Detection Human Papilloma Virus genotype (16/18) in Iraqi Women Patients with endometrial carcinoma by using Chromogen - Insitu Hybridization (CISH) Technique


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Abstract:

Aim: To investigate the possible association between human papillomavirus (HPV) and endometrial carcinoma. Does HPV play any role in the initiation or prognosis of endometrial adenocarcinomas among Iraqi women patients.

Method: To determine the relationship between HPV and endometrial carcinoma, a retrospective study was done. The study was carried out on 30 patients with histopathologically confirmed primary endometrial adenocarcinoma, samples were collected from each patients, as well as twenty (20) endometrial tissues from control individuals with no cancer. Chromogen In situ hybridization (CISH) was used to detect HPV DNA genotype 16/18 in endometrial tissues.

Results: HPV 16/18 was detected in 19/30 (63.3%) tumor sample while negative cases were 11/30 (36.7%) of tumor sample and HPV 16/18 was detected in 5/20(25.0%) of endometrial control group while negative in 15/20 (75.0%) cases of endometrial control group.

Conclusion: Our results revealed that high risk of HPV(16 / 18 ) being the most frequent type in Iraqi women patients with endometrial adenocarcinoma. Association between HPV infection and endometrial adenocarcinoma was observed and this research considered the first study which evaluated score, intensity and pattern of replication system of HPV by using CISH technique which reflected role of HPV in endometrial carcinogenesis.

Key words: endometrial cancer, HPV, chromogen in situ hybridization

Introduction:

Papillomaviruses are a highly diverse group of small double-stranded DNA viruses, which infect cutaneous and mucosal epithelia and causes a variety of lesions ranging from benign tumors (plantar, flat and common warts, genital condylomas and papillomas) to cervical neoplasia and cancer(1). Currently 120 different HPV genotypes that infect humans have been classified and are allocated a type number according to the order of discovery (2). Genital tract HPV types are classified by their relative malignant potential into low risk include (6, 11, 42, 43, 44, 55, 81, 83) and high risk types include (16,18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) (3, 4).

Persistent infection with high- subtype of HPV is associated with the development of cervical cancer (5). HPV16 is the predominant HPV type of squamous cell cervical carcinomas and HPV18 predominates within adenocarcinomas (6). The HPV infected epithelial cells and after integration with host DNA, the production of oncoprotein mainly, E6 and E7 disrupts natural tumor suppressor pathway and is required for proliferation of cervical cells (7).

Human papilloma virus also believed to play a role in other human cancer such as head and neck tumor (8), skin cancer (9), lung cancer (10). Anal cancer (11, 12), oropharyngeal squamous cell carcinoma (13), Breast cancer (14).

Endometrial carcinoma is the sixth most common cancer in women worldwide.fourteenth most common can-
Materials and Methods:

A- Study Design:
The study was designed as a retrospective study. Specimens belong to the period from June 2010 until November 2013.

B- Study groups
Endometrial tissues were obtained from thirty patients with endometrial cancer. From each patient two blocks were taken formalin fixed, paraffin embedded endometrial carcinoma and twenty cases from individual endometrial tissue were proved to be free from any significant pathological changes were considered as a negative control groups for this study. Tumor, control blocks were collected from the archives of histopathology laboratories of Teaching Laboratories of the Medical City/Baghdad and Teaching Alkarmaa hospital, Teaching AYarmouk hospital, AlWiya hospital for delivery as well as many private laboratories.

The diagnosis of these tissue blocks were based on the obtained pathologicological records of these cases from hospital files as well as histopathological laboratories records. A confirmatory histopathological re-examination of each obtained tissue blocks was done. Four μm thick sections were made and stuck on positively charged slides. Chromogenic in Situ Hybridization (CISH)/Detection system (Zytovisions GmbH. Bremerhaven, Germany) used to target DNA sequences using Digoxigenin – labelled long DNA probe for HPV types 16/18. Methods was conducted according to the instructions of manufacturing companies leaflet. Positive control reactions were performed by replacing the probe with Biotinylated and Digoxigenin housekeeping gene probe. For the negative control, all reagents were added except the probe. Proper use of this hybridization/detection system gave an brown blue signal at the specific site of the hybridization probe in positive test tissue.

C- Methods
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D- Evaluation of the In Situ Hybridization Signal
Quantification of different molecular markers in situ hybridization signal was evaluated under light microscopy and the counting of positive cells was performed at X1000. Chromogen In situ hybridization was given intensity and percentage scores, based on intensity of positive signals and number of signals, respectively.

