MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Allos *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 20 (2024) 639-647

RESEARCH ARTICLE

Association between Cervix Intraepithelial Neoplasia (CIN) and High-Risk Human Papillomavirus (HPV) Genotypes in Iraqi Women

Mina M. Allos[a,b*], **Hassan M. Naif**[a,c]

[a]Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq; [b]Department of Applied Sciences, University of Technology, Baghdad, Iraq, [c] Department of Medical Laboratories Technology, College of Health and Medical Technology, AlShaab University, Baghdad, Iraq

Abstract Human Papillomavirus (HPV) is an oncogenic virus that primarily causes premalignant and malignant cervical lesions particularly infection with high-risk types of viruses. Cervical cancer (CC) is a common gynecological tumor, ranking second in the female reproductive system tumor, particularly in women from developing countries. The prevalent and genotyping of high-risk HPV among Iraqi women during various stages of cervical lesions and cervical cancer were examined by using a real-time PCR. Results of this work revealed that the prevalence of HPV infection in women suffering from gynecological problem was 43%. The distribution of the high-risk HPV was relatively high for both HR-HPV-16 (44.90%) and HR-HPV-39 (14.29%), with HR-HPV-16 is being the most common. The finding of this study can be used to manage HPV infection and cervical cancer particularly at earlier stages of cervical lesions.

Keywords: High-Risk Human Papilloma virus; Genotyping; premalignant cervical lesion; Cervical Cancer; Iraq women.

Introduction

Cervical cancer is a common gynecological malignancy, ranking second in the hierarchy of female reproductive system tumors [1]. Human papillomavirus (HPV) is a viral infection that has been linked to a variety of cancers and diseases globally. These include cancers of the cervix, anus, vulva, penis, vagina, and head and neck squamous cell carcinomas (HNSCC) [2]. HPV are small DNA viruses that belong to the Papillomaviridae family. They are non-enveloped with double-stranded circular DNA. There are over 200 types of HPV that have been identified and classified into 29 genera, most of which affect humans[3,4]. The DNA of HPV is comprised of around eight open reading frames (ORFs). The genome of the human papillomavirus (HPV) is divided into three different regions: upstream regulatory region (URR), the early proteins (E1, E2, E4, E5, E6 and E7) and the late proteins L1 and L2 [5]. There are at least 14 different high-risk types of human papillomavirus (HPV) that have been identified as being associated with the risk of developing cervical cancer. These types are referred to as "carcinogenic" viral types and include HR-HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. HR-HPV-induced tumor types can be detected in over 90% of cervical cancer samples and in more than 60% of CIN (cervical intraepithelial neoplasia) tissues [6]. CIN is a condition that can be categorized into three types - low-grade lesion (CINI), high-grade lesion (CINII or CINIII), and carcinoma. The epithelial tissue in the cervix can be divided into three layers - basal, intermediate, and superficial. In CINI, proliferative lesions can be found in the lower third of the epithelial tissue. In CIN II, proliferative lesions can be found in the upper two-thirds of the epithelial tissue. In CIN III, the entire epithelium is affected by proliferative lesions [7]. HPV infection of the basal epithelial layer, which maintains the stratified ectocervix, results more often in regulated HPV expression that facilitates productive infection [8]. Persistent infection with high-risk HPV

***For correspondence:**
mina.m.eshaq
@uotechnology.edu.iq

Received: 10 Jan. 2024
Accepted: 6 May 2024

(particularly HPV-16 and HPV-18) is the most important risk factor for progression to high-grade dysplasia and these dysplastic lesions may persist, regress, or worsen, and in severe cases, can develop into invasive cancer. CIN is considered a precancerous condition with the potential to progress to malignancy [9,10].

Other risk factors for cervical cancer include: sexual activity at a young age, having sex with multi-partners, smoking, having many children, low socioeconomic position, usage of birth control pills (with negative or positive HPV), sexually transmitted infections, and immunological disorders. Cervical cancer predominantly affects young females and often presents with symptoms such as irregular vaginal bleeding, lower abdominal pain, infertility, and abnormal vaginal secretions. Although there has been a significant improvement in the treatment of recurrent and advanced cervical cancer in the past five years overall survival rate for all stages of cervical cancer is only 68% [11]. Therefore this study aimed to assess the prevalence and genotypes of HR-HPV spreading in Iraqi women.

