

## Prevalence and Correlation of Human Papilloma Virus and its Types with Prognostic Markers in Patients with Invasive Ductal Carcinoma of the Breast in Kuwait

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### انتشار وعلاقة فيروس الورم البشري الحلبي وأنواعه بمؤشرات الورم المعروفة بين مرضى أورام الثدي في الكويت

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**الملخص:** تهدف الدراسة إلى توثيق العلاقة بين فيروس الورم الحلبي البشري وأنواعه بأورام الثدي في عينات من أنسجة الثدي المصابة من النساء الكويتيات وعلاقته بمؤشرات التنبؤ بالورم المعروفة. الطريقة: تمت دراسة 144 عينة من الأنسجة المحفوظة من حالات سرطان الثدي الأقينية الغازية وأخذ البيانات المتاحة إكلينيكية وباثولوجية وتتضمن العمر، وحجم الورم، وانتشار المرض في العقد الليمفاوية، وحالة مستقبلات الإستروجين والبروجسترون وعامل نمو البشرة. وقد تم توثيق تكرار تواجد فيروس الورم الحلبي البشري باستخدام الكيمياء الهستولوجية المناعية ووسيلة التهجين اللونية. تم توثيق الأنواع الفرعية من فيروس الورم الحلبي البشري باستخدام المسابر لفيروس الورم الحلبي البشري CISH وقورنت نتائج CISH و IHC وارتباط فيروس الورم الحلبي البشري مع مؤشرات التنبؤ بالورم. النتائج: كان انتشار فيروس الورم الحلبي البشري حسب CISH و IHC من الحالات (35.4%) و 24 (16.7%) حالة تواترا. كانت الحساسية للكشف عن فيروس الورم الحلبي البشري باستخدام CISH 37.3%، والخصوصية كانت 94.6%. وجد أن المقارنة الإحصائية للحساسية والخصوصية بين CISH و IHC في الكشف عن فيروس الورم الحلبي البشري يعتد بها إحصائيا ( $P < 0.001$ ) باستخدام اختبار فيشر (HPV-CISH) وجدت في 51 من الحالات ومزيج من فيروس الورم الحلبي البشري 18، 6، 11، 16، وجدت في حالتين (3.9%): ومزيج فيروس الورم الحلبي البشري 33، 31، 11، 6، في 7 من الحالات (13.7%). فيروس الورم الحلبي البشري من الثلاث أنواع 18، 16، 11، 6، وكذلك 33، 13، كانت موجودة في حالتين (3.9%). وكان مدى انتشار HPV-CISH في الكويتيين وغير الكويتيين (52.9%) و 27 (37.2%)، على التوالي. لم تلاحظ أية رابطة مع مؤشرات الورم. الخلاصة: كان تواتر فيروس الورم الحلبي البشري في حالات سرطان الثدي في الكويت 35.4% (CISH) ومن هؤلاء 52.9% من الكويتيين ممن وجد عندهم فيروس الورم الحلبي البشري منخفض وعالي الخطر.

**مفتاح الكلمات:** فيروس الورم الحلبي؛ سرطان الثدي؛ الكيمياء الهستولوجية المناعية؛ التهجين الموضعي؛ الكويت.

**ABSTRACT: Objectives:** This study aimed to document the association of human papilloma virus (HPV) and its types in breast carcinoma tissues in Kuwaiti women, and correlate this with known prognostic markers. **Methods:** The clinicopathological data of archived tissue from 144 cases of invasive ductal breast carcinoma were studied (age, histological grade, size of tumour, lymph node metastases, oestrogen/progesterone receptors and human epidermal growth factor receptor 2 status). HPV frequency was documented using immunohistochemistry (IHC) and chromogenic in-situ hybridisation (CISH). HPV types were documented by CISH using HPV probes. CISH and IHC techniques were compared and HPV correlated with prognostic parameters. **Results:** The HPV prevalence as determined by CISH and IHC was 51 (35.4%) and 24 (16.7%) cases, respectively. The sensitivity of HPV by IHC was 37.3% and specificity was 94.6%. The sensitivity and specificity of HPV-CISH compared to HPV-IHC was statistically significant ( $P < 0.001$ ). HPV-CISH was seen in 51 cases. A combination of HPV 6 and 11, and 16 and 18 was seen in 2 (3.9%) cases, and a combination of HPV 6, 11, 31 and 33 was seen in 7 (13.7%) cases. All three HPV probes: 6 and 11, 16 and 18, as well as 31 and 33 were present in 2 (3.9%) cases. The prevalence of HPV-CISH in the Kuwaiti and non-Kuwaiti populations was 27 (52.9%) and 19 (37.2%), respectively. No correlation was observed with the prognostic parameters. **Conclusion:** The frequency of HPV in breast carcinoma cases in Kuwait was 35.4% (CISH). Of those, 52.9% were Kuwaitis in whom both low- and high-risk HPV types were detected.

**Keywords:** Human Papillomavirus; Breast Neoplasm; Immunohistochemistry; In Situ Hybridization; Kuwait.

**ADVANCES IN KNOWLEDGE**

- Reports on the distribution of human papilloma virus (HPV) infection in breast cancer patients are limited and highly controversial.
- This manuscript describes the prevalence of HPV in breast carcinoma cases in Kuwait and the distribution of the low risk (HPV 6 and 11) high risk (HPV 16 and 18) and intermediate risk (HPV 31 and 33) types in Kuwaiti and non-Kuwaiti women. Previous to this study, HPV and its subtypes in breast carcinoma in the Middle East had only been described in Turkey and Syria.

**APPLICATIONS TO PATIENT CARE**

- Molecular and epidemiological studies have shown that persistent infection with high-risk HPV is an important risk factor for cervical cancer. The causal role of HPV in the development of breast carcinoma remains controversial. The association of HPV and breast carcinoma, the commonest cancer in Kuwait, has not been studied before.
- Very few studies are available which correlate the presence of HPV with prognostic parameters in cases of breast carcinoma.
- The frequency of HPV-positivity in breast carcinoma could help guide the HPV vaccination policy.

**B**REAST CANCER IS THE MOST COMMON form of cancer among women in Kuwait. In 2001, the breast cancer age-standardised incidence rate (ASR) was 36 among Kuwaiti and 39.5 among non-Kuwaiti female residents.<sup>1</sup> Since then, its incidence has increased rapidly. Al-Shaibani *et al.* noticed that in Kuwait the majority of breast cancer patients were 40–49 years of age, and increased breast carcinoma risk was correlated with the menopause, recent hormone replacement therapy and family history.<sup>2</sup>

Well-known breast cancer risk factors associated with the development of breast cancer are age, familial history, hormonal intake, early *menarche*, late menopause, multiparity, first pregnancy at >30 years, obesity and personal history. Nevertheless, in 50–80% of cases, known risk factors have not been identified which has generated an interest in identifying new factors related to this cancer.<sup>2,3</sup> The three most studied viruses found to cause breast cancer in humans are mouse mammary tumour virus (MMTV), the Epstein-Barr virus (EBV) and the human papilloma virus (HPV), with MMTV and EBV occurring in up to 37% and 50% of breast carcinoma cases, respectively.<sup>4</sup> HPV is accepted as carcinogenic in human cervical, anogenital, and head and neck cancers. Molecular and epidemiological studies have shown that a persistent infection with high-risk types of HPV is the most important risk factor for both cervical cancer and its precursors.<sup>5</sup> Recently, the possibility of HPV being aetiologically-related to breast cancer has been debated. However, the causal role of HPV infection in the development of breast carcinoma remains controversial.<sup>6</sup>

Reports on the distribution of HPV infection in breast cancer are not only limited but also highly controversial.<sup>7</sup> Several authors have reported the

absence of HPV deoxyribonucleic acid (DNA) in breast cancer and suggested that it is improbable that integrated HPV is aetiologically-associated with the development of breast carcinoma.<sup>8–11</sup> However, a moderate frequency of 20–48% HPV infection and as high as 60–85% of HPV infection in breast carcinomas has been reported.<sup>7</sup>

The suspicion that HPV may also play a role in human breast cancer is based on the identification of HPV in human breast tumours and the immortalisation of normal human breast cells by HPV 16 and 18.<sup>12</sup> Previous studies have demonstrated the presence of HPV types 16, 18 and 33 in breast cancer specimens from diverse populations around the world: Italy, Norway, China, Japan, USA, Austria, Brazil, Australia, Taiwan, Turkey, Greece, Korea, Mexico, Hungary and Syria.<sup>6,13–23</sup> The prevalence of HPV-positive breast cancer in these studies was reported to vary from 4% in Mexican to 86% in American women.<sup>13</sup> In all the studies, high-risk HPV was found in tumour tissue only and not in the surrounding normal tissue, with the exception of the study from Turkey where the virus was also detected in normal tissue but at a lower expression than in the cancerous tissue.<sup>20</sup>

Most of the studies have used standard and nested polymerase chain reaction (PCR) techniques. The disadvantage of this technique is its inability to confirm whether or not a positive reaction is in the mammary epithelial cells.<sup>24</sup> Very few studies have used *in-situ* molecular methods for the demonstration of HPV, and in three of them a comparison between solution PCR and *in-situ* hybridisation methods for the same set of specimens was made and identified oncogenic HPV in mammary epithelium of 12% of the collective total *versus* 22% using standard or nested PCR.<sup>18,13–15,22,24</sup> *In-situ* methods have the potential to establish

more accurately the frequency of HPV in breast cancer tissue because of their ability to localise the signals in individual cells which can be categorised as benign or malignant.

The association of breast carcinoma with HPV has yet not been studied in Kuwait. The aim of this study was to document the association of HPV and its types (6, 11, 16, 18, 31 and 33) with breast carcinoma cases in Kuwait, both in Kuwaitis and non-Kuwaitis. The study also aimed to identify the correlation of HPV and its types with known prognostic and predictive markers, namely age, histological grade, tumour size, lymph node metastases, oestrogen (ER) and progesterone (PR) receptor expression and human epidermal growth factor receptor 2 (HER2/neu) amplification status. The secondary aim of this study was to compare HPV detection with immunohistochemistry (IHC) *versus* chromogenic *in-situ* hybridisation (CISH).

## Methods

Archived formalin-fixed paraffin-embedded tissue from 144 cases of invasive ductal breast carcinoma, from the Department of Pathology at Hussain Makki Al-Juma Center for Specialized Surgery (HMJCSS) in Kuwait, were studied over a 3-year period (2009 to 2011). The study was performed according to the guidelines of the local ethics committee which conforms to the Helsinki Declaration. Cases were selected in which the following clinicopathological data were available: age of the patient at the time of diagnosis, histological grade and size of tumour, lymph node status, ER, PR and HER2/neu status.

Histology slides were reviewed and histological grading of the tumour was done using the modified Scarff-Bloom-Richardson grading system.<sup>25</sup> The size of the tumour, the number of lymph nodes involved, and the ER, PR and HER2/neu status were reviewed for uniformity.

Paraffin-embedded tissue sections of the 144 breast cancer cases were analysed for HPV status by IHC and CISH. For IHC, the prefixed unstained tissue sections were used to demonstrate HPV by the standard IHC methodology using antibody from Dako (Carpinteria, California, USA), dilution of antibody (1:100) and the detection system, Dako EnVision, FLEX/HRP, High pH (Dako Global R & D, Glostrup, Denmark). Positive and negative controls were run with each batch. HPV staining

was considered positive when there was distinct nuclear staining.

For CISH, a CISH implementation kit AP-NBT/BCIP (Zytovision, Bremerhaven, Germany) was used. In CISH, all the cases were first screened for HPV status using the Zytovision biotin labelled HPV screening probe that allows the simultaneous screening of 7 HPV types (6, 11, 16, 18, 31, 33 and 35) without determination of specific HPV types. Positive and negative controls were run with each batch. HPV staining was considered positive when there was distinct nuclear staining.

For HPV typing, all positive cases were tested for the specific HPV types, namely the low-risk: 6 and 11; intermediate-risk: 31 and 33, and high-risk: 16 and 18, types using the specific biotin labelled 6 and 11, 31 and 33, and 16 and 18 Zytofast HPV DNA probes by the CISH technique.

The HPV types detected were correlated with the prognostic markers, namely age, histological grade, tumour size, lymph node metastases, ER, PR and HER2/neu. Data were analysed using Statistical Package for Social Sciences (SPSS), Version 17 (IBM, Corp., Chicago, Illinois, USA). Univariate analysis was used to compare the clinical and pathological features with the HPV types. Fisher's exact test was used to find the association between the two techniques of CISH and immunostaining. For all analyses,  $P < 0.05$  was considered statistically significant.

