Assessment of Human Papillomavirus Infection and Risk Factors in Egyptian Women With Breast Cancer

Breast Cancer: Basic and Clinical Research
Volume 15: 1–10

DOI: 10.1177/1178223421996279
SAGE

Nabila El-Sheikh[1]*, Nahla O Mousa[2,3]*, Amany M Tawfeik[1], Alaa M Saleh[1], Iman Elshikh[1], Mohamed Deyab[4], Faten Ragheb[5], Manar M Moneer[6], Ahmed Kawashti[4], Ahmed Osman[2,7] and Mohamed Elrefaei[8]

[1]Molecular Immunology Unit for Infectious Diseases, Department of Microbiology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. [2]Biotechnology Department, Egypt- Japan University of Science and Technology (E-JUST), Basic and Applied Sciences Institute (BAS), Alexandria, Egypt. [3]Biotechnology Program, Chemistry Department, Faculty of Science, Cairo University, Cairo, Egypt. [4]Department of Surgery, Faculty Medicine, Al-Azhar University, Cairo, Egypt. [5]Department of Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt. [6]Department of Epidemiology and Statistics, National Cancer Institute, Cairo University, Cairo, Egypt. [7]Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt. [8]Department of Laboratory Medicine and Pathology, Mayo Clinic, Jacksonville, FL, USA.

ABSTRACT: Numerous risk factors for breast cancer (BC) have been identified. High-risk human papilloma virus (HR-HPV) is the etiological agent of cervical cancer and in some cases of head and neck cancer, specifically oropharyngeal cancer, but the role of HR-HPV in evoking neoplasia in BC is still unclear. In this study, all women above the age of 18 visiting the oncology clinic at Al-Azhar university hospital and Ain Shams specialized hospital between the period of February 2017 and March 2018 were invited to participate. We determined the prevalence of HR-HPV genotypes 16, 18, and 31 in breast tissue samples from 72 women with treatment-naïve BC and 15 women with benign breast lesions (BBL) by quantitative real-time PCR (qRT-PCR) and primer sets targeting the E6 and E7 regions. High-risk human papilloma virus DNA was detected in 16 of 72 (22.2%) BC cases (viral load range = 0.3-237.8 copies/uL) and 0 of 15 women with BBL. High-risk human papilloma virus was detected in 14 of 16 (87.5%), 2 of 16 (12.5%), and 0 of 16 (0%) for genotypes 16, 18, and 31, respectively. Forty-three age-matched healthy Egyptian women were enrolled as controls for assessment of local risk factors that can be used to initiate a strategy of BC prevention in Egypt. Assessment of the risk factors demonstrated that low education level, passive smoking, lack of physical activity, family history of cancer, and use of oral contraception were significant risk factors for BC. In conclusion, our results lead us to postulate that HR-HPV infection may be implicated in the development of some types of BC in Egyptian women. In addition, identification of local risk factors can support practical prevention strategies for BC in Egypt.

KEYWORDS: Breast cancer (BC), high-risk human papillomavirus (HR-HPV), risk factors

RECEIVED: September 11, 2020. **ACCEPTED:** February 1, 2021.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was funded by the Science and Technology Development Fund (STDF), Egypt (Grant 22914).

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Mohamed Elrefaei, Department of Laboratory Medicine and Pathology, Mayo Clinic, Jacksonville, FL 32224, USA. Email: elrefaei.mohamed@mayo.edu

Introduction

Breast cancer (BC) is one of the most prevalent malignancies in women in both developed and developing world.[1] Breast cancer is the second-leading cause of death from cancer in Egyptian women which emphasizes the importance of identifying risk factors that are associated with BC development. The overall incidence of cancer is 157.0 per 100 000 Egyptian women with the highest incidence being BC (32%). A 3-fold increase in incidence of cancer in Egypt is predicted by 2050 with a tendency to occur in younger age groups with advanced stages.[2] This is, in part, due to a delayed diagnosis and advanced disease stage with a low cure rate. Most BC in Egypt is invasive duct subtype and the profile of hormone receptors is positive for estrogen receptor (ER) and/or progesterone receptor (PR) in less than half of cases.[3] Mastectomy is still performed in more than 80% of Egyptian women with BC. Several reasons for inadequate BC screening in Egyptian women have been identified, including lack of information about BC, low access to BC screening facilities, low priority of self-care, and fear of BC diagnosis.[4-6] Targeted intervention strategies are needed to improve early detection and better prognosis of BC.

While the cause of BC is still unknown, numerous risk factors have been identified. Virus infections including human parvovirus (B19), Epstein-Barr virus (EBV), and high-risk human papilloma virus (HR-HPV) have a causal role in cervical cancer and some head and neck tumors as thyroid and oropharyngeal cancers.[7-11] However, the role of HR-HPV in evoking neoplasia in BC is still unclear.[12-20] Previous studies have demonstrated the presence of HR-HPV types 16, 18, and

*Nabila El-Sheikh and Nahla O Mousa contributed equally to this work.

33 in BC specimens but not in the surrounding normal tissue.[12,21] In one study, HR-HPV was detected at lower levels in the normal tissue surrounding the BC tissue compared with the tumor tissue.[22] The development of modern molecular approaches based on quantitative real-time PCR (qRT-PCR) and sequence analysis has significantly improved the detection of HPV in BC tissue.[23] Recent studies have found the prevalence of HPV in BC tissue ranging from 4% to 86%.[24-31] One study has associated the detection of HPV in BC tissue with increased incidence of metaplastic breast carcinoma.[20] In contrast, HPV was not detected in BC tissue in other studies.[18,19]

HPV is a small, circular, double-stranded DNA virus that includes more than 120 known subtypes.[16] High-risk human papilloma virus encode a series of early (E1-E7) and late (L1 and L2) proteins. High-risk human papilloma virus E6 and E7 early proteins are oncoproteins that stimulate cell-cycle progression, cellular transformation, and cancer development.[15,32] The E6 protein binds to the p53 tumor suppressor protein resulting in accelerated ubiquitin-mediated degradation. E7 protein interacts with the so-called "pRb-associated pocket proteins," including the retinoblastoma protein pRb, which are negative cell-cycle regulators resulting in enhanced phosphorylation and degradation.[33] In addition, HR-HPV E5 and E6 early proteins are known to disrupt cytokeratin causing perinuclear cytoplasmic clearing and nuclear enlargement in koilocytes.[34-36]

