Human Papilloma Virus in a Sample of Iraqi Women with Normal and Abnormal Pap Smear

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ABSTRACT:

BACKGROUND:
World wide, cervical cancer is one of the most common cause of death from cancer in women, HPV testing have a key role in primary cervical screening and the currently accepted view is that HPV is an essential factor in the causation of the disease.

OBJECTIVE:
The objective of the study is to outline the presence of HPV infection in our community through sample of Iraqi's women who attended Central Health Laboratories with normal and abnormal Pap smear during the period from August 2006 to December 2008.

METHODS:
The data were collected from records of the laboratory which includes the information about patients who were referred to the central health laboratories to do HPV test, these data includes: age of the patients, marital state, years after marriage which represents the age of first sexual acts, also the records includes the smoking state, Pap smear results and lastly the HPV test results. HPV testing was done by PCR method, which initially required DNA extraction by MICROGENO DNA from AB Analitica, then DNA Amplification by PCR, and finally Electrophoresis for reading the UV light and translating it into certain molecular weight by Molecular Weight Markers.

RESULTS:
The percentage of HPV infection was 15.65%, the percentage of positive test was 20% among those aged between 20-30 years old, no viruses had been detected above 50 years old. All the cases that have been examined were married and had 15% infection rate among them. The maximum infection rate 20% was among those who had sexual activity for 5-9 years. 91.6% of the infected women were smokers. 5.5% of the women with negative cytological results was infected, 26.3% of those with ASCUS, 43.3% of those women with CIN I, 45.5% of those with CIN II, and 57% of those with CIN III; the highest infection rates were 100% among patients with micro-invasive cancer.

CONCLUSION:
- High rate of HPV infection is associated with age, smoking and multiple sexual partners (married more than once).
- High frequency of detection of oncogenic HPV infection are associated with increasing grade of cervical lesion.

KEY WORDS: human papilloma virus, cervical cancer, polymerase chain reaction, papanicolaou smear, premalignant disease.

INTRODUCTION:
About 80% of cervical cancer occurring in developing countries\(^\text{(1,2)}\). The overall life time risk is about 5% in parts of Africa, India, and Latin America, compared with 1% in Europe and North America\(^\text{(3)}\). Fortunately cervical cancer has a premalignant phase and among of the criteria for suitable screening programmed is fulfilled\(^\text{(4)}\). The aim of this screening is to detect premalignant cervical disease by means of Papanicolaou smear (Pap smear) and treat the premalignant disease before invasion occurs\(^\text{(5)}\).

The adolescent cervix is believed to be more susceptible to carcinogenic stimuli because of the active process of squamous metaplasia, which occurs within the transformation zone during periods of endocrine changes. This squamous
Human papilloma virus (HPV) infection HPV is among the most common sexually transmitted infections, a member of papilloma family of viruses that is capable of infecting humans. They are called "papilloma virus" because certain types may cause warts, or papillomas, which are benign (non-cancerous) tumors. Usually, HPV infections do not persist, those that do can remain latent for many years. Most women have no clinical evidence of the disease, and the infection is eventually suppressed or eliminated. Other women exhibit low-grade cervical lesions that may regress spontaneously due to natural immune processes. In most women, the infection will clear in 9 to 15 months. When infection with oncogenic HPV persist, especially with high viral loads, have higher likelihood of progressing to precancerous lesion or invasive cancer.

Viral Types Based on DNA sequencing, HPV are a group of more than 100 related viruses. More than 30-40 types can be based from 1 person to another through sexual contact and can infect anogenital region. The majority of known types of HPV cause no symptoms in most people, some types can cause warts (verrucae) which grow on hands and feet, and they are different from those types that cause growths in the throat and genital area, those viruses are called "low-risk viruses" which include types 6, 11, 42, 43 and 44. These are rarely associated with malignancies.