The intensity score included low, moderate, and high intensity of reaction. Positive cells were counted in ten different fields of 100 cells for each sample and the average of positive cells of the ten fields was determined assigning cases to one of the three following percentage score categories: Score(1) = 1- 25%, Score(2) = 26-50%, Score(3) >50% (10). Chi-square test was used to detect the significances between variables of our study. All the statistical analysis was done by SPSS program (version-18). P-value was considered significant when < 0.05.

Results:-

Specimens collected in this study were related to endometrial cancer patients whom ages were ranged from twenty five to seventy five years. The mean age of the patients with endometrial carcinoma was (56.9± 11.73) while endometrial control groups were ranged from twenty six to fifty eight years. The mean age of the endometrial control groups was (45.6± 8.34). Table (1).

The distribution of endometrial carcinoma cases according to histopathological grades was evaluated in the present study. It was found that Moderately differentiated grade in endometrial carcinoma cases constituted of 60% (18 out of total 30 cases), whereas cases with well and poorly differentiated grades constituted of 36% (11out of 30 cases) and (3.3%) (1 out of 30 cases) respectively. Statistical analysis of grading show no significant difference (p>0.05)(Table 2).

Regarding endometrial cancer group, Total percentage of positive HPV16/18 – CISH detection was 63.3% ((19 out of 30 cases were found to be positive for HPV16/18-CISH detection. Whereas in the control group it was 25.0% (5 out of 20 cases). Statistically, significant difference (p<0.05) was found on comparing the percentage of HPV16/18 among the study groups (Table 3). In the endometrial carcinoma group, The highest percentage of HPV 16/18 score signaling was 33.3% ( 10 out of 30
cases) found in the low score I, whereas 16.7% (5 out of 30 cases) and 13.3% (4 out of 30 cases) were found within high score III and moderate score II, respectively. In the control group HPV 16/18 DNA was found exclusively in low score I 25% (5 out of 20 cases). Statistically, significant difference (p<0.05) as shown in (Table 4) and (Figure 1) confirmed this data.

The percentage of HPV-infected cells that were evaluated for the intensity of HPV 16/18–CISH reactions was shown in current study. It was found that the higher percentage (30%: 9 cases out of 30) of HPV 16/18 DNA reaction show high signal intensity, whereas (20: 6 cases out of 30) and (13.3%: 4 cases out of 30) of HPV16/18 DNA reaction have moderate and low signal intensity, respectively. While in the endometrial control group, the highest percentage (25%: 5 out of 20) of CISH–reaction has low signal intensity. Statistically significant differences were found in percentage of HPV16/18 DNA in the study groups according to their signal intensity (Table 4).

The pattern of HPV16/18 replication in cancerous and non-cancerous endometrial tissues revealed that there was a significant difference (p<0.05) between the HPV16/18 positive cases and patterns of DNA replication. The HPV16/18 DNA signals were detected in endometrial carcinoma cases with high percentage (26.7%) as dot (integrated DNA) and mixed patterns (episomal and integrated DNA) in (20.0%) of the cases and (16.7%) of the cases as diffused (episomal DNA) and all the positive HPV16/18 DNA signals in endometrial control group were as a dot patterns of replication which represented in (25.0%) of them (Table 5).

Histopathological grades were studied between positive and negative HPV with endometrial carcinomas. The results are shown in (Table 6). It was found the positive results of CISH reactions of HPV16/18 HPV according to tumor grade of endometrial cancer tissues were found 77.8% that have moderate differentiated grade. While the positive results of CISH reactions of HPV16/18 according to tumor grade of endometrial cancer tissues were found 45.5% that have well differentiated grade. Statistically, no significant differences (p > 0.05) on comparing the results of HPV genotypes with tumor grade of cervical cancer (Table 6).

<table>
<thead>
<tr>
<th>The study groups</th>
<th>No.</th>
<th>Mean age</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial carcinoma</td>
<td>30</td>
<td>56.97*</td>
<td>25.00</td>
<td>75.00</td>
</tr>
<tr>
<td>Endometrial control</td>
<td>20</td>
<td>45.60</td>
<td>26.00</td>
<td>60.00</td>
</tr>
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</table>

*p(0.02)

<table>
<thead>
<tr>
<th>Histological grades</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly Differentiated</td>
<td>(3.3%)</td>
</tr>
<tr>
<td>Moderate Differentiated</td>
<td>(60.0%)</td>
</tr>
<tr>
<td>Well Differentiated</td>
<td>(36.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>(100%)</td>
</tr>
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</table>

*P(0.54)

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Total HPV16 CISH -ve results</th>
<th>Positivity according to score numbers</th>
<th>Total HPV16 CISH +ve results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Score I</td>
<td>Score II</td>
<td>Score III</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>(36.7%)</td>
<td>(33.3%)</td>
<td>(13.3%)</td>
</tr>
<tr>
<td>(30 cases)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial control</td>
<td>(75%)</td>
<td>(25.0%)</td>
<td>(0.0%)</td>
</tr>
<tr>
<td>(20 cases)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P(0.022)
Table (4): Frequency distribution of positive HPV16/18 DNA signal intensity among study groups:

<table>
<thead>
<tr>
<th>Study groups</th>
<th>HPV16 CISH -ve results</th>
<th>Positivity according to intensity</th>
<th>Total HPV16 CISH +ve results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>low</td>
<td>Moderate</td>
</tr>
<tr>
<td>Endometrial cancer (30 cases)</td>
<td>(36.7%)</td>
<td>(13.3%)</td>
<td>(20.0%)</td>
</tr>
<tr>
<td>Endometrial control (20 cases)</td>
<td>(75.0%)</td>
<td>(25.0%)</td>
<td>(0.0%)</td>
</tr>
</tbody>
</table>

*P(0.003)

Table (5): Frequency distribution of positive HPV16/18 DNA among study groups according to patterns of replication:

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Total HPV16/18 CISH -ve results</th>
<th>Positivity according to patterns</th>
<th>Total HPV16/18 CISH +ve results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dot</td>
<td>Diffused</td>
</tr>
<tr>
<td>Endometrial cancer (30 cases)</td>
<td>(36.7%)</td>
<td>(26.7%)</td>
<td>(16.7%)</td>
</tr>
<tr>
<td>Endometrial control (20 cases)</td>
<td>(75.0%)</td>
<td>(25.0%)</td>
<td>(0.0%)</td>
</tr>
</tbody>
</table>

* P(0.013)

Table (6): Relationships between HPV16/18 DNA CISH results in correlation with histopathological grades among study groups:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total HPV16/18 CISH RESULTS</th>
<th>No.</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Tumor Grades</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poorly</td>
<td></td>
<td>1</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td>18</td>
<td>14</td>
<td>77.8%</td>
</tr>
<tr>
<td>Well</td>
<td></td>
<td>11</td>
<td>5</td>
<td>45.5%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>30</td>
<td>19</td>
<td>63.3%</td>
</tr>
</tbody>
</table>

* P(0.088)
Figure (1): Chromogen In Situ Hybridization (CISH) For Detection HPV 16/18 in Endometrial Carcinoma Using Digoxigenin-Labeled HPV (16/18) Probe; Stained With DAB- Chromogen (Brown Signal) And Counter Stained By Nuclear Blue Solution (Blue).

A: Endometrial Carcinoma With Negative HPV - CISH Signal (10X)
B: Positive HPV - CISH Reaction With Low Score And Low Intensity Signal (40X)
C: Positive HPV - CISH Reaction With Moderate Score And High Intensity Signal (40X)
D: Positive HPV - CISH Reaction With High Score And High Intensity (40X) Signal (40X (40X))
Discussion:

In the current study, the mean age of patients with endometrial carcinoma was 56.9±11.73. The present results obtained are consistent with those reported worldwide which show that endometrial carcinoma where usually affecting females over fifty years of age. (27,28). In Iraq, study (29) observed that, endometrial cancer are more commonly diagnosed in women older than the age 50 years and those results are compatible with the results of current study.

According to histopathological grades, Our investigation was found that the most prominent grades among endometrial carcinoma cases were Moderate differentiated grade these finding agreement with other studies (30,31). In Iraq, research (32) also reported a similar results in the endometrial carcinoma studied patients and found that (35%) of them were found to be grade I while (40%) and (25%) were in grade II and grade III, respectively. In addition, our results obtained show disagreement with finding of other (33) who found that (77%) of endometrial carcinoma cases were well differentiated grade while (21%) were classified as moderate differentiated grade and (3%) of them were poorly differentiated grade. Perhaps the reason was related to sample size. However, a subset of endometrial cancer which displayed aggressive behavior characterized by high histological grade, as well as by lymph vascular myometrial invasion was associated with poor prognosis in about (25%) of cases (34).

In the present study, HPV DNA 16/18 was detected in 63.3% of endometrial carcinoma cases, this is comparable with others (23,35). In addition, other study (36) was detected HPV16/18 in (87.5%) of the endometrial adenocarcinoma cases by using PCR assays. Other scientific investigations showed results unlike with the results of the present study with non significant role of incidence of HPV16/18 infection in endometrial adenocarcinoma (37,38) and other (40) who failed to detected human papilloma virus family 16 by using CISH and PCR assay in endometrial cases. Those variation in results in between studies may be related to several factors may have been interfered in the different rates of HPV prevalence found in earlier studies and their actual methodology such as, the choice of detection method and different non appraised risk factors (low socioeconomic level, number of sexual partners, interaction with other sexual transmitted infection, smoking habits,different in HPV genotype among different countries).