## Results

Of 144 cases of invasive ductal breast carcinoma, 67 (46.5%) were Kuwaitis and 66 (45.9%) were non-Kuwaitis, comprising both non-Kuwaiti Arabs and non-Arabs including Asians and Caucasians. In 11 cases (7.6%), information on nationality was not available. The age range was 27–84 years with a median age of 52. There were 42 cases (29.2%) in the fourth and fifth decades of life. A histological grade of 1, 2 and 3 was documented in 5 (3.5%), 47 (32.6%) and 86 (59.7%) of the cases, respectively. Of the 144 cases, tumour sizes were documented in 139 (96.5%) cases and the size was less than 2 cm, 2–5 cm and 5 cm or more in 19 (13.2%), 98 (68.1%) and 22 (15.3%) cases, respectively. Lymph nodes were involved in 75 (52.1%) cases but not involved in 56 (38.9%) cases, and data were not available in 13 (9%) cases. For the 144 cases of breast carcinoma,

**Table 1:** Distribution by nationality of human papilloma virus (HPV) types in breast cancer cases testing positive for HPV by chromogenic *in-situ* hybridisation

HPV types	HPV status as determined by CISH				Total number of cases n = 51
	Nationality			N/A n = 5	
	Kuwaiti n = 27	Non-Kuwaiti n = 19			
		Arabs n = 9	Non-Arabs n = 10		
6, 11	11 [40.7]	3 [33.3]	5 [50]	4 [80]	23 [45.2]
16, 18	2 [7.4]	2 [22.3]	2 [20]	-	6 [11.7]
31, 33	5 [18.5]	3 [33.3]	2 [20]	1 [20]	11 [21.6]
6, 11 & 16, 18	2 [7.4]	0	0	0	2 [3.9]
6, 11 & 31, 33	6 [22.3]	1 [11.1]	0	0	7 [13.7]
6, 11, 16, 18 & 31, 33	1 [3.7]	0	1 [10]	-	2 [3.9]

HPV = human papilloma virus; CISH = chromogenic *in situ* hybridisation; N/A = not applicable; [ ] = percentage within HPV types.

ER status was available for 137 (95.1%) cases; 103 (75.2%) cases were ER-positive and 34 (24.8%) cases ER-negative. The PR status was available in 134 cases, of which 88 (61.1%) cases were PR-positive and 46 (31.9%) cases were PR-negative. HER2/neu, determined by IHC, was available in 135 cases of which 21 (15.6%) cases were positive and 114 (84.4%) cases were negative.

HPV was detected by CISH in 51 (35.4%) cases and by IHC in 24 (16.7%). The correlation of the HPV status in breast carcinoma cases by CISH and IHC. A good correlation was seen in 19 (13.2%) and 88 (61.1%) cases, being positive and negative, respectively, for HPV by both methods. The sensitivity and specificity of HPV-IHC was 37.3% and 95.6%. The sensitivity and specificity of HPV-CISH was 79.2% and 73.3%. This association was found to be significant by Fisher's exact test ( $P < 0.001$ ).

HPV typing performed on 51 cases found positive by CISH were HPV 6 and 11 positive in 23 (45.1%) cases, HPV 16 and 18 positive in 6 (11.8%) cases, and HPV 31 and 33 positive in 11 (21.6%) cases. A combination of HPV 6 and 11, and 16

and 18 was seen in 2 (3.9%); and a combination of HPV 6 and 11, and 31 and 33 was seen in 7 (13.7%) cases. The distribution by nationality of HPV types in breast carcinoma cases determined positive for HPV-CISH is shown in Table 1.

In the Kuwaiti population, HPV 6 and 11 were present alone in 11 of 27 (40.7%) cases. In 9 cases, HPV 6 and 11 were found in combination with HPV 16 and 18 (2 cases, 7.4%) and HPV 31 and 33 (6 cases, 22.3%). All three types were detected in one Kuwaiti woman. High risk HPV 16 and 18 were detected in 2 (7.4%) Kuwaiti women while the intermediate-risk HPV 31 and 33 alone were detected in 5 (18.5%) Kuwaiti women [Table 1].

The distribution of HPV types and the age groups of the cases with breast carcinoma are shown in Table 2. The low-risk HPV types 6 and 11 were seen in all decades of life. The prevalence was higher in patients greater than 30 years of age. The high-risk HPV types 16 and 18, and intermediate-risk HPV 31 and 33 were detected in the fifth, sixth and seventh decades of life.

The correlation of HPV types determined by CISH according to age, histological grade, tumour size, lymph node metastases, ER, PR and HER2/neu status is shown in Table 3. Univariate analysis between the HPV status determined by CISH was done with the histological grade of the tumour, size of the tumour, lymph node metastases, ER, PR and HER2/neu. No statistically significant differences were observed. Linear-by-linear association did not show significant correlation with the prognostic parameters although a trend was observed in that HPV types 16 and 18 were associated with lymphnodal metastases; however, the numbers were too limited to come to a definitive conclusion.

## Discussion

This study documented HPV and the various types (6, 11, 16, 18, 31 and 33) in Kuwaiti and non-Kuwaiti women. We found HPV determined by CISH in 51 (35.41%) cases of invasive ductal carcinoma of the breast.

The reported prevalence of HPV in breast tissue between 1992 and 2012 varies between 4% in Mexico to 86% in the USA.<sup>26</sup> HPV prevalence as determined by the PCR method was seen in 61.1% cases in Syria.<sup>23</sup> Simoes *et al.* conducted a comprehensive systematic review of studies

**Table 2:** Distribution of age according to human papilloma virus (HPV) types in breast cancer cases (n = 51)

Age group in years	Total number of HPV-positive cases	HPV types					
		6 & 11	16 & 18	31 & 33	6 & 11 + 16 & 18	6 & 11 + 31 & 33	6 & 11 + 16 & 18 + 31 & 33
n (% within HPV types); n [% within age groups]							
21–30	2 [3.9]	2 [8.7]	-	-	-	-	-
31–40	8 [15.7]	3 [13]	1 [16.7]	3 [27.3]	-	-	1 [50]
41–50	8 [15.7]	4 [17.4]	-	1 [9.1]	1 [50]	2 [28.6]	-
51–60	15 [29.4]	8 [34.8]	2 [33.3]	2 [18.2]	1 [50]	1 [14.3]	1 [50]
61–70	12 [23.5]	4 [17.4]	2 [33.3]	3 [27.3]	-	3 [42.8]	-
≥71	6 [11.8]	2 [8.7]	1 [16.7]	2 [18.2]	-	1 [14.3]	-
<b>Total</b>	<b>51</b>	<b>23 (45.2)</b>	<b>6 (11.7)</b>	<b>11 (21.6)</b>	<b>2 (3.9)</b>	<b>7 (13.7)</b>	<b>2 (3.9)</b>

addressing worldwide HPV prevalence rates.<sup>27</sup> In this meta-analysis of 29 studies comprising of 2,211 samples, the overall prevalence of HPV in breast carcinoma was 23% with great variation worldwide, ranging from 13.4–42.8%.<sup>27</sup> This large worldwide variability could be related to methodology. Most studies utilise the PCR technique, which is more sensitive but less specific as compared to *in-situ* hybridisation techniques, which show the virus location.<sup>24</sup>

The HPV types found in our study, as determined by CISH, were HPV 6 and 11 (45.1%); HPV 16 and 18 (11.8%); and HPV 31 and 33 (21.6%). More than one HPV type was seen in 11 cases (21.6%). A combination of HPV 6 and 11 and HPV 16 and 18 was seen in two cases (3.9%) while

a combination of HPV 6 and 11 and HPV 31 and 33 was seen in 7 cases (13.7%). In two cases (3.9%), all HPV types examined were identified [Table 1]. In the Kuwaiti population, the distribution of HPV types 6 and 11; 16 and 18, and 31 and 33 were 40.7%, 7.4% and 18.5%, respectively, while in non-Kuwaitis it was 42.1%, 21.1% and 26.2%, respectively [Table 1]. A combination of HPV 6 and 11, and 16 and 18 was seen in 7.4% of the Kuwaiti women while a combination of HPV 6 and 11, and 31 and 33 was found in 22.3%. All three HPV types were seen in one woman. In the non-Kuwaitis, only one (5.3%) case had a combination of HPV 6, 11, 31, 33 [Table 1]. The high- and intermediate-risk HPV types were found in 16 of the 27 (59.3%) Kuwaiti women and 11 of the 19 (47.9%) non-Kuwaitis