Some types of HPV are associated with certain types of cancer, so those are called "high-risk, oncogenic, or carcinogenic HPVs" e.g. cancer of the cervix, vulva, vagina, or anus in women or cancer of the anus and penis in men. The majority of research focus to date has been upon the high-risk viruses such as type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 or 59.
The high-risk viruses are also the most prevalent in the general population. Specifically, HPV 16 is the dominant cancer-related HPV, accounting for 40 to 70 percent of invasive squamous cell cervical cancers worldwide\(^5\). However, HPV16 genome detection alone may not be a useful predictor of precancerous progression\(^5\). There was a strong highly significant association between detection of high-risk HPV types and increasing severity of cervical intraepithelial neoplasia\(^18\). Like HPV 16, viral types 18, 45, and 56 are also highly oncogenic\(^18\).

**Viral Life Cycle**  
Completion of the viral life cycle takes place only within an intact squamous epithelium. Early genes are expressed in the lower layers and late genes are expressed in the more superficial layer, in synchrony with epithelial differentiation. Viral replication is completed within the most superficial epithelial layers. HPV is a nonlytic virus, and therefore infectiousness depends upon desquamation of infected cells. Intact virus is shed within superficial squamous. Late genes are not strongly expressed in high-grade neoplastic lesions\(^5\). The high-risk oncogenic viruses are potentially dangerous to human host, for they contain two early genes E6 and E7 that can result in vulvar, vaginal, or cervical neoplasias\(^17\). E6 and E7 encode transforming proteins that induce cell proliferation and prevent apoptosis of these infected epithelial cells by binding to the tumor suppressor gene products p53 and tumor suppressor retinoblastoma pRB\(^17\). The "p53" gene is important in repairing DNA and, if damaged, may predispose to malignant changes\(^3,18\).  

Viral integration results in overexpression of the E6 and E7 viral protein products with increase binding and inactivation of their respective tumor suppressor proteins. Removal of these two inhibitory influences on cellular proliferation as thought to provide HPV-infected cells with a growth advantage, ultimately leading to neoplastic transformation\(^17\). The cytological changes of HPV were given the term "koilocytosis"\(^17\).

**Diagnosis**  
A definitive diagnosis can be made only by the direct detection of HPV DNA\(^5\). Commercially available kits are available that will test for the common oncogenic virus subtypes. HPV testing combined with a cervical cytology smear has been approved as a primary screening approach in the patient age 30 years and older. The combination of HPV DNA testing with cytology increases the sensitivity of a single Pap test for high-grade neoplasia from 50 to 85 percent, to nearly 100 percent\(^4,5\).

Direct detection of HPV DNA can be done histologically by either:  
1- nucleic acid amplification via polymerase chain reaction (PCR)\(^5\), PCR has been employed in many clinical observational studies. It has the advantages of being very sensitive and specific in the identification of individual HPV types but more expensive\(^17\).  
2- Currently, the only FDA-approved testing system in the US is the second-generation hybrid capture (HC) system\(^5,17\), which screens five low-risk HPV types and 13 high-risk HPV types and it is less expensive\(^17\). Currently, Hybrid Capture 2 is the most common technique in clinical use. It is a chemiluminescent test that uses a mixture of RNA probes for the detection of 13 oncogenic HPV types. Clinical HPV testing by HC 2 can be carried out by collection of cervical cells using a small brush device or in conjunction with liquid-based cytology. It is primed to register a positive result when there are 5000 or more HPV copies present. The specimen collection is easy and the results are seemingly simple and straightforward\(^5,17\).

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**Figure 1a: Diagram of HPV.**
Study Design:
A retrospective analysis including total number of reported cases of positive testing for HPV genome in the central health laboratories from August 2006 through December 2008.

Study group:
Two-hundred thirty women who were referred to Central Health Laboratory from their local gynecologist (complaining from different gynecological problems such as vaginal discharge, postcoital bleeding or dyspareunia) for either Pap smear or for pap smear and HPV testing between August 2006 and December 2008.

METHOD:
The sample was obtained by laboratory staff, in Central Health Laboratory. Endocervical brush (Medscand) or Ayers spatula was used for obtaining a cytological sample. This sample was transferred to buffer media.

The HPV testing done in 3 basic steps:
1. DNA Extraction.
2. DNA Amplification by PCR.
3. DNA Electrophoresis.

