

## Distribution of human papillomavirus in women by genotype, age, education, and geography in Baghdad, 2021-2022

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### ABSTRACT

**Background:** Human papillomavirus (HPV) is the main causative agent of cervical cancer and genital warts. HPV infection can be persistent with high-risk HR-HPV genotype. **Objective:** Investigate the distribution of HPV genotype in Baghdad province, to afford a scientific source for initial detection policies and immunization for cervical tumors in the province. **Methodology:** From January 2021 to September 2022, 400 women who were tested for HPV genotyping, were referred to the Central Public Health Laboratory (CPHL) in Baghdad province. They have one or more of the following (intermenstrual bleeding, post-menopausal bleeding, post-coital bleeding, vaginal discharge, and warts). **Results:** The prevalence of HPV infection showed a high percentage in Rusafa about 74.17%, however, Al-karkh showed about 25.83%. Both regions appeared high distribution for genotypes (16&18) as predominant types for HR-HPV. Two major rates of HPV infection have been found in women aged group (20-30) years, about (45.31%) and (36.72%) in women aged (30-40) years, these groups consider reproductive age therefore HR-HPV risking persistent infection. In comparison, HPV infections show (13.28%) among women aged 40-50 years. finally, HPV infection in the elderly group 50-65 years was (4.69%) showing the lowest percentage, also women with secondary education level appeared higher percentage of HPV infection about (42.63%) compared with primary and higher education. **Conclusion:** The prevalence of high-risk-HPV infection showed a high percentage in Baghdad providence and there was an important for age specificity, in addition to an inverse relationship with the education level.

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**Keywords:** Human papillomavirus infection, HR-HPV genotype distribution, risk factor, Baghdad.

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### 1- Introduction

The most prevalent sexually transmitted infection that affects both men and women is the human papillomavirus. According to recent epidemiological data, the virus is strongly linked to anal canal, vulvar, penile, and cervical cancer (1). HPV is a small-scale DNA virus that has a double-strand DNA genome of about 8 (Kilo bases) KB in circumference and is grouped according to the DNA sequence of its genes. HPVs have less than 90% resemblance in the size of the genome (2).

Based on their correlation with malignant or non-malignant lesion consequences, such as cervical warts or condylomas, These viruses are identified as either oncogenic, also called high-risk HPV (HR HPV) which is found in 99.7% of uterine cancers, or non-oncogenic low-risk HPV (LR HPV) groups (3). According to Arbyn (2014), there are at least 12 HPV subtypes, i.e. HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, and HPV59, those are considered as high-risk and one less evident high-risk is HPV68 (4). Other studies recorded about 13 HPV subtypes have been proven to cause more than 96% of cervical cancers (5). In 2015, Iraq study recorded 12 HPV genotypes were identified, including HPV-33 (18.60%), HPV-

35 (18.60%), HPV-56 (18.60%), HPV-39(10.85%), HPV-52 (10.08%), HPV-18 (7.75%), HPV-16 (4.65%), HPV-59 (4.65%), HPV-58(2.32%), HPV-31 (1.55%), HPV-45(1.55%) and HPV-66 (0.77%), so that the number of HPV genotypes can change according to PCR detection (6). Several other HPV genotypes are unclassified regarding their epidemiologic oncogenic risk although few of them have been shown to bind and ubiquitinate p53 onco-suppressor with the same efficiency as the Group 1 oncogenic virus (7).

Because the HR-HPV strains' early oncoproteins (E6 and E7) can bind to and modify a variety of gene products, involving tumor suppressor proteins (p53 and pRb), they may be carcinogenic. These interactions lead to a disruption of the cell cycle, a deficiency in DNA repair, genomic instability, and an elevated risk of malignant transformation (8).

World Health Organization (WHO) in 2018 approximates that the worldwide predominance of HPV disease is between (9 and 13) % or approximately 630 million (9). Persistent infection with HPV could be an engrained reason for cervical cancer and there have been enormous developments within the characterization of the natural history of cervical HPV infection in females (10, 11). It is well documented that the worldwide incidence of HPV contagion in females without cervical abnormalities is (11 to 12) %, while in Asia as a whole and China, this data goes to 8% and (11.4–20.3) %, respectively (12). In 2018 local study regarding the prevalence of HPV genotypes among women in Iraqi provinces, they were (12.5, 11.8, 10.5, 5.1, 3.7 and 1.7)% in Kerbala, Babylon, Baghdad, Basrah, Diwaniya, and Diyala subsequently (13).