Materials and Methods

The cases examined in this study were gathered from the Gynecological Oncology Department of Baghdad Teaching Hospital between February and December 2022. A consultant Gynecologist Oncologist collected cervical swabs from 100 women with cervical lesion abnormalities or cancer, as well as from twenty healthy women as a control group. The sampling process involved removing excess mucus from the endocervix using a cotton swab, followed by inserting a cervical brush 1.0-1.5 cm into the cervix and rotating it three full turns counterclockwise. Each patient provided two swabs, with the first swab being immediately examined by a cytopathologist (Pap smear test). The second swab was inserted into 3 ml of virus transport media (VTM) and used for high-risk HPV molecular detection and genotyping. Samples from women with a history of cervix lesions and gynecological symptoms who tested positive for HR-HPV were included in this study. Whereas samples obtained from pregnant women, those under treatment and negative for both Pap smear and HR-HPV were excluded.

After collecting a cervical swab, it was evenly spread onto a glass slide. To prevent air-drying artifacts, the slide was immediately fixed using a mixture of 95% ethyl alcohol and ether. In cases where pap smears showed abnormalities in epithelial cells, a biopsy of the cervix was taken by applying 3% acetic acid to the acetowhite areas. These biopsies were fixed into a 10% formalin solution, routinely processed, and finally stained with hematoxylin and eosin.

Molecular detection of HR- HPVs was achieved by isolation and purification of DNA from specimens and followed by amplification of 14 specific high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) using real-time PCR (kit packed from Sacase, Italy) in the urogenital swabs and biopsies. Genomic DNA was extracted from cervical swabs in VTM by using the DNA-Sorb-A (Sacase, Italy) nucleic acid extraction kit according to the manufacturer's instructions. A genotyping kit (Sacase, Italy) was used to detect the presence of 14 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) in cervical swabs of Iraqi women by Real-Time PCR.

Results and Discussion

Age Distribution of Study Groups

The age of women with abnormal cervix lesion and healthy women the ranged between 20 and75 years old. The subject were grouped into women ≥30 years and those <30 years old. Results presented that 82.72% of women with abnormal Pap smear or cervix lesion age over 30, while only 17.28% women with age equal and below 30 with abnormal Pap smear were showed in Table 1.

Our finding are consistent with those previously published by Omoyeni and Tsoka-Gwegweni that found 47.8% of women with age between 30-44 had abnormal Pap smear [12]. Another study from Nigeria, was found that the age group of 40 and above had the highest percentage 28.9% of abnormal Pap smear results while the lowest rate was discovered in individuals aged 20-29 years (8.7%) [13]. The risk of uterine and cervical cancer increases due to an extended exposure to carcinogens and a weakened immune system caused by age [14]. Women's reproductive organs also undergo a natural aging process after the age of 35, which can increase the incidence of cervical cancer. Certain risk factors may be involved such as pre-menarche and post-menopause age [15]. The findings of the risk factor were closely associated with the clinical findings observed in the research conducted by Verma group 2017 [16].

Table 1. Age distribution of study groups

Age group	Women with abnormal pap smear	
	No.	Percentage
>30	67	82.72%
<30	14	17.28%
Total	81	100%
P value	<0.0001****	

Among the female participants of this study, the most common presenting symptom was abnormal vaginal discharge, reported by 54.5% of the women. This was followed by 19.5% of women reporting intermenstrual bleeding, 10.5% reporting postcoital bleeding, and 2.5% reporting an unhealthy looking cervix. Additionally a significant association was found between sexually transmitted infections (STIs) and the development of precancerous cervical lesions. Women who have previously contracted sexually transmitted infections (STIs) are twice as likely to develop precancerous cervical lesions compared to those who have not had any STIs. In fact, 38.2% of women with a history of STIs are at risk of developing precancerous cervical lesions [17,18].

Vaginal bleeding, especially post-coital bleeding, is a common gynecologic complaint seen by primary care clinicians. This symptom can cause distress as it may indicate underlying malignancy [19]. Post-coital bleeding is the initial symptom in 11% of women who are diagnosed with cervical cancer [20]. Smoking tobacco is another a risk factor for developing cervical cancer and its precursors, but the mechanism of how smoking leads to cervical carcinogenesis is unclear [21]. Immune suppression during pregnancy, hormonal effects on cervical epithelium, and physical trauma resulting from vaginal deliveries could be other contributing factor [14].