1. The first step DNA extraction from the cells of the tissue specimen by MICROGENO DNA Kits from AB Analitica, with MICROGENO DNA kit method a highly purified genomic DNA is extracted in short time (about 20 min.). It is based on the direct lysis of the sample in the presence of a mixture of cationic detergents. This lysis solution allows the complete solubilization of cellular components such as membranes and proteins. During the first extraction step only the high molecular weight genomic DNA binds to the purification resin. Low molecular weight DNA, RNA and proteins do not bind to the resin, and are eliminated in the following washing step. Purified DNA is resuspended in water and it could be used in PCR. If stored at +4 C, the DNA remains stable for some weeks; if stored at -20 C, it remains stable for at least 1 year.

2. PCR: It can be defined as an in vitro amplification reaction of a specific part of DNA (target sequence) by thermo-stable DNA polymerase. It is a process involving different temperatures, and including three main steps:
   A. Denaturation: at 90 C breaking of the strands of DNA nucleotides, then transforming to:
   B. Annealing of the target DNA which is specific three segment of nucleic acid (the double strand DNA template to be amplified) with "primers" which are two single-strand oligonucleotides, at 35-63 C.
   C. Extension of DNA at 72 C, this process repeated 30-40 times.

3. Electrophoresis for the amplified DNA then using the UV transilluminator for analyzing the results by comparing the size of the amplification products with reference DNA Molecular Weight Marker. According to the color of the UV, the molecular weight of DNA was identified, then HPV positive or negative can be determined.
Statistical analysis:
The data of records described as absolute numbers, mean, standard deviation, and statistical significant was considered whenever the P value (using Pearson Chi-squared test) is < 0.05.

RESULTS:

Figure -3:- The HPV results.

Pie chart showing the number of women with positive HPV tests, 36 case (15.65%) from total 230 case which represented by red color on Pie chart and the blue represents number of women with negative tests for HPV which is 194 case (84.35) from total number of cases enrolled through the previously mentioned period, and the percentage of each.

Table 1- shows 2 characteristics of the data collected from the records: first one, the age distribution of the patients enrolled and, the number and percentage of HPV positive tests in each age group. Patients age ranges between 15-53 years (mean=31.92+_± (SD=8.58), with the larger age group between 20-29 years, 81 cases (35.2%) which have 16 cases (20%) with positive HPV tests which is the maximum rate of infection among certain age group.

Table 1: The age distribution.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Total sample</th>
<th>HPV positive</th>
<th>HPV negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>&lt;20</td>
<td>16</td>
<td>7.0</td>
<td>2</td>
</tr>
<tr>
<td>20-29</td>
<td>81</td>
<td>35.2</td>
<td>16</td>
</tr>
<tr>
<td>30-39</td>
<td>79</td>
<td>34.3</td>
<td>15</td>
</tr>
<tr>
<td>40-49</td>
<td>45</td>
<td>19.6</td>
<td>3</td>
</tr>
<tr>
<td>50 and more</td>
<td>9</td>
<td>3.9</td>
<td>-</td>
</tr>
<tr>
<td>Mean±SD (Range)</td>
<td>31.92±8.58 (15-59)</td>
<td>28.31±6.31(16-42)</td>
<td>30.45+8.25(15-59)</td>
</tr>
</tbody>
</table>

P value=0.6204 (Not significant using Pearson Chi-squared test at 0.05 level of significance)

In table -2- ,we see the distribution of cytology according to HPV DNA test, (25%) of those with positive HPV test were found to have negative cytology. (13.9%) of those with positive HPV test were found to have ASC-US ,where (30.6%) had CIN I. (13.9%) of them had CIN II ,(11.1%) had CIN III and (5.5%) of those with positive HPV test were found to have micro- invasive cervical cancer.
Table 2: The distribution of cytology and histology according to HPV test.