Cervical Cancer (CC) caused by HR-HPV covers 5.2% of all cancers worldwide. This means that out of total cancer in high-income and low-income countries, 2.2 and 7.7% are cervical cancer patients, respectively (14). The high death rate from cervical cancer internationally (52%) might be decreased by active screening and treatment programs (15). Despite increased research into HPV, and the introduction of HPV vaccines to prevent infection and subsequent cervical cancer development, screening will remain important (16).

The study aims to investigate the distribution of high-risk HPV genotypes in Baghdad province, to provide a scientific basis for early detection strategies and vaccination for cervical cancer in the region.

## 2-Material and Method

### Study design

A cross-sectional population study was established by collecting clinical specimens from four hundred women from Baghdad who were suspected of cervical cancer in the period from January 2021 to December 2022 in the Central Public Health Laboratory (CPHL) /Public Health/ MOH, all women in the present study who have undergone HPV tests were among the inclusion criteria including (A) Aged 18-65 years (B) All patients resided in Baghdad province (C) No history of sexual life or vaginal medication in the past (5-7 days) (D) Clinical symptoms like (continual bleeding in some cases, Pain during sexual relationship, vaginitis as well as genital warts). (E) Education levels (Primary, secondary, and higher) for participants. Criteria in this study undergo to work ethics in CPHL that include patient consent to receive information. All methods were performed by relevant guidelines and standard operating procedures (SOPs).

### Specimen collection

The cervical specimens were collected by gynecological practitioners, using cervical swabs. First, the cervix was exposed using a sterile disposable speculum, remove excess mucus from the cervical and surrounding ectocervix with a cotton swab, cervical swab was inserted 1.0-1.5 centimeters into the cervical canal and rotated 4 to 5 times in a counterclockwise direction to obtain a sufficient amount of cervical epithelial cells. Then, the swab was placed into the nuclease-free tube with 2ml of Viral Transport Media (VTM) (Citect Labware Manufacturing, China). Cervical samples should be stored at (2-8) °C for no more than 7 days after collection and for long storage at -20°C.

### DNA extraction

DNA was extracted from cervical cells by using a DNA extraction kit (Sacace Biotechnologies, Italy). Samples on cervical swabs were eluted; 100µl of elution was transferred to a 1.5 ml Eppendorf centrifuge tube and added 300µl of Lysis solution, then vortex and incubated 5 min at 65°C after that centrifuged for 5 min at 12000-

16000xg. Twenty µl of the sorbent was added to the tube, vortexed for 5-7 sec, and incubated for 3 min at room temperature, then centrifuged at 5000xg/ 30 sec. After that 500µl of washing solution was added for each tube, vortex, and centrifuged for 30sec/10000xg then the supernatant was discarded, the wash step was repeated, and incubate tubes with an open cap for 5-10 min at 65°C. Finally, the pellet was re-suspended in 100µl of DNA eluent, incubated for 5 min/65°C, and vortexed periodically, then the tube was centrifuged for 1 min/12000xg, transfer the supernatant into a new sterile 0.5 ml tubes.

### **HPV testing**

DNA that was extracted from the cervical sample using PCR amplification performed by the HPV genotypes 14 Real-TM kit (Sacace Biotechnologies, Italy). The test used for quantitative or qualitative detection of the most widespread and oncogenic genotypes of HPV (14 genotypes) according to the kit that we use in PCR detection. Namely genotype-16, genotype-18, genotype-31, genotype-33, genotype-35, genotype-39, genotype-45, genotype-51, genotype-52, genotype-56, genotype-58, genotype-59, genotype-66, and genotype-68) with a determination of clinical significance. Amplification was performed using 7500 Applied Biosystems by Thermo Fisher Scientific PCR amplification instrument.

Reaction conditions were: The template DNA was amplified for 5 cycles of denaturation programmed for 5 second at 95°C, annealing of primers at 60°C programmed for 20 sec and extension at 72°C programmed for 15sec, and for 40 cycles of denaturation programmed for 5 second at 95°C, annealing of primers at 60°C programmed for 30 sec and extension at 72°C programmed for 15sec Fluorescent data were acquired during each extension phase. This program was followed according to the kit's instructions for optimum detection of the target.