Cytological Findings

Cytology was done in all the 100 women in the study Papanicolaou testing (Pap test) and colposcopy, followed by biopsy if necessary. The HPV DNA test was routinely recommended and performed for patients who consented. The cytological states of samples was shown in Table 2, where out of 80 there were 56 women (75.69%) had abnormal cytology. The highest parentage of twenty women (27.03%) had atypical squamous cells of undetermined significance (ASCUS), followed by fifteen with nonspecific cervicitis (20.27%), 16.22% of cases with LSIL, 5.41% had HSIL and 6.76% women diagnosed with cancer.

Table 2. Cytological examination result of pap-smear derived from Iraq patients women

Cytology	Number	Percentage
Cervicitis	15	20.27%
ASCUS	20	27.03%
LSIL	12	16.22%
HSIL	4	5.41%
Cancer	5	6.76%
Normal	24	30%

Colposcopy biopsy was also done for these patients with abnormal cervix lesions. Histological change results showed in Table 3 and Figure 1 that 53 of the patients with different stages of diseases which classified as squamous cell atypia was high and appeared in 41.51 % (22 out of 53) of tested samples, followed by low-grade squamous intraepithelial lesions (CIN I) constituted 26.42% (14 out of 53). Meanwhile, the high grades (CIN II & III) constituted 9.43% and 1.89 %, respectively. Squamous cell carcinoma appeared in 6.98 % (9 out of 53), and adenocarcinoma appeared in 3.77% of samples.

Table 3. Cervical lesion stages according to histological examination

Abnormal histopathological change	Number	Percentage
Atypia	22	41.51%
CIN I	14	26.42%
CIN II	5	9.43%
CIN III	1	1.89%
Adenocarcinoma	2	3.776%
Squamous carcinoma	9	16.98%