The expression of ER and PR in BC predicts the response to growth factors, and may be associated with better prognosis and improved response to hormonal therapy.[37,38] In contrast, low hormone receptor expression in metaplastic carcinoma which is an aggressive type of BC is characterized by poor prognosis.[39] The expression of Human Epidermal Growth Factor Receptor 2 (HER2) is associated with poor prognosis and increase disease recurrence.[40] About 20% of BC patients lack ER, PR, and HER2 expression and have poor clinical outcome.[41,42]

Numerous risk factors for BC have been identified including genetics, reproductive life, hormonal factors, diet, and exposure to radiation and selected chemicals.[43] However, the single most important risk factor is age. The incidence of BC increases with age, and doubling every 10 years until menopause with most BC developing in women older than 50.[44]

In addition, younger age at menarche, nulliparity, older age at first live birth, and natural menopause after the age of 55 are also implicated as risk factors for BC. Increased public awareness and regular screening can play a vital role in the prevention, early detection, and treatment of BC.[45] The aim of the study was to determine the prevalence of HR-HPV infection in breast tissue samples from Egyptian women with BC and assess local risk factors that can be used to initiate a strategy of BC prevention in Egypt.

Materials and Methods

Population and source of samples

All women above the age of 18 visiting the oncology clinic at Al-Azhar university hospital and Ain Shams specialized hospital between the period of February 2017 and March 2018 were invited to participate in the study. All new cases of histologically confirmed BC were enrolled. Women who were diagnosed with benign breast lesions (BBL) as well as women residing in the same areas and admitted to the hospital, for a wide spectrum of acute, non-neoplastic conditions unrelated to known or likely risk factors for BC were invited to volunteer as controls in the study. Women with gynecological, hormonal, or neoplastic diseases were excluded from the study. Frozen breast tissue samples were collected according to the ethical regulations at Al-Azhar university hospital. Demographic and clinical information was obtained from medical records. The study received approval from the Institutional Review Board at Al-Azhar University. Breast tissue samples including tumor core and normal tissue from the surgical margin adjacent to tumors were collected from 72 women with treatment-naïve BC and 15 women with BBL after providing informed written consent. In addition, 43 age-matched healthy Egyptian women were enrolled as controls for assessment of local risk factors. No breast tissue samples were collected from the 43 female controls.

Extraction of DNA from breast tissue

The DNA and RNA extractions were performed together from the same homogenized specimen. DNA was extracted using the genomic DNA extraction kit for tissues (ThermoFisher, USA), following the manufacturer's instructions. The yield and quality of isolated DNA were checked on microplate reader A_{260}/A_{280}. DNA was stored at −20°C until used in qRT-PCR. The quality of the isolated DNA was checked by PCR of β-*globin* gene as previously described.[21,46,47] Briefly, a 268 bp fragment of the β-*globin* gene was amplified using the 5′-GAA GAG CCA AGG ACA GGT AC-3′ and 5′-CAA CTT CAT CCA CGT TCA CC-3′ primers under the following PCR conditions: initial denaturation at 95°C for 15 minutes, 40 cycles with the cycling profile of 95°C for 1 minute, 52°C for 1 minute, 72°C for 1 minute, and final extension for 5 minutes at 72°C. The β-*globin* gene product was detected in all extracted DNA samples, indicating adequate quality of our DNA templates.

Quantitative real-time PCR

The qRT-PCR was carried out using the Genesig Advanced Kit (Genesig, England) following the manufacturer's protocol. Briefly, the assay is an in vitro real-time amplification test for qualitative and quantitative detection of HR-HPV genotypes. Three HR-HPV types 16, 18, and 31 were investigated. The assay is based on 2 major processes: isolation of DNA from tissue specimens as HR-HPV DNA was detected in BC tissue in previous studies and qRT-PCR amplification of the E1-E2 region of HPV that is necessary for HPV genome replication.[13,48] The PCR products were detected by the "Step One Plus RT-PCR System (Applied Biosystem, USA). An HPV

genotype-specific primer and probe mix is provided, which is detected through the FAM channel.

The copy number of each sample is determined based on the standard set/curve (S1 = 2×10^5/μL; S2 = 2×10^4/μL; S3 = 2×10^3/μL; S4 = 2×10^2/μL; S5 = 20/μL; S6 = 2/μL). Assay performance was validated using a set of positive and negative controls and a no-template control. Positive control template is expected to amplify with a C_T between 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised. On the contrary, the negative control may give an amplification curve with $C_T > 35$.

RNA extraction and detection of E6 and E7 oncogenes

RNA Extraction was performed with TRIzol reagent (ThermoFisher, USA) following the recommended vendor's instructions followed by treatment with RQ1-RNase-free DNase to remove contaminating genomic DNA. The quality of the isolated RNA was checked spectrophotometrically. To confirm the efficiency of RNA extraction, the ubiquitously expressed *phosphoglycerate kinase* (*PGK*) which acts as a control gene, was amplified from reverse transcribed RNA samples as previously described.[49] Briefly, after pre-denaturation at 94°C for 30 seconds, a 2-step program of qRT-PCR was set as follows: denaturing at 94°C for 5 seconds, subsequently annealing at 56°C for 30 seconds, and repeated for 40 cycles. Finally, melting curves of qRT-PCR amplifications were performed to confirm the specificity of the primers again by heating up the products from 56°C to 95°C.

The RNA (0.1-1.0 μg) from HR-HPV positive cases was reverse transcribed in 20 μL reactions using the high-capacity cDNA synthesis kit (ThermoFisher, USA) according to the manufacturer's instructions. The PCR amplification steps were 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 minutes, and hold at 4°C. The cDNA was stored at −20°C until use. The cDNA samples served as templates in qRT-PCR reactions using specific primers for the E6 and E7 oncogenes of HPV genotypes 16 and 18 (Supplementary Table 1) and Maxima SYBR Green qRT-PCR Master Mix (ThermoFisher, USA). The amplification of *E6* and *E7* genes was performed as previously described.[28] The amplification conditions were 2 minutes at 50°C, 15 minutes at 95°C, and a 2-step cycle of 95°C, for 15 seconds, and 60°C, for 1 minute for a total of 40 cycles. Melting curve analysis was performed at the end of the PCR run to ensure that only a single PCR amplicon of the expected length and melting temperature was produced using gel electrophoresis and PCR amplicon melting curve data, respectively. E6 and E7 are 2 major oncogenes that have been shown to be constitutively expressed leading to initial establishment and subsequent progression in cervical cancer.[33]

A DNA spike-in control was designed to assess the amplification efficiency process of the *E6* gene of HR-HPV type 16. This spike-in control is an 80-nucleotide long pair (Supplementary Table 1), which includes 3 segments with the middle sequence representing a 38-bp-long *Drosophila melanogaster* sequence that

Table 1. Demographic and disease characteristics of BC and BBL in Egyptian women.