### **Statistical Analysis:**

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors on study parameters. The chi-square test was used to significant compare between percentages (0.05 and 0.01 probability) in this study (17).

## **3-Results**

### **Age-specific HPV prevalence**

A total of 400 specimens were collected from women complaining from different gynecological problems, whose pap smears show abnormal findings, visited the CPHL and subjected to HPV DNA testing. These specimens were divided into two groups:

- Positive HPV group consist of 128 women
- Negative HPV group consist of 272 women

It was established that 128 (32%) of women had HPV infections, this percentage was divided into four groups according to the age of women that appears most HPV infections occur in women of the age group (20-30) (45.31%), highest rate showed for HPV genotype 16, then genotypes 68,39,51, 52 respectively. While lowest rate appeared in HPV genotypes 33, 35 and 59. A single type of HPV infection (one genotype of HPV detected in patients) was common among women aged (30-40) years (36.72%). In this age group HPV genotypes that appeared highest percentage was genotype-16, then followed by genotype-18, 56 and 58. Where the lowest percentage showed in genotype 68. While HPV infections show (13.28%) among women aged 40-50 years. Which showed high rate in genotype-31 and 51. But appeared low rate in genotype-18, genotype-39 and genotype-59. finally, HPV infection in the elder group 50-65 years was (4.69%) show the lowest percentage, high rate in genotype-16 and low rate in genotype-18,33 and 39 in addition to genotype 68. As shown in Table (1).

Table (1): Relationship between age and the distribution of Human papillomavirus genotypes

Genotyping	Age (20-30)	Age (30-40)	Age (40-50)	Age (50-65)	Total (%)
Genotype 16	8	11	2	2	23 (7.97%)
Genotype 18	3	5	1	1	10 (7.81%)
Genotype 45	3	2	0	0	5 (3.91%)
Genotype 31	4	2	3	0	9 (7.03%)
Genotype 33	1	2	0	1	4 (3.12%)
Genotype 35	1	2	0	0	3 (2.34%)
Genotype 39	6	4	1	1	12 (9.37%)
Genotype 51	6	0	3	0	9 (7.03%)
Genotype 52	6	2	2	0	10 (7.81%)
Genotype 56	4	5	2	0	11 (8.59%)
Genotype 58	3	5	0	0	8 (6.25%)
Genotype 59	2	3	1	0	6 (4.69%)
Genotype 66	4	3	0	0	7 (5.46%)
Genotype 68	7	1	2	1	11 (8.59%)
Total (%)	58 (45.31%)	47 (36.72%)	17 (13.28%)	6 (4.69%)	128
Chi-square	60.760 **				34.507 **
P-value	(0.0001)				(0.0001)
C.I. (95%)	0.86 -2.49				---
** (P≤0.01).					

### Prevalence of HPV genotype in Baghdad province

The geographic distribution of HPV infection appeared fourteen HPV genotypes were identified as HR-HPV (HPV 16, 18, 45, 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68) that ratios have been shown in Al-Rusafa and Karkh were 89 (74.17%) and 31 (25.83%) respectively. The highest percentage showed (15.83 %) in genotype-16, 10% for genotype 39 and 56, 8.33% for genotype-51 and 68. When the lowest percentage appeared in genotype-35 that recorded in Al-Rusafa. As shown in Table (2)

Table (2): Distribution of HPV genotype in women by residency

Genotyping	Al-Rusafa	Al-Karkh	Total (%)
Genotype 16	13	6	19 (15.83%)
Genotype 18	5	4	9 (7.50%)
Genotype 45	3	1	4 (3.33%)
Genotype 31	8	0	8 (6.67%)
Genotype 33	2	2	4 (3.33%)
Genotype 35	3	0	3 (2.50%)
Genotype 39	8	4	12 (10.00%)
Genotype 51	8	2	10 (8.33%)
Genotype 52	6	3	9 (7.50%)
Genotype 56	8	4	12 (10.00%)
Genotype 58	6	2	8 (6.67%)
Genotype 59	4	1	5 (4.17%)
Genotype 66	7	0	7 (5.83%)
Genotype 68	8	2	10 (8.33%)
Total (%)	89 (74.17%)	31 (25.83%)	120
Chi-square	28.033 **		24.692 **
P-value	(0.0001)		(0.0001)
C.I. (95%)	0.79 -2.08		---
** (P<0.01).			