*CIN: cervical intraepithelial neoplasia

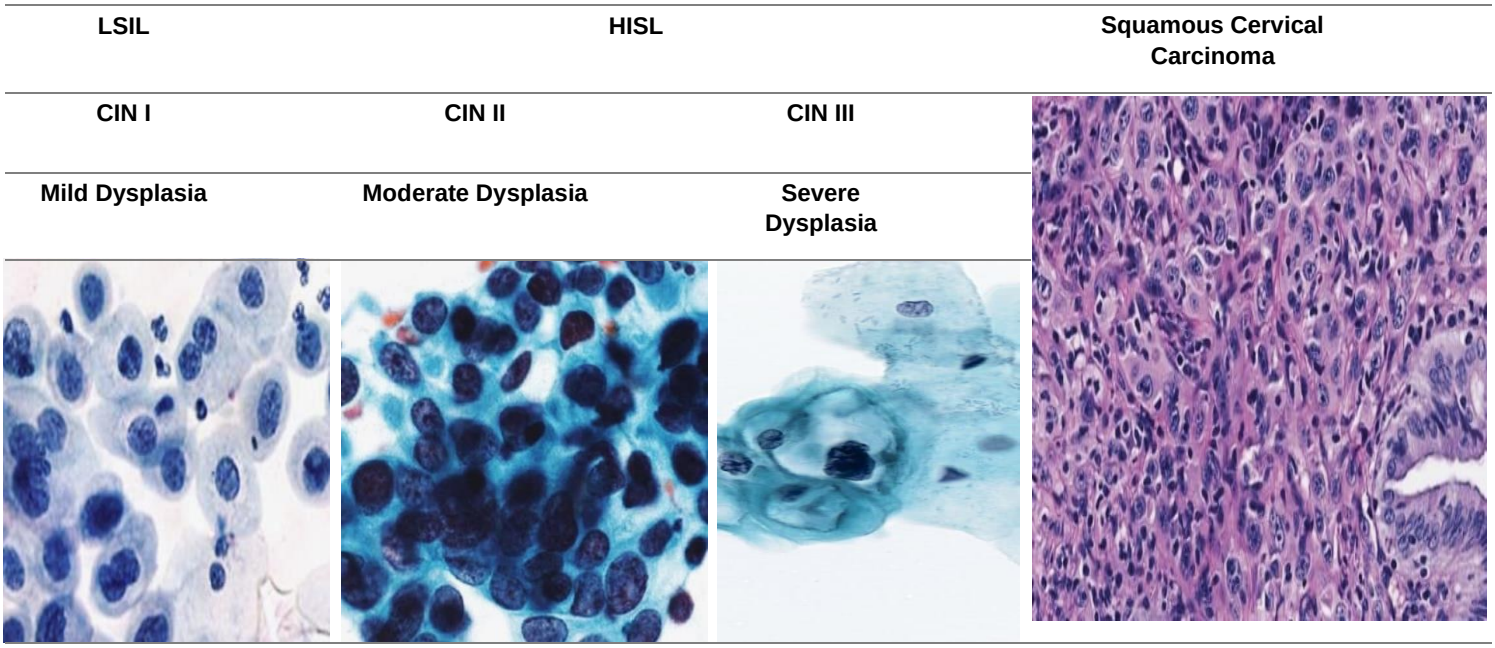

Figure 1. Histological sections of biopsies obtained from women with abnormal cytology

Previous study showed that, 68.9% of Pap smears showed negative results, 15.4% showed inflammation, 7% showed ASCUS, 5.9% showed LSIL, and only 2.9% showed HSIL [22]. Similar findings were also reported by Jihad *et al*. [23], where 30% of the most prevalent abnormal cases were at the ASCUS stage of the cervical lesion. Another study showed that 32.3% of tested Pap smear had abnormal lesions [24]. The overall of abnormal Pap smear results in this study were ASCUS (12.2%), LSIL (15.5%), and HSIL (3.7%). According to a recent study by Yousif *et al*. (2023) [25], abnormal histopathological changes of cervix lesions were observed in women in Iraq who were infected with HR-HPV. The study found that 55% of cases had CIN. Out of this, 25% had CIN II, 15% had CIN III, and 5% had invasive carcinoma. According to a study published by Adhikari *et al*. in 2019 were found to be 31.8% for CINI, 8.6% for CIN II, and 3.2% for CIN III. During the follow-up period, 17.1% had ASCUS, 20.7% had LSIL, 0.6% had HSIL, 4.4% had CIN I, 2.41% had CIN II, 0.9% had CIN III, and 21.6% women had cervical atypia [26]. CIN is a premalignant condition that can be classified into three grades, namely CIN I, CIN II, and CIN III, based on the severity of cellular abnormalities observed in histology. High-grade CIN, i.e., CIN II or CIN III, if not treated in time, can progress to cervical cancer.

Molecular Detection of High-risk HPV in Women

According to the patient's history, clinical diagnosis by (speculum and colposcopy), the results of cellular histopathology and cytological changes a total of 80 samples were tested for HPV DNA by real time PCR

and founded 43.75% were positive, while the rest of women who had abnormal cervix and healthy controls were negative for HPV (Table 4).

Table 4. Genotyping of detectable high risk-HPV in women with cervix lesion

HPV Genotype	Frequency	Percentage%
HR-HPV16	22	44.90 %
HR-HPV39	7	14.29%
HR-HPV66	4	8.16%
HR-HPV31	3	16.12%
HR-HPV56	2	4.08%
HR-HPV51	2	4.08%
HR-HPV52	2	4.08%
HR-HPV18	1	2.04%
HR-HPV35	1	2.04%
HR-HPV33	1	2.04%
HR-HPV45	1	2.04%
HR-HPV58	1	2.04%
HR-HPV59	1	2.04%
HR-HPV68	1	2.04%
Total	49	100%

Although, 80% of tested had single HPV genotypes, but 20% of cervical lesions had multiple co-infection with high-risk HPV (Table 5). Three patients with squamous cervical cancer were identified as having multiple co-infection genotypes the first had two HR-HPV genotypes (16+39); the second had three genotypes (HR-HPV-16+HR-HPV-31+HR-HPV-56) and the third had three genotypes (HR-HPV16+HR-HPV-56+HR-HPV-51). Two samples were from CIN I stage ended up with three genotypes (HR-HPV-16+HR-HPV-31+HR-HPV-52) and two genotypes (HR-HPV-39+HR-HPV-52), one samples from ACSUS stage had five genotypes (HR-HPV-16+HR-HPV-31+HR-HPV-39+HR-HPV-45+HR-HPV-51). The other samples was from CIN II stage had two genotypes (HR-HPV-39+HR-HPV-58). Later as highly, most prevalence genotypes that appeared with multiple infections are HR-HPV-16 and HR-HPV-31.