	BC (N = 72)[a]	BBL (N = 15)[a]	*P* VALUE
Age (years) mean ± SD	53.8 ± 11.5	55.0 ± 13.7	.72
Size (cm) (median, range)	3 (1-12)	7 (1-10)	<.005
<2	11 (15.3%)	1 (11.1%)	
2-5	54 (75.0%)	3 (33.3%)	
>5	7 (9.7%)	5 (55.6%)	
Unknown	0	6	
Stage			
Early (I and II)	45 (62.5%)		
Advanced (III and IV)	23 (31.9%)		
Unknown	4		
Tumor grade			
Grade I	2 (2.8%)		
Grade II	54 (76%)		
Grade III	15 (21.1%)		
Unknown	1		
Lymph node metastasis			
Positive	35 (50.0%)		
Negative	35 (50.0%)		
Unknown	2		
Estrogen receptor			
Positive	58 (84.1%)		
Negative	11 (15.9%)		
Unknown	3		
Progesterone receptor			
Positive	54 (78.3%)		
Negative	15 (21.7%)		
Unknown	3		
HER2			
Positive	43 (62.3%)		
Negative	26 (37.7%)		
Unknown	3		

Abbreviations: BBL, benign breast lesions; BC, breast cancer; HER2, Human Epidermal Growth Factor Receptor 2.
[a]Numbers in parenthesis represent the range or percent.

is flanked by two 21-bp sequences that represent the forward and reverse primers of *E6* gene of HPV genotype 16. A valid C_T value is considered between 16 and 32. The amplification of the target gene and the spike-in control in separate reactions can be distinguished by melt curve analysis. This internal control DNA served as a positive control for the E6 of HPV genotype 16 qRT-PCR reactions, which verified absence of intrinsic inhibition due to presence of endogenous inhibitors.

Assessment of risk factors for BC

Clinicopathological risk factors were assessed for age, lymph node metastasis, tumor size, histologic grade, tumor stage, and hormonal receptor status, including ER, PR, and HER2. Expression of ER, PR, and HER2 receptors was assessed by immunohistochemistry staining performed in Al-Azhar University hospital clinical laboratory as previously described.[50] Breast cancer is regarded to be hormonal receptor positive if at least 1% stained positive for ER or PR assays as previously described.[51] Test results were obtained from the patient medical records. Tumor stages were classified according to the American Joint Committee on Cancer (AJCC)-TNM (tumor, node, metastases) classification seventh edition.[52]

Several questionnaires were designed to assess local environmental risk factors for BC development. All interviews for BC cases and controls were conducted at Al-Azhar and Ain Shams university hospitals. The questionnaires included information on personal characteristics and lifestyle habits, including marital status and other socio-economic indicators, smoking, diet, physical activity, menstrual and reproductive factors (such as age at menarche, menstrual pattern, number of abortions and births, breast feeding, and age at each birth), selected medical conditions, history of benign breast disease, and family history of cancer.

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. For not normally distributed quantitative data, comparison between 2 groups was done using Mann-Whitney test (non-parametric *t*-test). All tests were 2-tailed. When more than 2 groups of data sets were compared, 1-way analysis of variance (ANOVA) was performed as described. A P value $< .05$ was considered significant.

Results

Patient demographics and disease characteristics

The demographic and disease characteristics of 87 Egyptian women including 72 treatment-naïve BC and 15 with BBL patients are shown in Table 1. The mean age of patients with BC and BBL was 53.8 ± 11.5, and 55.0 ± 13.7, respectively. There was no significant difference in age between patients with BC compared with BBL ($P = .728$). In addition, 70% of patients with BC were post-menopausal. Tumor size was significantly larger in patients with BBL compared with BC ($P < .005$). Forty-five of 72 patients with BC (62.5%) were diagnosed with early stage disease (stages I and II) while 23 of 72 (31.9%) were diagnosed with advanced disease (stages III and IV). High tumor grade III and lymph node metastasis were diagnosed in 15 of 71 (21.1%) and 35 of 71 (50%) patients with BC, respectively. The number of BC patients expressing ER, PR, or HER2 was 58 (84.1%), 54 (78.3%), and 43 (62.3%), respectively, while 5 BC patients lacked expression of all 3 (triple negative).

Table 2. Histopathologic subtypes of BC and BBL in Egyptian women.

HISTOPATHOLOGY	BC (N = 72)[a]	BBL (N = 15)[a]
Carcinoma in situ	3 (4.2%)	
Invasive duct carcinoma	54 (75%)	
Mammary carcinoma	8 (11.1%)	
Metaplastic carcinoma	4 (5.6%)	
Invasive lobular carcinoma	1 (1.4%)	
Neoplastic multifocal carcinoma	1 (1.4%)	
Papillary intraductal carcinoma	1 (1.4%)	
Benign mammary lesion		4 (26.6%)
Fibroadenoma		3 (20%)
Fibrocystic changes		1 (6.66%)
Benign ductal epithelial cells		1 (6.66%)
Benign mastitis		1 (6.66%)
Benign unspecified		5 (33.3%)

Abbreviations: BBL, benign breast lesions; BC, breast cancer.
[a]Numbers in parenthesis represent the percent.

Histopathologic subtypes

Among 72 treatment-naïve BC patients, 54 (75%) had invasive duct carcinoma (IDC), 8 (11.1%) mammary carcinoma, 4 (5.55%) metaplastic carcinomas, 3 (4.16%) had carcinoma in situ, 1 (1.38%) invasive lobular carcinoma (ILC), 1 (1.38%) neoplastic multifocal carcinoma, and 1 (1.38%) papillary intraductal carcinoma (Table 2). Regarding the 3 carcinoma in situ, 2 of 3 were ductal and 1 of 3 was lobular. All the 54 IDC and 8 mammary carcinoma were invasive. Among 15 BBL patients, 4 (26.6%) were benign mammary lesion, 3 (20%) fibroadenoma, 1 (6.66%) fibrocystic change, 1 (6.66%) benign ductal epithelial cell tumor, 1 (6.66%) benign mastitis, and 5 (33.3%) benign unspecified.