### HPV genotype and Education levels

The multivariate analysis suggested that education level, and annual income was independently associated with the rate of HR-HPV infection so low educational level, low income can be considered as risk factors.

Table (3): Relationship between education level and the genotype HPV distribution

Educational level	HPV infection (%)
Primary	31.78%
Secondary	42.63%
Higher	25.48%
P-value	( $P \leq 0.05$ )

The regression equation was  $(\log P = -1.103 - 0.333 \times 2 - 0.233 \times 3 + 0.813 X4 - 0.179 \times 7 + 0.205 X9.)$  Education level and annual income were protective factors for HR-HPV infection. For each 1-grade increase in education level, the risk of HR-HPV infection was reduced by 28.4%. For each 1-grade increase in annual income, the risk of HR-HPV infection was reduced by 20.8% (18). In this study the HPV infection appears variable results according to educational level and we noticed most of patients with Primary and secondary education level had low annual income so our study focused on education instead of income. The secondary level shows a higher percentage 42.63% compared with primary 31.78% and higher education 25.48%, at  $P \leq 0.05$  and C.I. (95%) 0.82-1.68 that show true value in population as shown in Table (3).

#### 4-Discussion

The majority of HPV infections occur immediately after the start of sexual activity, and the virus is primarily spread through sexual contact (19). This study was established the distribution of HPV genotypes among women, referred to CPHL for two years (2021-2022). Our research was discussed the relationship between high-risk HPV genotype that can cause many changes in cervical epithelial cells, which effect on health of women and cause many problems that lead to cervical cancer and age, education in addition to geographic. The study showed HPV infection rate among women was highest among women aged (20-30), mean 25.91 was (45.31%) with multiple genotypes of HR-HPV infection as shown in Table (1) appears the highest rate in HPV Genotype (16, 39, 51, 52, 68) respectively. When compared to the findings of research conducted in various nations, the observed prevalence of multiple infection is higher. In Brazil, there were no multiple infections (20), 16.7% in Paraguay (21), and 28% in The Netherlands (22) of the study population had multiple infections. These variations in the prevalence of multiple infections could be caused by variations in the methodology used or actual variations in the prevalence of the HPV types in the populations under study.

While women aged (30-40), mean (35.65) showed a Single genotype of HR-HPV infection where the most prevalent HR-HPV infections were HPV (16, 18, 56, 58, 39) receptively. This result agreed with other studies which give high results in the six most prevalent HPV, especially in 16 (20%), and 18 (12.7%) (23) \*.Concerning in older women, no women on age groups > 65 years old was HPV positive. This was disagree with other research revealed that the age-specific HPV prevalence in women was highest for those under 20 years old, fell in the middle age groups, and then increased once more for those 65 years of age and above.(24)

The prevalence of HPV was highest between the ages of 21 and 30, and then followed by age groups (31 to 40 years old). The prevalence of HPV in the United States decreases from roughly 40% at age 20 to 10% to 20% at age 30 to 40% (25). Our results show that HPV genotypes 16 and 18 being predominant in reproduction age, the risk in women whose age at first intercourse was less than 30 years old was 45.31% compared with women in age more than 30 years. When HPV 16 and 18 were considered one of the causes of cervical cancer in the world. Statically the result gives ( $P \leq 0.01$ ) significant differences between women age group and HPV infection. In local study, High-risk HPV DNA was detected in 19% among women population with the oncogenic HPV-16, -18 and -58 were being the most prevalent high risk genotypes among women at frequencies of 26.7%, 13.3% and 13.3% (26).

HPV infection plays an important role in the occurrence and development of cervical cancer, many studies have shown that HPV genotypes and infection rates differ among countries and regions (27) \*. Our study has divided the prevalence of HPV genotype in Baghdad city into two regions Karkh and Rusafa that appear high percentage of HPV infection in Rusafa (74.17%), which shows a higher percentage of HR-HPV genotype (16, 31, 39, 51, 56, 68, 66 and 18) respectively, while in Karkh (25.83%) HPV genotype 16 and 18 give higher results than other genotypes, which give low result compare with Rusafa may be related to population density or geographic distribution in Baghdad regions at P-value ( $P \leq 0.01$ ) that appear statically high significant differences between them as shown in Table (2). Local study show HPV 16 and 18 is predominate in cervical samples in Baghdad city which agreed with our study that appear the prevalence of HPV 16 and 18 (26) \*.

The high percentage in secondary may be related to low annual income and lack of awareness of correct lifestyle or other causes. The study was agreed with other studies that give the same result when women in junior middle school show high percentage in HR-HPV infection (28) \*. That make the above equation is correct whenever the education level is low, HPV infection risk is high.

### 5-Conclusion

Prevalence of Multiple high-risk HPV genotypes was detected in women; our study indicated that HPV (16 and 18) might be the major circulating types in Baghdad which considered one of the causes of cervical cancer in the world. Therefor our population need to provide HPV vaccines to reduce the rate of HR-HPV infection especially for women in reproductive age which promote strategies such as cervical screening and HPV vaccination to reduce the incidence of cervical cancer in this population. There is significant age specificity and educational level with high rate of infection.

### Recommendation:

In this study we recommend increase in samples to cover the remaining provinces, Take another Criteria like: number of pregnancies, presence of multiple partners, Smoking, presence suppressive disease and immune suppressive drug use.

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## توزيع فيروس الورم الحليمي البشري عند النساء حسب التركيب الجيني والعمر والتعليم والموقع الجغرافي في بغداد

٢٠٢٢-٢٠٢١

وسام جاسم محمد، ثامر عبد حسين، نورس عبد الكريم توفيق، دينا سامي ابراهيم

مختبر الصحة العامة المركزي/دائرة الصحة العامة /وزارة الصحة/ بغداد/ العراق

**خلفية البحث:** فيروس الورم الحليمي البشري (HPV) هو العامل المسبب الرئيسي لسرطان عنق الرحم والتآليل التناسلية. يمكن ان تكون عدوى فيروس الورم الحليمي البشري مستمرة مع النمط الجيني HR-HPV عالي الخطورة. **هدف البحث:** دراسة توزيع النمط الجيني لفيروس الورم الحليمي البشري في محافظة بغداد، لتوفير اساس علمي لاستراتيجيات الكشف المبكر والتطعيم ضد سرطان عنق الرحم في المنطقة **طرق ومواد العمل:** في الفترة من يناير ٢٠٢١ الى سبتمبر ٢٠٢٢ ، تم اختيار ٤٠٠ امرأة للكشف عن التتميط الجيني لفيروس الورم الحليمي البشري، واللاتي تم احالتهن الى مختبر الصحة العامة المركزي في محافظة بغداد اللاتي لديهن واحد او اكثر مما يلي (نزيف ما بين فترات الحيض، نزيف ما بعد انقطاع الطمث ، نزيف ما بعد الجماع والافرازات المهبلية والتآليل). **النتائج:** أظهرت نسبة انتشار فيروس الورم الحليمي البشري ارتفاعا في الرصافة حوالي 74.17% في حين أظهرت الكرخ حوالي 25.83% في كلا المنطقتين ظهر توزيع عالي للتتميط الجيني (16&18) كأشكال سائدة لفيروس الورم الحليمي البشري. تم الحصول على معدلين رئيسيين للإصابة بفيروس الورم الحليمي البشري في النساء بعمر (20-30) سنة، حوالي 45.31% والنساء في عمر (30-40) سنة، حوالي 36.72% حيث تعتبر هذه المجاميع هي سن الانجاب لذلك يعتبر فيروس الورم الحليمي البشري هو خطر للعدوى المستمرة. في حين تظهر نسبة الإصابة بالفيروس حوالي 13.28% بين النساء في الفئة العمرية (40-50) سنة. واخيرا، كانت الإصابة بالفيروس في الفئة الأكبر سنا (50-65) سنة هي الأقل نسبة حوالي 4.69%. كما أظهرت نساء بمستوى التعليم الثانوي نسبة عالية بالإصابة بالفيروس حوالي 42.63% مقارنة مع نساء في مستوى التعليم الابتدائي والعالي. **الاستنتاجات:** اظهر انتشار الإصابة بفيروس الورم الحليمي البشري عالي الخطورة نسبة عالية في محافظة بغداد وكان هناك اهمية لخصوصية العمر. بالإضافة الى العلاقة العكسية مع مستوى التعليم.

**الكلمات المفتاحية:** عدوى فيروس الورم الحليمي البشري، توزيع النمط الجيني لفيروس الورم الحليمي البشري، عامل الخطورة، بغداد.