<table>
<thead>
<tr>
<th>Cytology and histology</th>
<th>HPV positive</th>
<th>HPV negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous metaplasia, mixed</td>
<td>9</td>
<td>156</td>
<td>165</td>
</tr>
<tr>
<td>inflammatory reaction</td>
<td>25%</td>
<td>80.4%</td>
<td></td>
</tr>
<tr>
<td>ASC-US</td>
<td>5</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>CIN I (LSIL)</td>
<td>11</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>66CIN II (HSIL)</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>CIN III</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Micro-invasive Ca</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>194</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

P value=0.0001 (Significant using Pearson Chi-squared test at 0.05 level of significance)

DISCUSSION:
Eradication of the HPV infection is almost 100% protective against progression to cervical cancer, at least during a certain period of a few years. Because HPV infections are progressively becoming a major subject in gynecological malignancy literatures, as it is the most dangerous risk factor for most common genital tract tumor, and studies on the natural history of HPV infection in Iraq's women are lacking, we tried to point the light on the outlines of the problem in Iraq, especially with increasing number of patients who are discovered to have variable degrees of preinvasive or invasive cervical carcinoma. We depend on the available data to make an idea about the size of the problem in Iraq and we tried to determine whether HPV screening would help to identify high-risk groups to premalignant disease of the cervix.

In this study, the percentage of HPV infection was 15.65%, we had 36 cases out of total 230 case, from those 36 case only 11 case were having viral typing test, (31%) of those with positive test had typing, and all of them were found to have single genotype and all viral types were of the high risk genotype. In a meta-analysis summarizing the global literature for women with negative cytology results, de Sanjosé et al in July 2007 the worldwide prevalence of women harboring HPV DNA was 10.4%.

In other studies the rates range from 7.6% to 27%, depending on the region.

while in June 2010 a randomized trial in Turkey by Eren et al, found that the prevalence of HPV among Turkish women was 16.5%.

While in in Japan study done by Inoue in 2010, the prevalence was 14.5%. Another randomized trial done by Fernandes et al in Brazil in 2009, shows that the overall HPV prevalence in Brazil was 48%.

In this study 12.5% for women aged <20 years old was infected, 20% among those aged between 20-30 years old, no viruses had been detected above 50 years old, the highest incidence of HPV infection among patient aged between 20-29 years can be explained by the fact that early marriage in our country which is not very evident in other researches. Although age curves for HPV infection differ notably across regions, HPV prevalence is strongly associated with age worldwide. In all world regions, HPV prevalence was highest in women younger than 35 years of age, decreasing in women of older age. Although we noted a decline in HPV prevalence with increasing age, the rates for women aged between 20 and 29 years and those aged between 30-39 did not differ significantly, the difference in rates was significant for women younger or older than 40 years. In agreement with other studies were the highest prevalence is found for patients younger than 30 years.

While in Turkish study, the high prevalence of HPV for women aged between 31 and 40 years may be related to their mean age of 23.8 years at first intercourse which is older than in many other countries, and the sexual habits of their population. Contrary to other studies, we report no bimodal age curve for HPV infection, with a second higher-risk peak for older women, this results also found in Turkish study.

21
6
2
9
1
7
8
If we interpret the result of cytology as a percentage of HPV infection, we find that (25%) of patient with positive HPV infection have a negative cervical cytology , while in Turkey, of those participants who tested positive for HPV DNA , (79%) had negative cytology results . (23) If we interpret the result of cytology as a percentage of HPV infection, we find that (25%) of patient with positive HPV infection have a negative cervical cytology , while in Turkey, of those participants who tested positive for HPV DNA , (79%) had negative cytology results . (23) Also in this study (13.9%) of patient with positive HPV infection have (ASCUS), while in Turkey , of those participants who tested positive for HPV DNA, were found to have ASCUS (3.7%) and have ASC-H (1.2%). (23) Also in this study (30.6%) of patient with positive HPV infection have CIN I , while in Turkey , of those participants who tested positive for HPV DNA, (10%) were found to have LSIL. (23) Also in this study (13.9%) of patient with positive HPV infection have CIN II , and (11.1%) were found to have CIN III , while in Turkey , of those participants who tested positive for HPV DNA , (6%) were found to have HSIL. (23)

CONCLUSION:
The results of this study lead us to conclude that high rate of infection are associated with age , smoking and multiple sexual partners.
- High frequency of detection of oncogenic HPV infection is associated with increasing grade of cervical lesion.

REFERENCES: