Kufa Medical Journal | Vol. 19, No. 1, 2023

Original article | DOI: https://doi.org/10.36330/kmj.v19i1.11146

Submitted at: 29 January 23
Accepted at: 13 March 23

An Alarming Evidence Of Increased HPV Infection In Cervical Smear In Iraqi Patients

Zahraa Jabbar Samari [(1),] Nada Alibraheemi [(2)], Aseel Al-Quzweni [(3)] , Asaad Al- Janabi [(4)], Alaa Salah Jumaah [(5)]

[(1)] Al-Kufa Board Training center, Iraqi Board for medical specialization, Najaf, Iraq.
[(2)] Department of Obstetrics and Gynecology, Faculty of Medicine, University of Kufa, Najaf, Iraq.
[(3)] Al-Sadder teaching hospital, Iraq, Kufa, Najaf, Iraq.
[(4,5)] Department of Pathology and Forensic Medicine, Faculty of Medicine , University of Kufa, Najaf, Iraq.
Corresponding Author Email : alaa.alshammari@uokufa.edu.iq

ABSTRACT

Background: Cervical cancer is one of the common cancers among women worldwide, with increasing incidence. This study was designed to assess the role of cervical cytology in detecting the various cervical lesions predisposed to cervical cancer with the special emphasis on squamous intraepithelial lesions (SIL) and to predict the prevalence of these lesions and their association with Human papilloma virus (HPV).

Patients and methods: This study enrolled 3500 women aged 17 years and above, presented with cervical lesions and attended for cytological evaluation of cervical smears. The data was retrieved from the archives of these patients for the period from January 2017 to December 2021. All results were classified according to Bethesda system (2014) and statistically analyzed.

Results: the cytological examinations revealed that 91.7% of the submitted cases were negative for intraepithelial lesion or malignancy (NILM). Abnormal cellular changes were reported in 8.3% of the cases; Low-grade squamous intraepithelial lesion was the most common abnormality representing 4.7%, while High-grade squamous intraepithelial lesion constituted 0.5% of the total cases. A HPV-DNA test was done to 220 cases; 52 cases (23.6%) were positive, most of them (73%) were of low-risk genotypes while high risk genotypes were reported in 27% of the cases.

Conclusion: the cervical squamous abnormality has formed a low prevalence rate while HPV-DNA test has been detected in a considerable proportion of Iraqi patients. This fact discloses alarming evidence of a growing problem in the population that needs to introduce a screening program for early diagnosis of precancerous cervical lesions.

Keywords: HPV, Pap, And Smear.

INTRODUCTION

Squamous intraepithelial lesions (SIL) and cervical cancer remain important health problems for women with worldwide high morbidity and mortality for advanced lesion [(1)]. Cervical cancer is regarded as a growing burden globally, in both developing and developed nations. In 2012, cervical cancer resulted in an annual mortality of 266,000 and reported 528,000 new cases[(2)]. In 2019, in the United States, it was estimated that 13,179 women would be diagnosed with cervical cancer and that 4250 women would die of the disease[(3)]. An incidence rate for cervical cancer shows a wide geographic variation because of the wide spread differences in the availability of screening programs and the prevalence of risk factors[(4)] .The lowest reported incidence

rates are from the Middle East where the incidence is particularly low among Muslim countries, as compared to other religious groups[5]. According to Iraqi Cancer Registry Center records (2019), the incidence rate about 0.78 /100,000 of the female population, (0.99%) and mortality rate about 0.54 /100,000 (0.30%)[6].

No form of cancer better documents the remarkable benefit of effective screening, early diagnosis, and curative therapy than does the cervical cancer; most credit for these dramatic gains belongs to the effectiveness of Pap test [7]. Pap smear, named for its developer, Dr.George Papanicolaou M.D, is still one of the few tests which can detect the presence of premalignant lesions allowing for the prevention of cancer[8]. Persistent infection with certain types of Human Papilloma Virus (HPV) known as high risk HPV (HR-HPV) is now believed to be a major causal factor in the development of cervical cancer[9]. HPV testing was initially deployed as a triage test for patients with atypical cells that fell short of being diagnosable as SIL; results of HPV testing were used to guide referral for colposcopic examination in this setting. More recently, there has been a move towards either co-testing, i.e., performing both conventional cervical smear cytological examination and HPV testing, or primary HPV screening, where only HPV testing is performed as a screening test with no cervical smear cytology examination[10,11].

This study targeted the assessment of the association of HPV with various cervical abnormality in Iraqi women according to Bethesda system.

PATIENTS AND METHOD

This is a cross-sectional study performed in Kufa Training Centers/ Iraqi Board for Medical Specialization in Pathology. A letter of approval was obtained from / Iraqi Board for Medical Specialization in Pathology. The data sets reviewed for the last five years and clinical and pathological data were retrieved including age, complaint, and diagnosis and HPV status for the investigated cases.

A total of 3500 cases enrolled in the study. Demographic and clinicopathologic data were retrieved including Pap test diagnosis and HPV status. The enrolled cases were classified according to Bethesda system as follows[12]:

- NILM (Negative for intraepithelial lesion or malignancy).
- ASCUS (Atypical squamous cells of undetermined significance)
- ASCUS-H (Atypical squamous cells of undetermined significance cannot exclude HSIL)
- LSIL (Low-grade squamous intraepithelial lesion)
- HSIL (High-grade squamous intraepithelial lesion)
- Cervical carcinoma.

HPV- DNA test was done for 220 cases; the molecular detection and genotyping of HPV viral DNA was done by PCR and reverse dot blot hybridization using HPV direct flow ship. HPV Direct Flow CHIP Kit (Spain) is intended for simultaneous screening and genotyping of 36 HPV types (High risk- HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82-), and) low risk- HPV 6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81, 84 and 89) by PCR (polymerase chain reaction), followed by reverse dot blot automatic hybridization, based on DNA-Flow Technology. Clinical samples (fresh and paraffin embedded) are amplified directly, without the need of DNA extraction.

Statistical analysis: The results were analyzed by using Microsoft Excel version 2013, SPSS Version 26, and Chi-Squared test was used to test associations. A P-value of ≤ 0.05 was considered significant.

RESULTS

Mean age of the studied group is (34.309±9.161) and ranged between (20-73) yrs. Detailed Pap smear findings according to Bethesda system shown in Table. 1.

Table No.1. Detail Pap Smear Findings According to Bethesda System

Cellular changes	No.	Percentage
Benign cellular changes (NILM)	3210	91.7%
Abnormal cellular changes	290	8.3 %
Total	3500	100 %
Bethesda System Findings Classifications of Findings		
Pap Results	No. of Cases	Percentage
NLIM	3210	91.7%
ASCUS	99	2.8%
ASCUS-H	6	0.2%
LSIL	164	4.7%
HSIL	18	0.5%
SCC	3	0.1%
Total	3500	100%

On other hand, there is highly significant differences between HPV expression and Pap test results Table. 2 .

Table No.2. HPV expression and results of Pap Test According to Bethesda System.

	HPV-DNA test			P Value
	Positive	**Negative**	**Total**	
NLIM	0 (0.0%)	145 (65.9%)	145 (65.9%)	
ASCUS	16 (7.3%)	16 (7.3%)	32 (14.5%)	
LSIL	34(15.5%)	7 (3.2%)	41(18.6%)	
HSIL	2 (0.9%)	0 (0.0%)	2 (0.9%)	<0.001
Total	52 (23.6%)	168 (76.4%)	220 (100.0%)	

Table.3 displays the relation between HPV genotype and risk group which is highly significant.