Table 3. HR-HPV subtypes and viral load in Egyptian women with BC.[a]

HISTOPATHOLOGY	HR-HPV SUBTYPES			
	TYPE 16	VIRAL LOAD (RANGE)[b]	TYPE 18	VIRAL LOAD (RANGE)[b]
Carcinoma in situ	1	9.5	0	
Invasive ductal carcinoma	8	9.74 (0.9-30)	1	237.8
Metaplastic carcinoma	3	16.8 (6-35)	1	0.3
Invasive lobular carcinoma	1	1.4	0	
Mammary carcinoma	1	0.8	0	
Total	14[c]		2[c]	

Abbreviations: BC, breast cancer; HR-HPV, high-risk human papilloma virus.
[a]HR-HPV was detected in 16 of 72 (22.2%) BC patients.
[b]HR-HPV viral load is measured as mean number of viral copies per uL and range in between parenthesis.
[c]The prevalence of HR-HPV genotypes was 14 of 16 (87.5%), 2 of 16 (12.5%), and 0 of 16 (0%) for genotypes 16 and 18, respectively.

High-risk human papilloma virus subtypes and histopathology

High-risk human papilloma virus types 16, 18, and 31 were assessed because they are very common and may play a causal role in evoking neoplasia.[8,9,11,53] High-risk human papilloma virus was detected in 16 of 72 (22.2%) BC but in 0 of 15 of BBL patients. The prevalence of HR-HPV genotypes was 14 of 16 (87.5%), 2 of 16 (12.5%), and 0 of 16 (0%) for genotypes 16, 18, and 31, respectively. None of the patients were infected with more than 1 HR-HPV genotype. High-risk human papilloma virus genotype 16 was detected in either the center of the cancerous tissue or in the noncancerous safety margin in 12 of 14 and 2 of 14 patients, respectively (Table 3). Evaluation of mRNA of HR-HPV E6 and E7 early proteins may represent a good marker for HR-HPV involvement in malignant transformation.[54] However, no mRNA transcription was detected for HR-HPV E6 and E7 early proteins in our study (data not shown).

The distribution of HR-HPV genotype type 16 according to histopathology revealed predominance in metaplastic carcinoma 3 of 4 (75%) with a mean viral copy number of 16.8 (range = 6-35), and IDC 8 of 54 (14.8%), with a mean viral copy number of 9.74 (range = 0.9-30). High-risk human papilloma virus genotype type 16 was also found in 1 of 3, 1 of 1, and 1 of 8 of carcinoma in situ, ILC, and mammary carcinoma, respectively, with a viral copy number of 9.5, 1.4, and 0.8, respectively. In contrast, HR-HPV genotype type 18 was found in only 2 patients, 1 of 54 invasive ductal carcinoma and 1 of 4 metaplastic carcinoma with a viral copy number of 237.8 and 0.3, respectively.

The clinicopathological features of BC patients with and without HR-HPV infection are shown in Table 4. There was no significant difference in tumor size, disease stage, tumor grade, and lymph node metastasis between BC patients with and without HR-HPV infection ($P > .05$). Invasive duct

Table 4. HR-HPV and clinicopathological features in Egyptian women with BC.

	HR-HPV POS (N = 16)	HPV NEG (N = 56)	*P* VALUE
Stage			.5
Early (I and II)	11 (68.75%)[a]	34 (60.7%)	
Advanced (III and IV)	4 (25%)	19 (33.9%)	
Unknown	1 (6.25%)	3 (5.4%)	
Histopathology			.13
Invasive duct carcinoma	9 (56.25%)	45 (80.4%)	
Other pathologic types	7 (43.75%)	11 (19.6%)	
Size (cm)			.88
<2	3 (18.75%)	8 (14.3%)	
2-5	11 (68.75%)	43 (76.8%)	
>5	1 (6.25%)	5 (8.9%)	
Unknown	1 (6.25%)	0	
Grade			.9
I and II	12 (75%)	44 (78.6%)	
III and IV	3 (18.75%)	12 (21.4%)	
Unknown	1 (6.25%)	0	
Lymph node metastasis			.77
Negative	7 (43.75%)	28 (50%)	
Positive	8 (50%)	27 (48.2%)	
Unknown	1 (6.25%)	1 (1.8%)	

Abbreviations: BC, breast cancer; HR-HPV, high-risk human papilloma virus.
[a]Numbers in parenthesis represent the percent.

carcinoma was the most prevalent BC subtype and was found in 9 of 16 (56.25%) and 45 of 56 (80.4%) BC patients with and without HR-HPV infection, respectively. However, there was no significant difference in frequency of IDC between BC patients with and without HR-HPV infection ($P = .13$).

Risk factors for BC

Several questionnaires were designed to assess local risk factors for BC development. The risk of developing BC was significantly higher with low education level (illiterate and primary or secondary school) compared with university graduate (Table 5). Egyptian women who were not working also had a higher risk of developing BC than working women ($P < .001$). However, marital status was not significant a risk factor for BC because most of the BC patients (84%) and controls (95.3%) were married. The risk of developing BC was significantly higher with the lack of any physical activity (⩾10 minute walk per day). Although the habit of active smoking among Egyptian women is uncommon, passive smoking, which is the involuntary inhaling of smoke from other people's cigarettes, was found to significantly influence BC risk ($P < .001$).

Most Egyptian women with BC (60%) were postmenopausal ($P < .001$). In addition, the risk of developing BC was significantly higher in women with history of oral contraception use ($P < .001$). In contrast, other hormonal factors including age of menarche and regularity of menses were not associated with a higher risk of developing BC. Age at delivery of first baby was not significant a risk factor for BC because most of the BC patients (98%) and controls (93%) had their first child under the age of 35.

The risk of developing BC was significantly higher in women with family history of any type of cancer ($P = .02$) and any type of benign tumors including BBL ($P < .001$). In contrast, positive family history of Diabetes Miletus and Heart Disease were not associated with a higher risk of developing BC. In addition, the risk of developing BC was not increased by intake of fast food, poultry, or the use of charcoal in cooking. Most Egyptian women cook with homemade butter and vegetable oil (60% and 83% in BC and controls, respectively).