Other study (40) observed that (8%) of endometrial adenocarcinoma contain HPV DNA type (16,18,13) by PCR technique, he expected to find a large number of positive cases of HPV in women with endometrial carcinoma, but heterogeneity among the groups regards to age, weight, hormonal alteration and histological types may be impact those results.

A number of other studies had not detected HPV DNA in endometrial adenocarcinoma due to the low sensitivity of the technique used, however HPV DNA detection sequence in tissues originally fixed in formaldehyde and embedded in paraffin wax may prove difficult by PCR methods and the results abstained were inconsistent, as both specificity and sensitivity of various HPV PCR primer sets were not affected by intermeshed variation (35). In current study, high percentage of HPV 16/18 signal in the endometrial tissue were found in low score categories (Table 3) this may reflect a low reproduction (replication) rate of the virus in endometrial tissue as well as the low intensity and high intensity of HPV16/18 DNA signals may also reflect viral latency or past infection and continuous replication (42). Our results revealed that positive cases in endometrial control groups were in a low score with low intensity categories (Table 3 & Table 4) This may reflect that HPV16/18 may have precancerous effect and considering problem in the future which could be development of malignancy (43).

It was evident from our data that dot or mixed chromogenic in situ hybridization signals were highest in the HPV positive in endometrial carcinoma cases (Table 5) which revealed that the integration of the viral genome is considered a critical step in the progression to cancer (44). The integration of HPV DNA into the host DNA increases cellular proliferation and the chance of malignancy (45). On the other hand, in the present study, all positive cases in endometrial control groups were found as dot patterns (integrated patterns) as shown in Table (5). This may reflects that HPV16/18 DNA in endometrial carcinoma has been shared in carcinogenic process as co factor and other factor are necessary for progression endometrial HPV infection to cancer like life style, hormonal factors, genetic mutation, environmental factor and immune deficient states.

Our results have been revealed that no correlation was demonstrated between the presence of viral genome with histopathological grading of endometrial carcinoma as shown in (Table 6). These results are in keeping with others findings (35, 37).

In Summary high risk HPV16/18 infection seems to be common in endometrial cancer tissues. Our findings confirm the hypothesis of HPV involvement in the genesis of the endometrial carcinoma detected and can be studied effectively by chromogen in situ hybridization (CISH) technique.
References:


الكشف عن وجود فايروس الورم الحليمي البشري نوع 16/18 بمرضى النساء العراقيات المصابات بسرطان بطانة الرحم بواسطة تقنية التهجين الموضعي المولد

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2 علم الفيروسات الجزئي / الكلية التقنية الصحية والطبية
3 رئيس قسم المختبرات / وزارة الصحة
4 رئيس وحدة النسيج المرضي / مختبرات الصحة العام المركزي / بغداد

الفحص: 

الهدف من الدراسة: لتحري عن العلاقة المحتملة بين فايروس الورم الحليمي البشري وسرطان بطانة الرحم و وهذا الهدف من الدراسة ذو اثر رجعي . وهذه الدراسة شملت 30 مريضة اُكتسبت اصابتها بسرطان بطانة الرحم نسيجيا . العينات تم جمعها من كل المرضى بالإضافة إلى 20 عينة من نسيج من اشخاص غير مصابين بسرطان بطانة الرحم .

النتائج: فايروس الورم الحليمي البشري تم الكشف عن 16/18 من اصل 30 (30% ) عينة مصابة بالسرطان بينما الحالات السالبة كانت 4/18 من اصل 30 (13.3% ) عينة غير مصابة بالسرطان من مجموعة السيطرة بينما كان غير موجود في 5 من اصل 20 (25% ) عينة غير مصابة بالسرطان من مجموعة السيطرة.

الاستنتاجات: نتائج هذه الدراسة تشير الى أن فايروس الورم الحليمي البشري نوع 16/18 البشري هو من الانواع الأكثر انتشارا بالنسبة للنساء العراقيات المصابات بسرطان بطانة الرحم الغدي وقد يفتح تحقيق العلاقة بين اصابة فايروس الورم الحليمي البشري وسرطان بطانة الرحم الغدي و هذا البحث يعتبر أول دراسة تشير الى كمية وكثافة ونمط التكاثر للفايروس باستخدام تقنية التهجين الموضعي المولد للذين تعمل دور فايروس الورم الحليمي البشري بسرطان بطانة الرحم .

الخلاصات:

1. العلاقة المحتملة بين فايروس الورم الحليمي البشري وسرطان بطانة الرحم.
2. استخدام تقنية التهجين الموضعي المولد للذين تعمل دور فايروس الورم الحليمي البشري بسرطان بطانة الرحم .