Table No.3. HPV Genotype in Correlation to Risk Groups

Group	Genotype			P Value
	Single infection	Multiple infection	Total	
		High Risk		
16	1 (1.9%)	1(HPV16+HPV45) (1.9%)	2 (3.8%)	
18	0 (0.0%)	0 (0.0%)	0 (0.0%)	
33	1 (1.9%)	0 (0.0%)	1 (1.9%)	
39	1 (1.9%)	1(HPV39+HPV56) (1.9%)	2 (3.8%)	
45	2 (3.8%)	1(HPV45+HPV16) (1.9%)	3 (5.8%)	
51	1 (1.9%)	0 (0.0%)	1 (1.9%)	
52	0 (0.0%)	1(HPV52+HPV68) (1.9%)	1 (1.9%)	
53	1 (1.9%)	1(HPV53+HPV11) (1.9%)	2 (3.8%)	
56	0 (0.0%)	1(HPV39+HPV56) (1.9%)	1 (1.9%)	
68	0 (0.0%)	1(HPV52+HPV68) (1.9%)	1 (1.9%)	
		Low Risk		
6	14 (26.9)	0 (0.0%)	14 (26.9%)	
11	20 (38.5)	2(HPV53+HPV11) (HPV11+HPV43) (3.8%)	22 (42.3)	<0.001
40	1 (1.9%)	0 0.0%	1 (1.9%)	
43	0 (0.0%)	1(HPV11+HPV43) (1.9%)	1 (1.9%)	
Total	42 (80.8%)	10 (19.2%)	52 (100.0%)	

Meanwhile, there is a high significant correlation between molecular genotype of HPV and results of Pap test (Table. 4).

Table 4. Molecular Risk of HPV in Correlation to Pap Test results.

Pap Test	Risk Group			P Value
	Low risk	High risk	Total	
ASCUS	12(23.1%)	4(7.7%)	16(30.8%)	
LISL	26(50.0%)	8(15.4%)	34(65.4%)	0.059
HISL	0(0.0%)	2(3.8%)	2(3.8%)	
Total	38(73.1%)	14(26.9%)	52(100.%)	

This study shows that there are high significant differences in specific HPV genotypes and Pap test results (Table. 5).

Table 5. Specific HPV Genotype and Pap Test Results.

HPV Genotype	Pap Test				P value
	ASCUS	LSIL	HSIL	Total	
		High Risk			
16	0(0.0%)	2(3.8%)	0(0.0%)	2(3.8%)	<0.001
33	0(0.0%)	1(1.9%)	0(0.0%)	1(1.9%)	
39	0(0.0%)	2(3.8%)	0(0.0%)	2(3.8%)	
45	2(3.8%)	1(1.9%)	0(0.0%)	3(5.8%)	
51	1(1.9%)	0(0.0%)	0(0.0%)	1(1.9%)	
52	0(0.0%)	0(0.0%)	1(1.9%)	1(1.9%)	
53	1(1.9%)	1(1.9%)	0(0.0%)	2(3.8%)	
56	0(0.0%)	1(1.9%)	0(0.0%)	1(1.9%)	
68	0(0.0%)	0(0.0%)	1(1.9%)	1(1.9%)	
		Low Risk			
6	4(7.7%)	10(19.2%)	0(0.0%)	14(26.9%)	
11	8(15.4%)	14(26.9%)	0(0.0%)	22(42.3%)	
40	0(0.0%)	1(1.9%)	0(0.0%)	1(1.9%)	
43	0(0.0%)	1(1.9%)	0(0.0%)	1(1.9%)	
Total	16(30.8%)	34(65.4%)	2(3.8%)	52(100.0%)	

DISCUSSION

Prevention of cervical cancer gains a worldwide effort. Screening program was developed to diagnose and manage cancer in its early stages or even in the precancerous state then preventing cancer progression[13]. Historically, Pap test is the exclusive screening program and its was effective in detecting 50-70% of precancerous cervical lesion. Recently, HPV testing is introduced as a screening program and this test is more effective and more reproducible than Pap test[14].

This study showed that prevalence of carcinogenic HPV in Iraqi women was relatively low (6.4%) and this finding agrees what was found by others[15.16]. The Islamic principles and morals committed to by the Iraqi community could explain the low incidence of HPV infection and other sexually transmitted diseases in Iraqi patients.