Discussion

In this study, the use of qRT-PCR confirmed the presence of HR-HPV in BC tissue and was comparable with previous standard PCR results.[12] The frequency of infection with HR-HPV genotypes in Egyptian women with BC was reported (22.2%) and is consistent with previously published worldwide data.[12,28,55-63] The lower HR-HPV detection rate reported in some studies may be a result of difficulties in PCR detection due to low viral load in BC in different populations.[64-66] We detected HR-HPV in cancerous tissue or noncancerous safety margin adjacent to BC tissue as previously reported.[21] These results suggest that HR-HPV may have a tumorigenic role in BC.[12,22,67] High-risk human papilloma

Table 5. Risk factors for BC in Egyptian women.

	BC (N = 50)	HC (N = 43)	*P* VALUE
Education			<.001
Low level[a]	19 (38%)[b]	2 (4.65%)	
University graduate	27 (54%)	41 (95.35%)	
Unknown	4 (8%)	0	
Occupation			<.001
Yes	17 (34%)	43 (100%)	
No	30 (60%)	0	
Unknown	3 (6%)	0	
Menopause			<.001
Pre	16 (32%)	34 (79.07%)	
Post	30 (60%)	8 (18.6%)	
Unknown	4 (8%)	1 (2.33%)	
Physical activity[c]			<.001
Yes	23 (46%)	33 (76.74%)	
No	27 (54%)	9 (20.93%)	
Unknown	0	1 (2.33%)	
Passive smoking[d]			<.001
Yes	28 (56%)	9 (20.93%)	
No	20 (40%)	33 (76.74%)	
Unknown	2 (4%)	1 (2.33%)	
Family history of cancer			.02
Yes	23 (46%)	10 (23.26%)	
No	27 (54%)	33 (76.74%)	
Unknown	0	0	
Family history of benign tumor			<.001
Yes	38 (76%)	2 (4.65%)	
No	8 (16%)	37 (86.05%)	
Unknown	4 (8%)	4 (9.3%)	
Family history of diabetes mellitus			.57
Yes	25 (50%)	24 (55.8%)	
No	25 (50%)	19 (44.2%)	
Unknown	0	0	
Family history of heart disease			.09
Yes	33 (66%)	35 (81.4%)	

(Continued)

Table 5. (Continued)

	BC (N = 50)	HC (N = 43)	*P* VALUE
No	17 (34%)	8 (18.6%)	
Unknown	0	0	
Oral contraception			<.001
Yes	33 (66%)	12 (27.9%)	
No	12 (24%)	31 (72.1%)	
Unknown	5 (10%)	0	
Marital status			.99
Married	42 (84%)	41 (95.3%)	
Widow	3 (6%)	2 (4.7%)	
Divorced	5 (10%)	0	
Age (years) of menarche			.99
⩽12	(40%)	17 (39.53%)	
>12	28 (56%)	25 (58.14%)	
Unknown	2 (4%)	1 (2.33%)	
Regular menses			.78
Yes	38 (76%)	33 (76.74%)	
No	9 (18%)	9 (20.93%)	
Unknown	3 (6%)	1 (2.33%)	
Fast food			.02
Yes	23 (46%)	34 (79.06%)	
No	24 (48%)	8 (18.6%)	
Unknown	3 (6%)	1 (2.33%)	
Charcoal use			.03
Yes	1 (2%)	33 (76.74%)	
No	43 (86%)	10 (23.26%)	
Unknown	6 (12%)	0	
Vegetable oil use			<.001
Yes	30 (60%)	36 (83.72%)	
No	17 (34%)	1 (2.33%)	
Unknown	3 (6%)	6 (13.95%)	

Abbreviations: BC, breast cancer; HC, healthy control.
[a]Illiterate, primary or secondary school.
[b]Numbers in parenthesis represent the percent.
[c]⩾10 minute walk per day.
[d]Involuntary inhaling of smoke from other people's cigarettes.

virus was shown to influence the cell-cycle control enzyme APOBEC causing genomic instability and may lead to BC.[68] Other infectious agents such as *Helicobacter pylori* in gastric cancer and hepatitis viruses in hepatocellular carcinoma act as indirect carcinogens while their genes were undetected in the respective cancer cells.[69,70] The assessment of HR-HPV in BC is complicated by the very low viral load, viral infection precede BC development by many years, and the prevalence of HR-HPV is more common than BC. The prevalence of BC is not increased in immunocompromised AIDS patients or organ transplant recipients which suggest that the oncogenic influence of HR-HPV in BC development may be indirect.[71] High-risk human papilloma virus alone may not be sufficient for full tumorigenic transformation and further cumulative changes occur over time leading to BC.

A strong association between the presence of mRNA for HR-HPV E6 and E7 early proteins and risk of neoplastic progression in cervical lesions has been described.[72] In addition, HR-HPV transcriptional activity was also found in most oropharyngeal squamous cell carcinoma.[73] However, in our study, the role of HR-HPV in BC oncogenesis is still unclear because no mRNA transcription was detected for HR-HPV E6 and E7 early proteins as previously reported.[74] We postulate that HR-HPV infection may be implicated in the development of some types of BC in Egyptian women. In addition, prevention of some BC may be possible by vaccination against HR-HPV types 16 and 18.[75] However, providing conclusive evidence for the role of HR-HPV in BC may be difficult because the HPV viral load in BC tissue is very low compared with cervical cancer.[21]

In Egypt, there is no mandatory BC case reporting, nor is there national or local health information on BC patients. In this study, data were collected using questionnaires from patients admitted to the university hospitals of Al-Azhar and Ain Shams universities. The risk for an Egyptian woman developing BC increases with age, with most BC occurring in women older than 50. Women who went through menopause after the age of 55 had a higher risk of BC. This may be due to prolonged exposure of breast cells to estrogen and progesterone.[76]

The cause of increased risk of developing BC in women with low education level compared with university graduates is unclear but may be due to variations in diet, obesity, environment, and comorbidities. Illiterate women living in poverty are more likely to be diagnosed at an advanced stage and are less likely to survive BC compared with more affluent educated women. Access to health care and the availability of treatment are additional factors.[77]

In this study, passive smoking and the lack of physical activity were significant risk factors for BC. Smoking tobacco is well known to be carcinogenic for most types of cancers including BC.[78,79] The carcinogenic effects are caused by aromatic hydrocarbons contained in tobacco, which, together with genetic polymorphisms in the N-acetyltransferase-2, may influence BC development.[80] Lack of physical activity is a stand-alone risk factor for colon, breast, and endometrial cancers.[81] Regular

physical activity may provide protection from BC by helping women maintain a healthy body weight, lowering hormone levels, and indirectly supporting the immune system.[81,82]

Our results are consistent with previous studies that demonstrated a significantly higher risk for BC development in women with close relatives diagnosed with any type of cancer or benign tumors including BBL.[83] A strong family history of BC may be linked to inheritance of the *BRCA1* or *BRCA2* genetic mutations.[84] Mutations in *BRCA1* and *BRCA2* genes are identified in approximately 10% of BC patients.[85] BRCA1 mutation is associated with 43% to 75% lifetime risk for breast or ovarian cancer.[86] The mechanism of BRCA1 associated malignancy is unclear. BRCA1 is involved in DNA damage repair by homologous and non-homologous recombination.[87] It has been shown that BRCA1 dictates the type of DNA repair mechanism at double strand breaks together with p53. The interaction of BRCA1 and p53 may increase DNA sensitivity to damaging agents as cytotoxic drugs and radiation.[88] Egyptian women tend to be part of large and extended families. The effect of size and age-structure of the woman's family on BC development risk should be assessed in larger future studies.[89]

Prolonged oral contraceptive use beginning at a young age was shown to be associated with higher risk of BC.[90] The use of oral contraception significantly increased the risk for BC in Egyptian women and is a major concern that limits their use for birth control in Egypt. Other factors associated with prolonged exposure to endogenous estrogen such as early menarche, late menopause, and older age at first childbirth were not associated with a higher risk of developing BC which may be due to the relatively small number of patients in the study.

The differences in dietary habits observed in this study between women with and without BC including consumption of fast food, poultry, and vegetable oil was not associated with a higher risk of BC. However, eating more fruits and vegetables and less red meat is linked with many health benefits. A Mediterranean diet that includes vegetables, fruits, whole grains, fish, and olive oil has been associated with a decreased risk of BC.[91]

Conclusions

Our results lead us to postulate that HR-HPV infection may be implicated in the development of some types of BC in Egyptian women. Further studies are warranted to elucidate the etiological role and pathogenesis of HR-HPV in Egyptian women with BC. In addition, identification of local risk factors can support practical prevention strategies for BC in Egypt.

Author Contributions

AMT, NOM, AMS, IE, FR, AK, and AO acquired, analyzed, and interpreted the patient data. MMM performed statistical analysis. AMT, NE-S, and ME were major contributors in writing the manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The study received approval from the Institutional Review Board at Al-Azhar University.

ORCID iD

Mohamed Elrefaei https://orcid.org/0000-0002-2030-4779

Supplemental Material

Supplemental material for this article is available online.

REFERENCES

1. Torre LA, Islami F, Siegel RL, Ward EM, Jemal A. Global cancer in women: burden and trends. *Cancer Epidemiol Biomarkers Prev*. 2017;26:444-457.
2. Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in Egypt: results of the national population-based cancer registry program. *J Cancer Epidemiol*. 2014;2014:437971.
3. Omar S, Khaled H, Gaafar R, Zekry AR, Eissa S, el-Khatib O. Breast cancer in Egypt: a review of disease presentation and detection strategies. *East Mediterr Health J*. 2003;9:448-463.
4. Donnelly TT, Khater AH, Al-Bader SB, et al. Arab women's breast cancer screening practices: a literature review. *Asian Pac J Cancer Prev*. 2013;14:4519-4528.
5. Uddin N, Fateem E, Hablas A, et al. Public and professional educational needs for downstaging breast cancer in Egypt. *J Cancer Educ*. 2012;27:149-155.
6. Stapleton JM, Mullan PB, Dey S, et al. Patient-mediated factors predicting early- and late-stage presentation of breast cancer in Egypt. *Psychooncology*. 2011;20:532-537.
7. You EL, Henry M, Zeitouni AG. Human papillomavirus-associated oropharyngeal cancer: review of current evidence and management. *Curr Oncol*. 2019;26:119-123.
8. Etemadi A, Mostafaei S, Yari K, Ghasemi A, Minaei Chenar H, Moghoofei M. Detection and a possible link between parvovirus B19 and thyroid cancer. *Tumour Biol*. 2017;39:1010428317703634.
9. Moghoofei M, Mostafaei S, Nesaei A, et al. Epstein-Barr virus and thyroid cancer: the role of viral expressed proteins. *J Cell Physiol*. 2019;234:3790-3799.
10. Nahand JS, Mahjoubin-Tehran M, Moghoofei M, et al. Exosomal miRNAs: novel players in viral infection. *Epigenomics*. 2020;12:353-370.
11. Sadri Nahand J, Moghoofei M, Salmaninejad A, et al. Pathogenic role of exosomes and microRNAs in HPV-mediated inflammation and cervical cancer: a review. *Int J Cancer*. 2020;146:305-320.
12. Heng B, Glenn WK, Ye Y, et al. Human papilloma virus is associated with breast cancer. *Br J Cancer*. 2009;101:1345-1350.
13. Khodabandehlou N, Mostafaei S, Etemadi A, et al. Human papilloma virus and breast cancer: the role of inflammation and viral expressed proteins. *BMC Cancer*. 2019;19:61.
14. Joshi D, Buehring GC. Are viruses associated with human breast cancer? Scrutinizing the molecular evidence. *Breast Cancer Res Treat*. 2012;135:1-15.
15. McLaughlin-Drubin ME, Munger K. Oncogenic activities of human papillomaviruses. *Virus Res*. 2009;143:195-208.
16. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. *J Clin Virol*. 2005;32:S16-S24.
17. Di Lonardo A, Venuti A, Marcante ML. Human papillomavirus in breast cancer. *Breast Cancer Res Treat*. 1992;21:95-100.
18. Bonlokke S, Blaakaer J, Steiniche T, et al. Evidence of no association between human papillomavirus and breast cancer. *Front Oncol*. 2018;8:209.
19. Vernet-Tomas M, Mena M, Alemany L, et al. Human papillomavirus and breast cancer: no evidence of association in a Spanish set of cases. *Anticancer Res*. 2015;35:851-856.
20. Herrera-Goepfert R, Vela-Chavez T, Carrillo-Garcia A, et al. High-risk human papillomavirus (HPV) DNA sequences in metaplastic breast carcinomas of Mexican women. *BMC Cancer*. 2013;13:445.
21. Khan NA, Castillo A, Koriyama C, et al. Human papillomavirus detected in female breast carcinomas in Japan. *Br J Cancer*. 2008;99:408-414.
22. Gumus M, Yumuk PF, Salepci T, et al. HPV DNA frequency and subset analysis in human breast cancer patients' normal and tumoral tissue samples. *J Exp Clin Cancer Res*. 2006;25:515-521.

23. Gao G, Chernock RD, Gay HA, et al. A novel RT-PCR method for quantification of human papillomavirus transcripts in archived tissues and its application in oropharyngeal cancer prognosis. *Int J Cancer*. 2013;132:882-890.
24. Francis IM, Al-Ayadhy B, Al-Awadhi S, Kapila K, Al-Mulla F. Prevalence and correlation of human papilloma virus and its types with prognostic markers in patients with invasive ductal carcinoma of the breast in Kuwait. *Sultan Qaboos Univ Med J*. 2013;13:527-533.
25. Hossein R, Behzad S, Tahar M, Azadeh NA. Prevalence of human papillomavirus genotypes associated with cervical and breast cancers in Iran. *Monoclon Antib Immunodiagn Immunother*. 2013;32:399-403.
26. Liang W, Wang J, Wang C, et al. Detection of high-risk human papillomaviruses in fresh breast cancer samples using the hybrid capture 2 assay. *J Med Virol*. 2013;85:2087-2092.
27. Manzouri L, Salehi R, Shariatpanahi S, Rezaie P. Prevalence of human papilloma virus among women with breast cancer since 2005-2009 in Isfahan. *Adv Biomed Res*. 2014;3:75.
28. Pereira Suarez AL, Lorenzetti MA, Gonzalez Lucano R, et al. Presence of human papilloma virus in a series of breast carcinoma from Argentina. *PLoS ONE*. 2013;8:e61613.
29. Liu Y, Klimberg VS, Andrews NR, et al. Human papillomavirus DNA is present in a subset of unselected breast cancers. *J Hum Virol*. 2001;4:329-334.
30. Choi J, Kim C, Lee HS, 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*. 2016;50: 442-450.
31. Bae JM, Kim EH. Human papillomavirus infection and risk of breast cancer: a meta-analysis of case-control studies. *Infect Agent Cancer*. 2016;11:14.
32. Pang CL, Thierry F. Human papillomavirus proteins as prospective therapeutic targets. *Microb Pathog*. 2013;58:55-65.
33. Pal A, Kundu R. Human papillomavirus E6 and E7: the cervical cancer hallmarks and targets for therapy. *Front Microbiol*. 2019;10:3116.
34. Fernandes A, Bianchi G, Feltri AP, Perez M, Correnti M. Presence of human papillomavirus in breast cancer and its association with prognostic factors. *Ecancermedicalscience*. 2015;9:548.
35. Lawson JS, Glenn WK, Heng B, et al. Koilocytes indicate a role for human papilloma virus in breast cancer. *Br J Cancer*. 2009;101:1351-1356.
36. Krawczyk E, Suprynowicz FA, Liu X, et al. Koilocytosis: a cooperative interaction between the human papillomavirus E5 and E6 oncoproteins. *Am J Pathol*. 2008;173:682-688.
37. Arpino G, Weiss H, Lee AV, et al. Estrogen receptor-positive, progesterone receptor-negative breast cancer: association with growth factor receptor expression and tamoxifen resistance. *J Natl Cancer Inst*. 2005;97:1254-1261.
38. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet*. 2005;365:1687-1717.
39. Zhang Y, Lv F, Yang Y, et al. Clinicopathological features and prognosis of metaplastic breast carcinoma: experience of a major Chinese Cancer Center. *PLoS ONE*. 2015;10:e0131409.
40. De Laurentiis M, Arpino G, Massarelli E, et al. A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer. *Clin Cancer Res*. 2005;11:4741-4748.
41. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. *Cancer*. 2007; 109:1721-1728.
42. Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res*. 2007;13:4429-4434.
43. Sasco AJ, Kaaks R, Little RE. Breast cancer: occurrence, risk factors and hormone metabolism. *Expert Rev Anticancer Ther*. 2003;3:546-562.
44. Bilimoria MM, Morrow M. The woman at increased risk for breast cancer: evaluation and management strategies. *CA Cancer J Clin*. 1995;45:263-278.
45. El Saghir NS. Responding to the challenges of breast cancer in Egypt and other Arab countries. *J Egypt Natl Canc Inst*. 2008;20:309-312.
46. Li J, Ding J, Zhai K. Detection of human papillomavirus DNA in patients with breast tumor in China. *PLoS ONE*. 2015;10:e0136050.
47. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*. 1988;239:487-491.
48. Salman NA, Davies G, Majidy F, et al. Association of high risk human papillomavirus and breast cancer: a UK based study. *Sci Rep*. 2017;7:43591.
49. Cai J, Li P, Luo X, et al. Selection of appropriate reference genes for the detection of rhythmic gene expression via quantitative real-time PCR in Tibetan hulless barley. *PLoS ONE*. 2018;13:e0190559.
50. Park C, Park K, Kim J, et al. Prognostic values of negative estrogen or progesterone receptor expression in patients with luminal B HER2-negative breast cancer. *World J Surg Oncol*. 2016;14:244.
51. Hammond ME, Hayes DF, Wolff AC, Mangu PB, Temin S. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Oncol Pract*. 2010;6:195-197.
52. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*. 2010;17:1471-1474.
53. Kobayashi K, Hisamatsu K, Suzui N, Hara A, Tomita H, Miyazaki T. A review of HPV-related head and neck cancer. *J Clin Med*. 2018;7:241.
54. Liu S, Minaguchi T, Lachkar B, et al. Separate analysis of human papillomavirus E6 and E7 messenger RNAs to predict cervical neoplasia progression. *PLoS ONE*. 2018;13:e0193061.
55. de Leon DC, Montiel DP, Nemcova J, et al. Human papillomavirus (HPV) in breast tumors: prevalence in a group of Mexican patients. *BMC Cancer*. 2009;9:26.
56. Aguayo F, Khan N, Koriyama C, et al. Human papillomavirus and Epstein-Barr virus infections in breast cancer from Chile. *Infect Agent Cancer*. 2011;6:7.
57. Herrera-Romano L, Fernandez-Tamayo N, Gomez-Conde E, et al. Absence of human papillomavirus sequences in epithelial breast cancer in a Mexican female population. *Med Oncol*. 2012;29:1515-1517.
58. Yu Y, Morimoto T, Sasa M, et al. Human papillomavirus type 33 DNA in breast cancer in Chinese. *Breast Cancer*. 2000;7:33-36.
59. Antonsson A, Spurr TP, Chen AC, et al. High prevalence of human papillomaviruses in fresh frozen breast cancer samples. *J Med Virol*. 2011;83:2157-2163.
60. Glenn WK, Heng B, Delprado W, Iacopetta B, Whitaker NJ, Lawson JS. Epstein-Barr virus, human papillomavirus and mouse mammary tumour virus as multiple viruses in breast cancer. *PLoS ONE*. 2012;7:e48788.
61. Lawson JS, Gunzburg WH, Whitaker NJ. Viruses and human breast cancer. *Future Microbiol*. 2006;1:33-51.
62. Tawfeik AM, Mora A, Osman A, Moneer MM, El-Sheikh N, Elrefaei M. Frequency of CD4+ regulatory T cells, CD8+ T cells, and human papilloma virus infection in Egyptian women with breast cancer. *Int J Immunopathol Pharmacol*. 2020;34:2058738420966822.
63. EISheshtawy NS, Shakweer MM, EL-Ghamrini YM. Is HPV virus associated with human breast cancer? Time to re-examine the postulate. Egyptian study. *Ann Pathol Lab Med*. 2017;4:1-9.
64. Choi YL, Cho EY, Kim JH, et al. Detection of human papillomavirus DNA by DNA chip in breast carcinomas of Korean women. *Tumour Biol*. 2007;28:327-332.
65. Peng J, Wang T, Zhu H, et al. Multiplex PCR/mass spectrometry screening of biological carcinogenic agents in human mammary tumors. *J Clin Virol*. 2014;61:255-259.
66. Herrera-Goepfert R, Khan NA, Koriyama C, Akiba S, Perez-Sanchez VM. High-risk human papillomavirus in mammary gland carcinomas and non-neoplastic tissues of Mexican women: no evidence supporting a cause and effect relationship. *Breast*. 2011;20:184-189.
67. Lawson JS, Salmons B, Glenn WK. Oncogenic viruses and breast cancer: mouse mammary tumor virus (MMTV), bovine leukemia virus (BLV), human papilloma virus (HPV), and Epstein-Barr virus (EBV). *Front Oncol*. 2018;8:1.
68. Ohba K, Ichiyama K, Yajima M, 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*. 2014;9:e97787.
69. Handa O, Naito Y, Yoshikawa T. Helicobacter pylori: a ROS-inducing bacterial species in the stomach. *Inflamm Res*. 2010;59:997-1003.
70. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. *Nat Rev Cancer*. 2003;3:276-285.
71. Lawson JS, Glenn WK, Whitaker NJ. Human papilloma viruses and breast cancer—assessment of causality. *Front Oncol*. 2016;6:207.
72. Yeo-Teh NSL, Ito Y, Jha S. High-risk human papillomaviral oncogenes E6 and E7 target key cellular pathways to achieve oncogenesis. *Int J Mol Sci*. 2018;19:1706.
73. Tanaka TI, Alawi F. Human papillomavirus and oropharyngeal cancer. *Dent Clin North Am*. 2018;62:111-120.
74. Frega A, Lorenzon L, Bononi M, et al. Evaluation of E6 and E7 mRNA expression in HPV DNA positive breast cancer. *Eur J Gynaecol Oncol*. 2012;33:164-167.
75. Rambout L, Hopkins L, Hutton B, Fergusson D. Prophylactic vaccination against human papillomavirus infection and disease in women: a systematic review of randomized controlled trials. *CMAJ*. 2007;177:469-479.
76. Ban KA, Godellas CV. Epidemiology of breast cancer. *Surg Oncol Clin N Am*. 2014;23:409-422.
77. Dal Maso L, Zucchetto A, Talamini R, et al.; and Prospective Analysis of Case-Control Studies on Environmental Factors and Health (PACE) Study Group. Effect of obesity and other lifestyle factors on mortality in women with breast cancer. *Int J Cancer*. 2008;123:2188-2194.
78. Johnson KC, Hu J, Mao Y; and Canadian Cancer Registries Epidemiology Research Group. Passive and active smoking and breast cancer risk in Canada, 1994-97. *Cancer Causes Control*. 2000;11:211-221.

79. Wells AJ. Lung cancer from passive smoking at work. *Am J Public Health*. 1998;88:1025-1029.
80. Krajinovic M, Ghadirian P, Richer C, et al. Genetic susceptibility to breast cancer in French-Canadians: role of carcinogen-metabolizing enzymes and gene-environment interactions. *Int J Cancer*. 2001;92:220-225.
81. Friedenreich CM, Neilson HK, Lynch BM. State of the epidemiological evidence on physical activity and cancer prevention. *Eur J Cancer*. 2010;46:2593-2604.
82. Dieterich M, Stubert J, Reimer T, Erickson N, Berling A. Influence of lifestyle factors on breast cancer risk. *Breast Care (Basel)*. 2014;9:407-414.
83. Barnard ME, Boeke CE, Tamimi RM. Established breast cancer risk factors and risk of intrinsic tumor subtypes. *Biochim Biophys Acta*. 2015;1856:73-85.
84. Kotsopoulos J. BRCA mutations and breast cancer prevention. *Cancers (Basel)*. 2018;10:524.
85. Armstrong N, Ryder S, Forbes C, Ross J, Quek RG. A systematic review of the international prevalence of BRCA mutation in breast cancer. *Clin Epidemiol*. 2019;11:543-561.
86. Mavaddat N, Peock S, Frost D, et al. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. *J Natl Cancer Inst*. 2013;105:812-822.
87. Gudmundsdottir K, Ashworth A. The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. *Oncogene*. 2006;25:5864-5874.
88. Navaraj A, Mori T, El-Deiry WS. Cooperation between BRCA1 and p53 in repair of cyclobutane pyrimidine dimers. *Cancer Biol Ther*. 2005;4:1409-1414.
89. Brewer HR, Jones ME, Schoemaker MJ, Ashworth A, Swerdlow AJ. Family history and risk of breast cancer: an analysis accounting for family structure. *Breast Cancer Res Treat*. 2017;165:193-200.
90. Morch LS, Skovlund CW, Hannaford PC, Iversen L, Fielding S, Lidegaard O. Contemporary hormonal contraception and the risk of breast cancer. *N Engl J Med*. 2017;377:2228-2239.
91. Albuquerque RC, Baltar VT, Marchioni DM. Breast cancer and dietary patterns: a systematic review. *Nutr Rev*. 2014;72:1-17.