**Table 3:** Distribution of human papilloma virus types in breast cancer cases with prognostic parameters (n = 51)

HPV types	HPV-positive cases n = 51	*Tumour size (cm) <2/2–5/>5 n = 50	**Histological grade 1/2/3 n = 51	†Lymph node metastasis n = 48	‡ER (IHC) n = 50	§PR (IHC) n = 48	¶HER2/neu (IHC) n = 49
6, 11, n (%)	23 (45.1)	4/16/3	0/7/16	12 (52.2)	17 (73.9)	15 (65.2)	4 (17.4)
16, 18, n (%)	6 (11.8)	0/4/2	1/3/2	6 (100)	4 (80)	2 (66.7)	0 (0)
31, 33, n (%)	11 (21.6)	1/8/2	0/6/5	7 (77.8)	10 (90.9)	10 (90.9)	2 (8.7)
6, 11 & 16, 18, n (%)	2 (3.9)	0/1/1	0/0/2	2 (100)	0 (0)	0 (0)	1 (50)
6, 11 & 31, 33, n (%)	7 (13.7)	1/4/1	0/3/4	3 (50)	4 (57.1)	3 (42.9)	1 (14.3)
6, 11 16, 18 & 31, 33, n (%)	2 (3.9)	0/2/0	0/0/2	2 (100)	1 (50)	1 (50)	0 (0)
<b>Total</b>	<b>51</b>	<b>6/35/9</b>	<b>1/19/31</b>	<b>32(62.7)</b>	<b>36 (72)</b>	<b>31 (64.6)</b>	<b>8 (16.3)</b>

\*P = 0.895, \*\*P = 0.225, †P = 0.134, ‡P = 0.129, §P = 0.125, ¶P = 0.400.

HPV = human papilloma virus; ER = oestrogen receptors; IHC = immunohistochemistry; pos = positive; neg = negative; PR = progesterone receptors; HER2/neu = human epidermal growth factor receptor 2.

[Table 1]. This is the first time that the association of HPV types with breast carcinoma has been documented in Kuwaitis and non-Kuwaitis in Kuwait. We have no explanation for these differences, and more research work needs to be undertaken.

Di Lonardo *et al.* were the first to report HPV 16 DNA using PCR in 29.4% of 40 breast cancer specimens.<sup>14</sup> Using PCR, HPV types 11, 16 and 18 have been reported with increasing frequency from women living in USA and Brazil, while HPV type 18 was present in the majority of Australian women.<sup>12,16,17</sup> HPV 33 was seen in 34.1% of breast carcinoma cases in Japan.<sup>28</sup> Studies from the Middle East reveal that HPV types 18, 33 and 35 were present in cancerous and normal breast tissue in Turkish women.<sup>20</sup> In Syria, a prevalence of HPV 16 (9%), HPV 33 (56%) and HPV 35 (37%) was observed.<sup>23</sup> In 24 (34.78%) women, more than one HPV type was detected.<sup>23</sup> Only two studies have been reported where *in-situ* PCR (IS-PCR), which involves amplification to detect HPV in breast tissue, was used.<sup>13,24</sup>

Very few studies have tried to correlate the presence of HPV in breast carcinoma cases with other prognostic markers.<sup>12,29,30</sup> In our study, 51 HPV DNA-positive cases, as determined by CISH, were correlated with the age of the patients, histological grades, tumour sizes, lymph nodal metastases, ER, PR and HER2/neu expression. We did not find any significant correlation as our numbers were limited; thus, more work needs to be done in this regard. Kan *et al.* using PCR for detecting HPV, did not find any significant correlation between grade of tumour, mortality of patients, ER, PR, ERB-B2, *p53* expression and the presence of *p53* mutations.<sup>12</sup> Cantu de Leon, using PCR for HPV determination, found that the greater the tumour size the greater the probability of finding viral DNA in the tumour, but not in tumours larger than 4 cm where the viral DNA appeared to be lost.<sup>29</sup> However, there was no correlation with the histological grade, ER, PR or clinical stage.<sup>29</sup> Mou *et al.*, in a retrospective study from China of 62 breast cancer patients tested for HPV status by nested PCR, did not find any significant correlation between the presence of HPV and patient age, tumour size, metastases, or ER and PR status.<sup>30</sup>

There are several limitations to this study apart from the limited sample size. While the nucleic acid

is degraded in archival paraffin-embedded tissue, CISH should still be considered a relevant technique when used on such material. Although IHC is not as sensitive a technique as PCR, we did not perform PCR in this study as *in-situ* methods have the advantage of detecting HPV in individual cells, which can be categorised as benign or malignant under conventional light microscopy.

## Conclusion

The frequency of HPV in breast carcinoma cases in Kuwait was 35.4% when determined by the CISH method. The CISH method was useful in detecting HPV in malignant cells and should be considered as part of a standard investigation. Of the 51 HPV-positive cases, 27 (52.9%) were Kuwaitis. Both low-risk and high-risk HPV types, along with a combination of HPV types, were detected in the Kuwaiti population. No correlation was observed with the prognostic parameters.

## DECLARATION

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