Table 5. Frequency of single and multiple high-risk HPV infection

Type of infection	NO.	Percentage
Single infection	28	80 %
Co-infection	7	20 %
Total	35	100%

However, the prevalence and genotype distribution of HPV varies worldwide due to differences in demographics, geography, and ethnicity [27]. The prevalence of HPV subtypes is constantly changing, and the introduction of HPV vaccination has played a role in this. In recent years, different HPV subtypes have shown different trends in their prevalence. The rates of infection for two high-risk HPV subtypes (HPV16 and HPV26) and two low-risk HPV subtypes (HPV11 and HPV83) have decreased, while the rate of HPV39 infections has increased. Therefore, it is essential to analyze the annual prevalence and distribution of HPV in order to develop effective vaccination strategies [28]. The most common HR-HPV infection in Iraqi women is HPV16. This prevalence has been reported in numerous studies over the past decade [29 ,23, 30]. In this study revealed that after HPV-16, the second most frequently identified was HPV-39, while HPV-66 was the third most commonly detected genotype. A study conducted by Liao *et al.*, [31] in Ghana revealed that HPV31 co-exists more frequently with HR-HPV16 and 18. The study also found that HR-HPV-positive women were more likely to have single infections, while multi-infections accounted for a small proportion (25.8%). The risk of developing precancerous lesions and invasive

tumors in women with more than one type of HPV is not higher than that of women with an infection from a single genotype [32] . A meta-analysis study revealed that 70% of cervical cancers are linked to HPV16 and 18, and 20% are linked to one or more of these genotypes 31, 33, 45, 52, 58 [33]. HR-HPV 16 was found to be the genotype with the highest oncogenic potential among the HR-HPV genotypes, but it is not always that HPV type 16 was detected in women with CIN III or carcinoma. Infection with HR-HPV 16 resulted more often in the development of an intraepithelial neoplasia than other HR-HPV genotypes [34]. Whether co-infection with more than one genotype influences diseases outcome is not yet understood and needs further investigations.

Correlation of HPV Genotypes with Cervical Lesions

Distribution of 14 high-risk HPV genotypes among different cervical lesion revealed that seven HR-HPV genotypes infections were detected at the early stages of disease (ASCUS) six had single infections with either HR-HPV-16, HR-HPV-66, HR-HPV-39, HR-HPV-59 and one had multiple genotypes co-infections (HR-HPV-16 + HR-HPV-31 + HR-HPV-39 + HR-HPV-45 + HR-HPV-51). And five cases of (cervicitis) with single infection genotypes HR-HPV-16, HR-HPV-35, HR-HPV-39 and HR-HPV68 and only one case with multiple co-infection (HR-HPV-39+ HR-HPV-52).

Table 6. Carcinogenic HPV genotypes distribution among HPV-positive squamous intraepithelial lesions

Cytological group	Type of infection			
	Single genotypes infection with HR-HPV	Cases no.	Multiple genotype co-infection with HR-HPV	Cases No.
Cervicitis	35	1	39 + 52	1
	16	2		
	39	1		
	68	1		
ASCUS	66	2	16+ 31 + 39+ 45+ 51	1
	39	1		
	59	1		
	16	2		
LSIL	39	1	39+ 58	1
	66	1		
	16	5	16+ 31 + 52	1
	33	1		
HSIL	16	3	16+ 39	1
	18	1		
Cancer	16	5	16+ 56+ 51	1
			16 + 31+ 56	1

Low-grade squamous intraepithelial lesions (LSIL) had eight single genotypes by HR-HPV-39, HR-HPV-66, HR-HPV-16, HR-HPV-33 and two cases had multiple co-infection genotypes (HR-HPV-39+HR-HPV-58) (R-HPV-16+ HR-HPV-31+ HR-HPV-52) while, In high-grade squamous intraepithelial lesions (HSIL), four single infections had HR-HPV-16, HR-HPV-18, and one multiple genotypes co-infections with (HR-HPV-16 + HR-HPV-39) were found Five cases of cancer had single genotypes infection genotypes includes HR-HPV-16 and two cases with multiple genotypes co-infection (HR-HPV-16 + HR-HPV56 + HR-HPV-51) and (HR-HPV-16+ HR-HPV-31, HR-HPV-56). Itis with noted that the prevalence of HR-HPV 16 was increased with advancing stages of higher grades of disease. Also, HR-HPV-16 appeared as the most frequent a single high-risk genotype detected compared with other high-risk HPV genotypes. On the contrast, HR-HPV-31, HR-HPV-51, HR-HPV-52, HR-HPV-56 and HR-HPV-58 appeared to be the least likely exist a single high-risk genotypes as shown in Table 6. Researchers found In Africa, the five most common HR-HPV genotypes were HPV-16, HPV-52, HPV-35, HPV-18, and HPV-58, while the most common HR-HPV genotypes in Asia are HPV-16, HPV-52, HPV-58, HPV-33, and HPV-53 [35]. Previous study founded the most common HR-HPV genotypes infected Korean women at HSIL stages was HPV-16, followed by HPV-52, HPV-58, and HPV-33 [36]. Another study showed that persistent HPV-16 and HPV-58 infections are risk factors for cervical lesions in Korea [37]. Similarly, to our finding the most aggressive HR-HPV in the development of cervical precancerous lesions and malignant cancer was HPV16 [38]. According to a recent study showed that among women in China, founded that

HHPV16, HPV39, HPV51, and HPV52 the moist common high risk HPV were associated with CIN and HPV16 infection was especially worrying since it aggravates cervical lesions [39].

It is worth noting that access to clinical samples was limited to what was available in clinics during the collection time; therefore, with consideration of the exclusion criteria, the `recruited number eroded up to what was stated in the study. A larger sample size would be useful and initially sought from various stages of cervical lesions to be employed for further statistical analysis.

Conclusion

This study aimed to examine the pattern change in the frequency of prevalence and genotypes distribution of HPV among Iraqi women attending gynecological clinics in Baghdad. Results showed a 43% prevalence of high risk HPV among tested samples with HR-HPV16 (44.9%0 and HR-HPV-39 (14.29%) were relatively high in Iraqi women. The finding of this study indicated the importance of frequent screening of the high-risk HPV to assess the change on epidemiology pattern of spread which may assist in the management of HPV vaccines. Interestingly, the current monovalent vaccines dose not contain HR-HPV-39 in its current compositions, therefore, this study highlighted the importance of including this oncogenic genotype in the future vaccine preparation.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

Authors would like to thank the Gynecology and Oncology Consultation Department, Baghdad Teaching Hospital, Ministry of Health in Iraq. Dr. Nawfal Fawzi Anwer (Consultant Gyneoncologis).

References

[1] Zou, M., Zhang, Q. (2021). miR-17-5p accelerates cervical cancer cells migration and invasion via the TIMP2/MMPs signaling cascade. *Cytotechnology, 73*(4), 619-27.
[2] Castro-Oropeza, R., Piña-Sánchez, P. (2022). Epigenetic and transcriptomic regulation landscape in HPV+ cancers: biological and clinical implications. *Front Genet.*, 1494.
[3] Cubie, H. A. (2013). Diseases associated with human papillomavirus infection. *Virology, 445*(1-2), 21–34.
[4] Tulay, P., Serakinci, N. (2016). The role of human papillomaviruses in cancer progression. *J Cancer Metastasis Treat, 2*(6), 201.
[5] Ryu, W. S. (2016). *Molecular virology of human pathogenic viruses*. Academic Press.
[6] Zhang, L., Bi, Q., Deng, H., Xu, J., Chen, J., Zhang, M., *et al*. (2017). Human papillomavirus infections among women with cervical lesions and cervical cancer in Eastern China: genotype-specific prevalence and attribution. *BMC Infect Dis.*, *17*, 1-9.
[7] Wei, L., Gan, Q., Ji, T. (2017). Cervical cancer histology image identification method based on texture and lesion area features. *Comput Assist Surg., 22*(sup1), 186-99.
[8] Chumduri, C., Gurumurthy, R. K., Berger, H., Koster, S., Brinkmann, V., Klemm, U., *et al*. (2018). Transition of Wnt signaling microenvironment delineates the squamo-columnar junction and emergence of squamous metaplasia of the cervix. *BioRxiv*, 443770.
[9] Deng, H., Hillpot, E., Mondal, S., Khurana, K. K., Woodworth, C. D. (2018). HPV16-immortalized cells from human transformation zone and endocervix are more dysplastic than ectocervical cells in organotypic culture. *2018*(October), 1-13.
[10] Agustiansyah, P., Sanif, R., Nurmaini, S. (2021). Epidemiology and risk factors for cervical cancer. *Biosci Med J Biomed Transl Res., 5*(7), 624-31.
[11] Kagabu, M., Nagasawa, T., Sato, C., Fukagawa, Y., Kawamura, H., Tomabechi, H., *et al*. (2020). Immunotherapy for uterine cervical cancer using checkpoint inhibitors: future directions. *Int J Mol Sci., 21*(7), 2335.
[12] Omoyeni, O. M., Tsoka-Gwegweni, J. M. (2022). Prevalence of cervical abnormalities among rural women in KwaZulu-Natal, South Africa. *Pan Afr Med J., 41*(1).

[13] Duru, C. B., Oluoha, R. U., Uwakwe, K. A., Diwe, K. C., Merenu, I. A., Emerole, C. A., *et al*. (2015). Pattern of PAP smear test results among Nigerian women attending clinics in a teaching hospital. *Int J Curr Microbiol App Sci., 4*(4), 986-98.
[14] Sharma, G. D., Adhikari, R., Parajuly, S. S., Adhikari, K. G. (2019). Cervical Cancer screening in a tertiary care centre by Pap smear and its clinical correlation. *Med J Pokhara Acad Heal Sci., 2*(1), 5.
[15] Imelda, F., Nasution, D. L. (2020). Correlations among age, parity, and contraception using with Pap smear results in Medan Sumtera Sumatera. *Indian J Public Heal Res Dev., 11*(6), 1247-51.
[16] Verma, A., Verma, S., Vashist, S., Attri, S., Singhal, A. (2017). A study on cervical cancer screening in symptomatic women using Pap smear in a tertiary care hospital in rural area of Himachal Pradesh, India. *Middle East Fertil Soc J., 22*(1), 39-42.
[17] Misgina, K. H., Belay, H. S., Abraha, T. H. (2017). Prevalence of precancerous cervical lesion and associated factors among women in North Ethiopia. *J Public Heal Epidemiol., 9*(3), 46-50.
[18] Kassa, R. T. (2018). Risk factors associated with precancerous cervical lesion among women screened at Marie Stops Ethiopia, Adama town, Ethiopia 2017: A case control study. *BMC Res Notes. 11*(1), 1-5.
[19] Tarney, C. M., Han, J. (2014). Postcoital bleeding: A review on etiology, diagnosis, and management. *Obstet Gynecol Int.*
[20] Anorlu, R. I., Abdul-Kareem, F. B., Abudu, O. O., Oyekan, T. O. (2003). Cervical cytology in an urban population in Lagos, Nigeria. *J Obstet Gynaecol (Lahore), 23*(3), 285-8.
[21] Nagelhout, G., Ebisch, R. M., Van Der Hel, O., Meerkerk, G. J., Magnée, T., De Bruijn, T., *et al*. (2021). Is smoking an independent risk factor for developing cervical intra-epithelial neoplasia and cervical cancer? A systematic review and meta-analysis. *Expert Rev Anticancer Ther.,* 21(7), 781-94.
[22] Padmini, C. P., Indira, N., Chaitra, R., Das, P., Girish, B. C., Nanda, K. M., *et al*. (2015). Cytological and colposcopic evaluation of unhealthy cervix. *J Evid Med Heal., 2*, 6920-7.
[23] Jihad, N. A., Naif, H. M., Sabri, E. H. (2020). Prevalence of high risk human papilloma virus among Iraqi women with abnormal cervical cytology. *Gene Reports*, *21*, 100871.
[24] Hasan, M. Y. A., Al Hashimi, B. A., Al Niyazee, A. A., Al Ameen, M. I. (2020). Prevalence of abnormal Pap smears among sample of Iraqi women attending Al Elweiya Cervical Screening Unit in Baghdad. *J Cardiovasc Dis Res., 11*(2), 120-4.
[25] Yousif, M. G., Al-Amran, F. G., Sadeq, A. M., Yousif, N. G. (2023). Prevalence and associated factors of human papillomavirus infection among Iraqi Women. *arXiv Prepr arXiv230714806.*
[26] Adhikari, I., Eriksson, T., Luostarinen, T., Apter, D., Lehtinen, M. (2019). Is the risk of cervical atypia associated with the interval between menarche and the start of sexual activity? A population-based cohort study. *BMJ Open, 9*(9), e030091.
[27] Yu, H., Yi, J., Dou, Y. L., Chen, Y., Kong, L. J., Wu, J. (2021). Prevalence and genotype distribution of human papillomavirus among healthy females in Beijing, China, 2016-2019. *Infect Drug Resist., 14*, 4173-82.
[28] Hou, Y. P., Fan, C., Jiang, Q. Q., Wu, L., Luo, Y. (2023). Prevalence and genotype distribution of human papillomavirus in 92,932 cases in Shanghai, China. *Future Virol*.
[29] Sabah, T. (2018). Expression level of miRNA 744, Bcl- 2, Caspase-3 apoptosis regulator genes and human papilloma virus-HPV in cervical abnormalities.Thesis. University of Baghdad.
[30] Daniel, S., Mohammed, A. S., Ibrahim, N., Hussein, N. R., Balatay, A. A., Naqid, I. A., *et al*. (2022). Human papillomavirus (HPV) genotype prevalence and impact of COVID-19 on the HPV prevention program in Duhok city. *Dialogues Heal.,* 1, 100055.
[31] Liao, G., Jiang, X., She, B., Tang, H., Wang, Z., Zhou, H., *et al*. (2020). Multi-infection patterns and co-infection preference of 27 human papillomavirus types among 137,943 gynecological outpatients across China. *Front Oncol., 10*, 449.
[32] Muñoz, N., Bosch, F. X., De Sanjosé, S., Herrero, R., Castellsagué, X., Shah, K. V., *et al*. (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med.*, *348*(6), 518-27.
[33] Brotherton, J. M. L., Tabrizi, S. N., Phillips, S., Pyman, J., Cornall, A. M., Lambie, N., *et al*. (2017). Looking beyond human papillomavirus (HPV) genotype 16 and 18: defining HPV genotype distribution in cervical cancers in Australia prior to vaccination. *Int J cancer. 141*(8), 1576-84.
[34] So, K. A., Lee, I. H., Lee, K. H., Hong, S. R., Kim, Y. J., Seo, H. H., *et al*. (2019). Human papillomavirus genotype-specific risk in cervical carcinogenesis. *J Gynecol Oncol., 30*(4), e52.
[35] Okoye, J. O., Chukwukelu, C. F., Okekpa, S. I., Ogenyi, S. I., Onyekachi-Umah, I. N., Ngokere, A. A. (2021). Racial disparities associated with the prevalence of vaccine and non-vaccine HPV types and multiple HPV infections between Asia and Africa: A systematic review and meta-analysis. *Asian Pacific J cancer Prev APJCP. 22*(9), 2729.
[36] Seong, J., Ryou, S., Lee, J., Yoo, M., Hur, S., Choi, B. S., *et al*. (2021). Enhanced disease progression due to persistent HPV-16/58 infections in Korean women: a systematic review and the Korea HPV cohort study. *Virol J., 18*, 1–13.

[37] Sigurdsson, K., Taddeo, F. J., Benediktsdottir, K. R., Olafsdottir, K., Sigvaldason, H., Oddsson, K., *et al*. (2007). HPV genotypes in CIN 2-3 lesions and cervical cancer: A population-based study. *Int J cancer. 121*(12), 2682-7.
[38] Saglam, H., Atalay, F. (2023). Effect of Pap smear cytology, HPV genotyping on the concordance of colposcopy and conization results. J *Coll Physicians Surg Pakistan. 33*(9), 972-8.
[39] Wang, J., Li, H., Zhang, J., Wang, H., Li, Y., Liu, Z., *et al*. (2024). Epidemiology and genotypes analysis of human papillomavirus infection in Beijing, China. *Virol J*., 21(1), 19. Available from: https://doi.org/10.1186/s12985-024-02292-3.