In respect to viral genotypes, the percentages of carcinogenic HPV genomes are (5.8%, 3.8%, 3.8%, 3.8%, 1.9%, 1.9%, 1.9%, 1.9% and 1.9%) in (HPV 45, 16, 39, 53, 33, 51, 52, 56 and 68) respectively. Genotype-45 is the most common type (5.8%), followed by HPV16 (3.8%). Several studies in Iraq displayed contradictory findings stating that type 16 is the commonest type in the country[17]; others state that HPV-33 (18.60%) is predominant type in Iraq (16). Further, Al-Awadhi et al[17] state that HPV16 is the first predominant type in Kuwait, followed by HPV66, HPV33, and HPV-53 constituting (54.6%) of the women with high risk genotypes. Tjalma WAA et al (2012)[18] reported that type-specific screening tests should focus not exclusively on HPV-16 and HPV-18, but also on HPV-45. HPV types 16, 18, and 45 are regarded as the commonest types in cervical cancer. At the same time, they

represent about 75% of squamous cell carcinoma and 94% of adenocarcinoma. Sanjose et al[19] suggested that screening test based on high risk type HPV testing should focus on HPV types 16, 18 and 45.

The difference in HPV genotypes prevalence could be attributed to differences in samples size, geographic area, techniques used and different primer pairs. Besides, maybe some genotypes of HPV virus is widespread at that time, and over the years other HPV genotypes may be circulated around the country and dominate in particular regions.

The prevalence of HPV in different ages worldwide vary according to economic status, moral, social and religious situation, age of women at first intercourse, lifetime number of partners of women as well as their male sexual partners[20]. This study showed high percentages of HPV infection in women in their childbearing age (20-39 years). The same has been found by others in Iraq (Alizi S et al 2018)[15] and (Faik AJ et al 2015)[16].

In the present study, the detection of high risk HPV especially in ASCUS lesions highlights the benefit of HPV genotyping to predict unscreened women at risk of developing cervical cancer especially in the absence of perfect screening program; the same findings have been found by others [16, 17].

Several studies showed controversial findings in detecting rates of HPV-DNA in patients with cervical lesions. Here, several factors may be proposed as genetic make-up variation, age group of patients, targeted DNA region and different circulating viral genotype as well as investigation methodology. On other hand, other risk factors as biological predisposition of immature cervix as well as immunodeficiency could influence the prevalence of HPV[20].

Limitations of the Study

Inadequate clinical data like age at marriage, number of parity, any history regarding intrauterine contraceptive devices, smoking, exposure to radiations and others did not permit the study to adequately analyze the prognostic variables, and management.

CONCLUSION

Cervical squamous abnormality has formed a low prevalence rate, while HPV DNA test was detected in a considerable proportion of patients in the region of the study. This fact discloses alarming evidence of growing problem in the studied population that needs to introduce a screening program for early diagnosis of precancerous cervical lesions.

Author contributions

(I) Conception and design: ZJS, NA**I.**

(II) Administrative support: ASJ, AAQ

(III) Provision of study materials or patients: AAQ, AAJ.

(IV) Collection and assembly of data: ASJ, ZJS, AAI**.**

(V) Data analysis and interpretation: ZJS, NAI**,** AAJ.

(VI) Manuscript writing: All authors

(VII) Final approval of manuscript: All authors

Acknowledgement: not applicable

Funding: no funding were received by the authors.

"Conflicts of Interest: The authors have no conflicts of interest to declare.

