar, et al., Human papillomavirus is associated with breast cancer in the north part of Iran, ScientificWorldJournal 2012 (2012) 837191.
[44] W. Liang, J. Wang, C. Wang, et al., Detection of high-risk human papillomaviruses in fresh breast cancer samples using the hybrid capture 2 assay, J. Med. Virol. 85 (2013) 2087–2092.
[45] S.H. Ali, N.A. Al-Alwan, S.H. Al-Alwany, Detection and genotyping of human papillomavirus in breast cancer tissues from Iraqi patients, East Mediterr. J. 20 (2014) 372–377.
[46] M. Ahangar-Oskouee, S. Shahmahmoodi, S. Jalilvand, et al., No detection of "high-risk" human papillomaviruses in a group of Iranian women with breast cancer, Asian Pac. J. Cancer Prev. 15 (2014) 4061–4065.
[47] L. Manzouri, R. Salehi, S. Shariatpanahi, et al., Prevalence of human papilloma virus among women with breast cancer since 2005–2009 in Isfahan, Adv. Biomed. Res. 3 (2014) 75.
[48] J. Peng, T. Wang, H. Zhu, et al., Multiplex PCR/mass spectrometry screening of biological carcinogenic agents in human mammary tumors, J. Clin. Virol. 61 (2014) 255–259.
[49] L. Fu, D. Wang, W. Shah, et al., Association of human papillomavirus type 58 with breast cancer in Shaanxi province of China, J. Med. Virol. 87 (2015) 1034–1040.
[50] J. Li, J. Ding, K. Zhai, Detection of human papillomavirus DNA in patients with breast tumor in China, PLoS One 10 (2015) e0136050.
[51] A. Fernandes, G. Bianchi, Feltri, et al., Presence of human papillomavirus in breast cancer and its association with prognostic factors, Ecancermedicalscience 9 (2015) 548.
[52] D. Wang, L. Fu, W. Shah, et al., Presence of high risk HPV DNA but indolent transcription of E6/E7 oncogenes in invasive ductal carcinoma of breast, Pathol. Res. Pract. 212 (2016) 1151–1156.
[53] S. Delgado-García, J.C. Martínez-Escoriza, A. Alba, et al., Presence of human papillomavirus DNA in breast cancer: a Spanish case-control study, BMC Cancer 17 (2017) 320.
[54] N.A. Salman, G. Davies, F. Majidy, et al., Association of high risk human papillomavirus and breast cancer: a UK based study, Sci. Rep. 27 (7) (2017) 43591.
[55] W. Naushad, O. Surriya, H. Sadia, Prevalence of EBV, HPV and MMTV in Pakistani breast cancer patients: a possible etiological role of viruses in breast cancer, Infect. Genet. Evol. 54 (2017) 230–237.
[56] D. Wrede, Y.A. Luqmani, R.C. Coombes, et al., Absence of HPV 16 and 18 DNA in breast cancer, Br. J. Cancer 65 (1992) 891–894.
[57] G.L. Bratthauer, F.A. Tavassoli, T.J. O'Leary, Etiology of breast carcinoma: no apparent role for papillomavirus types 6/11/16/18, Pathol. Res. Pract. 188 (1992) 384–386.
[58] K. Czerwenka, F. Heuss, J.W. Hosmann, et al., Human papilloma virus DNA: a factor in the pathogenesis of mammary Paget's disease? Breast Cancer Res. Treat. 41 (1996) 51–57.
[59] V. Gopalkrishna, U.R. Singh, P. Sodhani, et al., Absence of human papillomavirus DNA in breast cancer as revealed by polymerase chain reaction, Breast Cancer Res. Treat. 39 (1996) 197–202.
[60] K. Lindel, A. Forster, H.J. Altermatt, et al., Breast cancer and human papillomavirus (HPV) infection: no evidence of a viral etiology in a group of Swiss women, Breast 16 (2007) 172–177.
[61] P. De Cremoux, M. Thioux, I. Lebigot, et al., No evidence of human papillomavirus DNA sequences in invasive breast carcinoma, Breast Cancer Res. 109 (2008) 55–58.
[62] M. Hachana, S. Ziadi, K. Amara, et al., No evidence of human papillomavirus DNA in breast carcinoma in Tunisian patients, Breast 19 (2010) 541–544.
[63] S. Hedau, U. Kumar, S. Hussain, et al., Breast cancer and human papillomavirus infection: no evidence of HPV etiology of breast cancer in Indian women, BMC Cancer 11 (2011) 27–37.
[64] R.G. Silva, B.B. Da Silva, No evidence for an association of human papillomavirus and breast carcinoma, Breast Cancer Res. Treat. 125 (2011) 261–264.
[65] P. Chang, T. Wang, Q. Yao, et al., Absence of human papillomavirus in patients with breast cancer in north-west China, Med. Oncol. 29 (2012) 521–525.
[66] M. Vernet-Tomas, M. Mena, L. Alemany, et al., Human papillomavirus and breast cancer: no evidence of association in a Spanish set of cases, Anticancer Res. 35 (2015) 851–856.
[67] A. Kouloura, E. Nicolaidou, I. Misitzis, et al., HPV infection and breast cancer. Results of a microarray approach, Breast. 40 (2018) 165–169. Aug.
[68] P.den Hollander, M.I. Savage, H. Powel, Targeted therapy for breast cancer prevention, Brown Front. Oncol. 3 (2013) 250.
[69] S.R. Ghaffari, T. Sabokbar, Z. Meshkat, et al., Tracing human papilloma virus in breast tumors of Iranian breast cancer patients, Breast J. 17 (2) (2011) 218–219.