REFERENCES

1. Vasnik GK,Jain G,Hussainy FA,Bansal V. Correlation of Cervical Squamous Intraepithelial Lesions with Human Papillomavirus in Women Infected with the Human Immunodeficiency Virus. Indian Journal of Gynecologic Oncology,2019;17:(6):5-7.
2. Vu M, Yu J, Awolude OA, Chuang L. Cervical cancer worldwide. *Curr Probl Cancer*. 2018;42(5):457-465.

doi:10.1016/j.currproblcancer.2018.06.003

3. Cervical Cancer Statistics. Centers for Disease Control and Prevention. Cervical Cancer Statistics | CDC. Last accessed on February 26, 2023
4. Terán-Hernández M, Ramis-Prieto R, Calderón-Hernández J, Garrocho-Rangel CF, Campos-Alanís J, Ávalos-Lozano JA, Aguilar-Robledo M. Geographic variations in cervical cancer risk in San Luis Potosí state, Mexico: A spatial statistical approach. International Journal for Equity in Health,2016;15(16):3-5.
5. Gamaoun R. National cervical cancer prevention program in the Arab States: Strategies and cost-minimization study of the Tunisian case.Science direct Journal,2018;2-5.
6. Annual Report,Iraqi Cancer Registry.Statistical records,2019.
7. Petry UK. HPV and cervical cancer. Scandinavian Journal of Clinical & Laboratory Investigation,2014;74:60-61.
8. Bispo Pereira EH, Camilo-Júnior DJ, Correa Garcia Pires D'ávilla S, Xavier-Júnior JC. Cervical cytology results among pregnant and non-pregnant women in Brazil. *Eur J Obstet Gynecol Reprod Biol*. 2023;282:161-167. doi:10.1016/j.ejogrb.2023.01.027
   Goldblum JR,Lamps LW,McKenny JK,Myers JL.Rosai and Ackermans surgical pathology.11th edit,2018:1281.
9. Avila JP, Carvalho BM, Coimbra EC. A Comprehensive View of the Cancer-Immunity Cycle (CIC) in HPV-Mediated Cervical Cancer and Prospects for Emerging Therapeutic Opportunities. *Cancers*. 2023; 15(4):1333. https://doi.org/10.3390/cancers15041333.
10. Bhatla N,Singhal S. Primary HPV Screening for Cervical Cancer. Best Practice & Research Clinical Obstetrics & Gynaecology Journal,2020;65:1-30.
11. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*. 2002;287(16):2114-2119. doi:10.1001/jama.287.16.2114.
12. World Health Organizations ;Global strategy to accelerate the elimination of cervical cancer as a public health problem, Geneva:World Health Organization 2020:28.
13. Cervical cancer screening programs: making the case for risk-based guidelines,2018:5.
14. Alizi S,Jasim W,Sami H, Alkhafaji B. Detection and Genotyping of Human Papillomavirus DNA in Cervical Neoplasia of Iraqi Women Using Real Time PCR Technique .Journal of dental and Medical Sciences,2018;17(8):p2-4.
15. Faik AJ,Saber M Q,Mohammed W J,Ibraheem B Z,Lateef K R,Hassan A S. Genotyping of High-risk Human

Papilloma virus (HPV) among Iraqi women in Baghdad by Multiplex PCR. Journal of Biotechnology Research Center,2015;9(1):p 40-42.

16. Jihad NA,Naif HM,Sabri EH. Prevalence of high risk human papilloma virus among Iraqi women with abnormal cervical cytology.Science Direct Journal,2020;2.
17. Al-Awadhi R,Chehadeh W,Jaragh M,Al-Shaheen A,Sharma P,Kapila K. Distribution of Human Papillomavirus Among Women with Abnormal Cervical Cytology in Kuwait.Diagnostic cytology Journal,2011;0(0):6.
18. Tjalma WAA,Depuydt CE.Don't Forget HPV-45 in Cervical Cancer Screening .American Journal of Clinical Pathology,2012;137(1):161.
19. Sanjose SD,Quint WGV,Alemany L,Geraets DT,Klaustermeir JE,Tous S et al . Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study.Science Direct Journal,2010;11(11):1053.
20. Vinodhini K ,Shanmughapriya S ,Das BC ,Natarajaseenivasan K. Prevalence and risk factors of HPV infection among women from various provinces of the world. Archives of Gynecology and Obstetrician,2012;285:771-777.